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Mobay

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Bayer



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INIT 07/14/94

Mobay Corporation
A Bayer USA INC. Company

Health, Environment
& Safety Department

March 31, 1987



84940000122

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Ms. Roberta Wedge
Dynamac Corporation
11140 Rockville Pike
Rockville, MD 20852

Dear Ms. Wedge:

In response to your request, please be advised we do not recognize the product name as written for DISPERSE BLUE 79. We have several products with this Color Index Number. Please give us the correct Mobay product name, so that we can send you the appropriate Material Safety Data Sheet.

If we may be of any further assistance, please feel free to contact us. Thank you for your interest in our products.

Sincerely,

Janet Mostow
Regulatory Compliance Rep. Sr.

JM2497ps

cc: J. Gerulis

Writer's Direct Dial Number

p. 11

- acute oral + skin
lethal doses
- skin irritation

TDI
Purification

TDI

MDI

MDI

Fluro

triphenylmethane triisocyanate
levels
important

acute irritation
skin irritation
eye irritation

TOXICITY AND SAFE HANDLING OF ISOCYANATES

*A Review of the Literature (TDI) and
Results of Toxicity Screening Studies on Additional Compounds*

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Published in the interest of the urethane chemical industry by

MOBAY CHEMICAL COMPANY

PENN. LINCOLN PARKWAY WEST
PITTSBURGH, PA. 15205

1961

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→ N/A, see p. 5

TOXICITY AND SAFE HANDLING OF ISOCYANATES: A Review of the Literature (TDI) and Results of Toxicity Screening Studies on Additional Compounds

I. Introduction

Many industrial chemicals are capable of producing damage to man if potential health hazards are not recognized, and if means are not provided to protect those who may be exposed. The usual methods of reducing exposure to workers, i.e. exhaust ventilation or personal protective equipment, obviously do not change the toxicity or possible toxicological effects of the chemical; it is the exposure or hazard which must be controlled or eliminated. Too frequently there is misunderstanding or misinterpretation of toxicity data—particularly when known toxic effects in animals form the basis for predicting possible toxic effects in man. As a result, potential hazards may be overestimated or—more important from the standpoint of the workers' health—underestimated.

Animal toxicity data are important only because they allow intelligent evaluation of potential hazards to man. For example, a chemical which is highly toxic to laboratory animals when administered orally, but is not absorbed readily through the unbroken skin or through the respiratory tract, presents no hazard in industry except for accidental ingestion. At the same time, a chemical which may demonstrate a low order of toxicity in animals, from the standpoint of oral administration and skin absorption, may be hazardous to man because of physiological effects following repeated exposures or breathing of low vapor concentrations. The latter comment appears applicable to tolylene di-

isocyanate and probably to those other isocyanates whose vapor pressures are such that significant vapor concentrations may be encountered in workrooms.

II. Industrial Experience

More important than animal toxicity data is industrial experience in the United States and abroad which indicates that the isocyanates may be manufactured, shipped, handled and used without difficulties if proper precautions are followed.

Vapor inhalation must be avoided not only because of the bronchial irritation resulting from exposures to high concentrations for short durations, but also because a significant percentage of workers may develop sensitization and subsequent temporarily disabling asthma-like attacks. Apparently such sensitization can follow a few exposures of short duration to relatively high vapor concentrations or a larger number of exposures to concentrations below levels of detection by odor or irritation.

Precautions to avoid skin and accidental eye contact are also in order. The vapors of the more volatile compounds cause severe lacrimation. The handling of isocyanates is safe, provided the standard safety precautions for handling hazardous chemicals are established and followed.

III. Safe Handling and Use Precautions

In brief, precautions recommended for the safe handling of tolylene diisocyanate (TDI) include:

- A. Avoidance of spills and the use of adequate respiratory protective masks in cleaning up spills if they do occur.
- B. Provision of adequate exhaust ventilation to remove vapors during the handling or usage of TDI in permanently fixed processing equipment (e.g. polyurethane foam machines, wire coatings, spray applications, etc.).
- C. Provision and use of organic cartridge respirators in operations such as foam insulation spraying, some surface coating applications and in temporary small scale handling of TDI in open containers where the cost of mechanical exhaust ventilation cannot be justified.
- D. Medical screening of employees to prevent exposure of those known to be susceptible to allergies or asthma.
- E. Sampling and analysis of the workroom atmosphere to indicate the adequacy of control ventilation.*
- F. Immediate removal and reassignment to other jobs when any worker develops symptoms of sensitivity due to inadequate control, accidental exposure or pre-existing sensitivity.

For an excellent, recent publication on the safe handling of tolylene diisocyanate, the reader is referred to the following: *Properties and Essential Information for Safe Handling and Use of Tolyene Diisocyanate*, Chemical Safety Data Sheet SD-

*An available test kit for the determination of low concentrations of vapors of tolylene diisocyanate and tolylene diisocyanate urea can be obtained from Mine Safety Appliance Company, 201 North Braddock Avenue, Pittsburgh 9, Pennsylvania.

73, 1959, Manufacturing Chemists' Association, Incorporated, Washington 9, D. C.

From the standpoint of potential health hazards, prepolymer resins containing excess isocyanate are usually more free from discernible toxicological effects than the raw isocyanate itself, since part of the isocyanate is consumed by reaction with a polyol or other active hydrogen containing materials. It should be pointed out, however, that isocyanate prepolymer or adduct resins can vary widely in their monomeric (unreacted) isocyanate content. As may be expected, the potential health hazard is in proportion to the monomeric TDI content in the prepolymer (See Section V below).

Until more complete data (or handling experience) with other isocyanates develop, precautions similar to those pointed out above, should be exercised with mixtures or prepolymers containing free TDI and with other liquid isocyanates. The solid materials have insignificant vapor pressures at room temperature, but precautions to avoid breathing dust or vapors of these isocyanates when melted are similar to those for avoiding inhalation of vapors of the more volatile materials.

IV. Polyurethane Foams

Animal toxicity studies and human experience indicate that the polyurethane end-products are physiologically inert in all common applications. (One series of animal experiments related to a highly specialized application, viz., prosthetic appliances, has indicated a reaction when a polyurethane was implanted within body tissues over an extended period. We do not recommend this application based on the present state of our knowledge.) To indicate the low degree of hazard if polyurethane foam is accidentally ingested, a typical polyester foam was fed daily to rats over a five day period in amounts

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Manufacturing Chemists' Association, Washington 9, D. C. From the standpoint of potential health hazard, prepolymer resins containing isocyanate are usually more free from the toxicological effects than the isocyanate itself, since part of the isocyanate is consumed by reaction with other active hydrogen containing materials. It should be pointed out, however, that isocyanate prepolymer or resin can vary widely in their residual (unreacted) isocyanate content. Therefore, the potential hazard is in proportion to the monomeric TDI content in the prepolymer (see Table V below).

More complete data (or handling instructions) with other isocyanates and urethane adducts, should be exercised with mixtures of prepolymer containing free TDI and other liquid isocyanates. The materials have insignificant vapor pressure at room temperature, but precautions should be exercised with mixtures of prepolymer containing free TDI and other liquid isocyanates. The materials have insignificant vapor pressure at room temperature, but precautions should be exercised with mixtures of prepolymer containing free TDI and other liquid isocyanates. The materials have insignificant vapor pressure at room temperature, but precautions should be exercised with mixtures of prepolymer containing free TDI and other liquid isocyanates.

Polyurethane Foams

Toxicity studies and human experiments indicate that the polyurethane foams are physiologically inert in most applications. (One series of experiments related to a highly volatile application, viz., prosthetic devices, has indicated a reaction when polyurethane was implanted within the body over an extended period. We recommend this application based on the present state of our knowledge.) The low degree of hazard if polyurethane foam is accidentally ingested, as in the case of a child who ate a piece of polyester foam was fed daily for a five day period in amounts

equivalent to 7.5 grams per kilogram. No outward evidence of toxicity was noted and the foam was excreted unchanged. Furthermore, rabbits ate a total of 10.75 grams per kilogram over the same period with no apparent effect. In all feedings, the foam was coarsely ground.

Additional samples of the same polyester foam were used for the standard Schwartz patch test with 200 human subjects. No cases of irritation or sensitization resulted. These results, however, do not preclude the possibility that residual additives and catalysts other than those used in preparing the standard polyester foam may cause irritation and sensitization in contact with the skin.

Data are available demonstrating that the decomposition products when polyurethane foams are pyrolyzed are no more toxic than the products of combustion of other commonly used foams.¹

It must be mentioned, however, that the products of incomplete combustion, from burning urethane foams or from soldering urethane enameled wire, may contain isocyanate groups. Thus burning of polyurethane foams and elastomers or soldering of urethane enameled wire should be done in a well ventilated area and personnel within the immediate vicinity of such an operation should be equipped with a proper respirator in order to minimize any hazard.

V. Polyurethane Adducts Mondur® CB Isocyanate

As discussed above, isocyanate terminated adducts or prepolymer resins are potentially less hazardous than the raw isocyanate itself, but can vary widely in their monomeric or unreacted isocyanate content. Mondur CB, an isocyanate adduct of toluene diisocyanate with trimethylolpropane at an NCO/OH ratio of 2:1, has been specially processed to remove any undesirable excess of free TDI. Mondur CB is marketed by Mobay in a solvent solution at a 60% or 75% solids solution (Mondur CB-60 and CB-75) for urethane surface coatings applications.

Careful studies of the vapor present in rooms where painting operations were carried out have been made to determine the actual amount of free TDI present when Mondur CB was used in the paint vehicle. An air analysis method somewhat similar to that reported by Zapp was used.¹

In one test, painting was carried out in a room with restricted ventilation, representing an unfavorable condition. Air samples were taken near the mouth of the painter by attaching the flask containing the TDI-detecting reagent to the chest of the painter. Four different types of isocyanate and isocyanate adducts were used and the analyzed air showed the presence of TDI vapor in the air after painting in the amounts shown in Table I.

TABLE I

	Monomeric TDI in Isocyanate Component (%)	Amount of Paint Employed, g.	Air Analysis TDI parts per Million
TDI	100	330	0.19
Mondur C	9	320	0.03
Mondur CA	2	375	0.013
→ Mondur CB	1	380	0.006

In other tests 100 liters of air were blown through containers of paper coated with fresh urethane varnishes for a period of 90 minutes. The amount of TDI detected in the air issuing was determined. The results in Table II were obtained.

In both experiments, therefore, the measured concentration of free TDI vapor found in the air when Mondur CB was used, was far below the permissible threshold of 0.1 ppm.* Experience in Mobay's coating laboratories in spraying and brushing many hundreds of test panels over several years has never at any time given any indication of ill-effects upon operators. For example, the concrete floor of a completely closed room was painted using a standard Mondur CB/Multron® Polyester coating for concrete. No odor of TDI was detectable in the room at any time and the Mine Safety Appliance TDI analyzer showed no indication of TDI in the air.

Similar experience concerning the lack of hazard with Mondur CB type coatings was reported by Pfeuger.¹¹

Although the above data indicate that Mondur CB type urethane coating systems are relatively hazard free, it is recommended that the coatings applicator and others in the immediate area be equipped with a suitable respirator in order to minimize hazards from solvent vapors. This precaution is certainly necessary for spray operations which may be carried out in

*Refer to p. 7—paragraph 4.

a confined area or those operations that make use of an isocyanate component which has a monomeric TDI content significantly greater than Mondur CB. Likewise, when curing urethane surface and wire coatings, foams and elastomers in a confined area by means of heat, an exhaust system should be installed since isocyanate vapors will collect, and respirators should be worn by personnel who must enter the curing area.

VI. Review of Literature— Toxicological Effects of Tolylene Diisocyanate

There have been instances where failure to provide adequate ventilation control or personal respiratory protective devices has resulted in cases of disability. Woodbury² in 1936 reported eight cases of broncho-pulmonary symptoms ranging from mild attacks of cough and tightness in the chest when the employee was exposed briefly to high concentrations of tolylene diisocyanate vapor, to incapacitating asthma whenever a sensitized employee was exposed to the slightest odor of the substance. Johnstone³ has discussed symptoms in seventeen workers (exposed to tolylene diisocyanate vapors) describing the "severe cases" as characterized by a constriction of the chest, cough and dyspnea." Sands⁴ in describing "Engineering and Medical Control of Exposures in Polyurethane Foam Manufacturing" mentions that during two months (September to November 1955) follow-

TABLE II

<u>Polyisocyanate Used</u>	<u>TDI in Varnish milligrams</u>	<u>TDI found in Air parts per million</u>
Mondur C	652	0.49
Same after 2 hours	652	0.3
Same + 1% Zinc naphthenate	652	0.035
Mondur CA	150	0.1
Mondur CB-75	52	0.03
Mondur CB-75 + 2% Zinc Naphthenate	52	0.0086

and area or those operations that contain an isocyanate component as a monomeric TDI content is usually greater than Mondur CB. When curing urethane surface coatings, foams and elastomers in a confined area by means of heat, an exhaust system should be installed since the vapors will collect, and respirators should be worn by personnel who enter the curing area.

**Review of Literature—
Toxicological Effects of
Toluene Diisocyanate**

There have been instances where failure to provide adequate ventilation control and no respiratory protective devices used in cases of disability. Woodworth¹ in 1956 reported eight cases of pulmonary symptoms ranging from mild attacks of cough and tightness of the chest when the employee was exposed briefly to high concentrations of toluene diisocyanate vapor, to severe asthmalike attacks when a sensitive employee was exposed to the slightest concentration of the substance. Johnstone² has reported symptoms in seventeen workers exposed to toluene diisocyanate vapors (including the "severe cases" as characterized by a constriction of the chest, cough and wheezing). Sands³ in describing "Engineering and Medical Control of Exposure in Polyurethane Foam Manufacturing" mentions that during two months (October to November 1955) follow-

ing the introduction of this type of manufacturing, "forty-two cases of respiratory irritation were ascribed to TDI exposures. Of these forty-two cases, nine were serious enough to require hospitalization."

Walworth and Virchow⁴ reporting similar experiences in a plant making flexible polyurethane foam emphasize the lack of correlation between effects of TDI and atmospheric concentration of vapors to which workers were exposed.

The references cited above relating to experience in the United States were preceded by publications^{5,6,7,8,9} in foreign journals discussing symptoms in workers exposed to TDI and including summaries of animal toxicity studies. In the first publication describing industrial exposures⁵ Swensson⁵ points out that Fuchs and Valade in 1951 stated "that during a first period, varying between eight days and two months, the workers had no serious trouble but merely complained of a slight irritation of the mucous membrane of the conjunctiva, slight lacrimation and prickling sensations in the throat. After this, appears progressive bronchial irritation with an irritating dry cough and dyspnea, which may be at its worst during the night and prevent sleep. Asthmatic attacks may also occur. The symptoms recede quite rapidly when the exposure is discontinued, only to return in many cases in the form of an acute attack on renewed, even very short and slight, exposure." Further, Fuchs and Valade pointed out that animal studies indicate that the mixed toluene diisocyanates were respiratory irritants if inhaled, although not highly toxic by subcutaneous injection or skin absorption.

Zapp¹ has published the results of animal toxicity studies with TDI including effects following oral administration (acute and subacute), skin and eye application, and acute and subacute inhalation experiments. The latter led to his suggestion that the maximum concentrations of TDI in the atmosphere of the working

environment should not exceed 0.1 part per million parts of air. On the basis of observations made by twenty-four men, Zapp reported the least detectable odor to 50% of them as 4 times this level or 0.4 ppm and that irritation of the nose and throat occurred at 0.5 ppm.

Subsequent review of industrial experience by the American Conference of Governmental Industrial Hygienists has led to a suggested maximum hygienic threshold limit of 0.02 ppm. This recommendation has arisen from a report to this group of a limited number of cases of respiratory difficulty, where atmospheric concentrations were never found to exceed 0.05 ppm. It is believed, however, that where such cases occurred, the individuals must have been exposed to concentrations in excess of 0.1 ppm for indeterminate periods where these higher concentrations were not detected. Pending further evaluation of the data, and development of improved air sampling techniques, it is urged that maximum effort be exercised to keep exposure to a minimum and not to exceed the 0.02 ppm level.

The respiratory difficulties following inhalation of TDI vapors as discussed above could occur following exposures to other isocyanate compounds such as hexamethylene diisocyanate or molten phenylene diisocyanate. Our knowledge of isocyanates other than TDI is limited by the following factors:

- A. Industrial experience and thus exposure to isocyanates other than TDI has been limited since production and usage have not developed as widely.
- B. The vapors of several of the isocyanates, including ethyl isocyanate, cause lacrimation at such low levels that workers will not voluntarily expose themselves to concentrations sufficient to cause any other than lacrimatory effects.

Marsh Rams	TDI found in Air parts per million
52	0.43
52	0.3
52	0.035
50	0.1
52	0.03
52	0.0086

C. Several of the promising di- and triisocyanates have such low vapor pressures that atmospheric vapor concentrations are insignificant.

The chemical similarity and responses of animals to large doses of most of the isocyanates suggest that the respiratory effect of vapors of the liquid isocyanates and possibly dusts of the solid isocyanates may be the same as in the case of TDI. Until evidence to the contrary develops from animal toxicity research or from more extensive industrial experience, this assumption appears to be justified.

VII. Results of Animal Toxicity Studies

Mobay Chemical Company has sponsored animal toxicity studies to determine the acute effect of eleven isocyanates and one isocyanate adduct having a slight amount of free TDI. The isocyanates include the following:

2, 4-tolylene diisocyanate
ethyl isocyanate
n-butyl isocyanate
octadecyl isocyanate
phenyl isocyanate
p-chlorophenyl isocyanate
p-phenylene diisocyanate
hexamethylene diisocyanate
1, 5-naphthalene diisocyanate
4, 4'-diphenylmethane diisocyanate
triphenylmethane triisocyanate
isocyanate adduct*

A. Acute Oral Toxicity

As shown in Table III, approximate lethal doses were determined. These dosages were found by the introduction of measured single doses of the compound (undiluted, in corn oil or aqueous suspension) into the stomachs of female and male Sprague Dawley strain rats by means of

*The isocyanate adduct investigated was Moudur CB, a reaction product of tolylene diisocyanate with trimethylolpropane at an NCO/OH ratio of 2:1.

a rubber catheter attached to a hypodermic syringe. In the case of ethyl isocyanate, n-butyl isocyanate, para-chlorophenyl isocyanate and hexamethylene diisocyanate, sufficient number of animals were used with dosage levels limited to a narrow range so that the dose indicated represents a fairly accurate LD₅₀. In the case of triphenylmethane triisocyanate, the sample was provided as a 20% solution in methylene chloride. The lethal dose of 4.9 grams per kilogram indicated in the table undoubtedly represents the lethal dose of this chlorinated hydrocarbon rather than the triisocyanate.

In general, physiological and pathological effects were similar, varying in degree in relation to the magnitude of the lethal dose. With the more toxic materials (ethyl and phenyl and n-butyl isocyanates and hexamethylene diisocyanate), outward symptoms of toxicity included lethargy, marked discomfort, convulsions, labored breathing and coma. Macroscopic examination revealed irritation of the stomach and the intestinal and urinary tracts; discoloration and congestion of the liver and kidneys. Although considerably less toxic than the four compounds mentioned above, parachlorophenyl isocyanate caused similar effects.

Octadecyl isocyanate fed undiluted in doses up to and including 30.0 grams per kilogram caused moderate discomfort, diarrhea, loss of appetite and temporary weight losses.

Diphenylmethane diisocyanate, as a 75% dilution in corn oil, was fed in varying doses up to 31.6 grams per kilogram. This highest dose produced one death from a single dose. Autopsy revealed a substantial quantity of the chemical in the stomach and since this was the only death, it was concluded that death could have resulted from overloading the stomach. Multiple doses fed over a two or three day period, at a concentration of 25.1 grams per kilogram, resulted in diarrhea and loss

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attached to a hypoder-
the case of ethyl isocya-
yanate, para-chlorophen-
yl hexamethylene diisocya-
nate, number of animals were
levels limited to a nar-
row range indicated rep-
resentative LD₅₀. In the case
of triisocyanate, the
was fed as a 20% solution
in corn oil. The lethal dose of
100.4 grams per kilogram
indicated in the
table represents the lethal
dose of chlorinated hydrocarbon
diisocyanate.

Physiological and pathologi-
cal, varying in degree
of magnitude of the lethal
dose of toxic materials (ethyl
hexamethylene diisocyanates and
hexamethylene diisocyanate), outward
toxicity included lethargy,
stiffness, convulsions, labored
breathing. Macroscopic exami-
nation of the stomach
and urinary tracts;
congestion of the liver
though considerably less
for compounds mentioned
para-chlorophenyl isocyanate
effects.

Isocyanate fed undiluted in
corn oil including 30.0 grams per
kilogram moderate discomfort,
loss of appetite and temporary
diarrhea. In the case of
para-chlorophenyl diisocyanate, as a
solid in corn oil, was fed in vary-
ing amounts from 1.6 grams per kilogram
to 100.4 grams per kilogram. The
highest dose produced one death
out of five. Autopsy revealed a
concentration of the chemical in the
stomach. This was the only death,
and that death could have
been prevented by overloading the stomach.
The animals died over a two or three day
period with a concentration of 25.1 grams
per kilogram resulted in diarrhea and loss

of weight without death. The greatest mul-
tiple dose was 100.4 grams per kilogram
which was divided into four doses of 25.1
grams per kilogram each, fed every other
day. No deaths resulted and no signs of
systemic toxicity were observed through-
out the entire feeding tests.

Although deaths resulted in animals re-
ceiving higher doses than those indicated
for para-phenylene diisocyanate and 1,5-
naphthalene diisocyanate, death appeared
to be due to caking of the material causing
blockage of the stomach. In lower doses,
the compound appeared to be excreted
practically unchanged resulting in no out-
ward signs of toxicity.

The isocyanate adduct, Mondur CB,
was fed as a 50% suspension of the solid
adduct in corn oil to Sprague-Dawley
strain albino rats. Dosages up to and in-
cluding 25.0 grams per kilogram fed over
a period of three days were non-lethal.
Much of the compound appeared to be
eliminated with little or no change.

B. Subacute Oral Toxicity

Following the acute oral studies, each of
a group of six rats per sample was fed
daily for 10 days over a two-week period,
a dose approximately one-fifth of the
lethal dose or one-fifth of the dose toler-
ated without death in the case of those
samples where lethal doses were not ob-
tained. Table IV indicates the daily doses
fed, the percentage weight changes at ter-
mination and deaths occurring during
feeding of samples. Observations were
made for outward signs of toxicity during
the feeding and 10 days following the last
administration at which time animals were
sacrificed for microscopic examination of
the heart, lungs, liver, spleen, kidneys and
intestinal tract.

C. Skin Absorption Toxicity

Determination was made of the Minimum
Lethal Dose following application of the
material to the skin of rabbits. In the case

of the liquid samples, the material was
applied undiluted. Samples of solid mate-
rial were ground to a fine powder and ap-
plied as a solution-suspension in corn oil.
For those materials where lethal doses
were obtained (phenyl isocyanate, para-
chlorophenyl isocyanate and hexamethy-
lene diisocyanate), the animals showed
common signs of toxic effects including
lethargy, loss of appetite, convulsions and
coma, with generally local reaction to the
skin as well as a systemic injury. Local
effects included discomfort and dehydra-
tion of the skin, which extended to sub-
cutaneous layers, in the case of hexa-
methylene diisocyanate. Macroscopic ex-
amination indicated dehydration of the
viscera, suggested liver damage, and in the
case of hexamethylene diisocyanate, ex-
tensive pulmonary congestion. The ani-
mals which survived the applied doses
exhibited only minor discomfort due to
the large body area covered with the
sample.

D. Vapor Inhalation

Rats were placed in a metal chamber of
85 or 150 liters capacity and exposed for
six hours to the vapors of each of five of
the isocyanates. The vapors were pro-
duced by passing the air supplied to the
chamber through a flask containing the
liquid sample. The rate of air flow was
approximately 3 1/4 liters per minute.

The results in Table III indicate that
vapors of the liquid isocyanates may be
extremely irritating to the eyes, the nasal
passage and the intestinal tract. At au-
topsy the lungs were found to be hemor-
rhagic and congested and there was some
damage to the liver and kidneys.

Since the triphenylmethane triisocya-
nate was in solution in methylene chloride,
the effects of the latter appeared to be pre-
dominant in that the animals appeared to
be anesthetized in 10-12 minutes and de-
veloped labored breathing (with only mild
ocular and nasal irritation) until death.

Macroscopic examination revealed pulmonary edema and hemorrhagic areas in the liver.

E. Irritation Potential

The last two columns in Table III indicate the degree of irritation following application of the sample to the skin and eyes of rabbits. The technique of Draize, Woodward and Calvery¹⁰, was employed in these studies. Although the effect or degree of irritation was measured by a numerical score in accordance with the Draize technique, the results are reported in common terms for simplification.

The skin irritation test consists of applying the sample undiluted or in aqueous suspension to the shaven skin of rabbits

and allowing it to remain for 24 hours. In Table III, a reported "slight" degree of irritation indicates slight erythema with very slight to no edema. "Moderate irritation" represents well defined erythema with edema persisting for 48-72 hours after application of the sample. The "severe" reaction indicated for hexamethylene diisocyanate represents severe edema and swelling with shallow subcutaneous damage.

The degree of eye injury from the samples ranged from mild conjunctivitis, with moderate lacrimation, to dulling of the iris, swelling and closing of the eyelids with copious discharge of fluid. Parachlorophenyl and n-butyl isocyanates, however, produced corneal damage.

F. Summary of Animal Toxicity Tests for Each Isocyanate Product

TDI 1. Toluene diisocyanate, 2,4-isomer

- | | |
|-----------------------------|----------------|
| a. Single oral lethal dose: | 4.9-6.7 g./kg. |
| b. Single skin lethal dose: | 16 g./kg. |
| c. Primary skin irritation: | severe |
| d. Eye injury: | severe |

2. Ethyl isocyanate, 10% in corn oil

- | | |
|-----------------------------|-------------|
| a. Single oral lethal dose: | 0.23 g./kg. |
|-----------------------------|-------------|

3. n-Butyl isocyanate

- | | |
|---|------------------|
| a. Single oral lethal dose, 25% in corn oil: | 0.85 g./kg. |
| b. Single skin lethal dose, 75% in corn oil: | 6.0-8.0 g./kg. |
| c. Single vapor inhalation, deaths/no. exposed: | 6/6 (10-15 min.) |
| d. Primary skin irritation: | moderate |
| e. Eye injury: | severe-permanent |

4. Octadecyl isocyanate

- | | |
|---|------------------------------------|
| a. Single oral lethal dose: | 30.0 g./kg. |
| b. Single skin lethal dose: | 12.3 |
| c. Single vapor inhalation, deaths/no. exposed: | 0/4 (2/4 died hours after removal) |
| d. Primary skin irritation: | slight |
| e. Eye injury: | moderate |

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wing it to remain for 24 hours. In 11, a reported "slight" degree of n indicates slight erythema with ght to no edema. "Moderate irri- represents well defined erythema lema persisting for 48-72 hours plication of the sample. The "se- action indicated for hexamethyl- ocyanate represents severe edema elling with shallow subcutaneous

egree of eye injury from the sam- ged from mild conjunctivitis, with te lacrimation, to dulling of the elling and closing of the eyelids cious discharge of fluid. Parachlo- l and n-butyl isocyanates, how- oduced corneal damage.

imate Product

9-6.7 g./kg.
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vere
vere

23 g./kg.

85 g./kg.
0-8.0 g./kg.

'6 (10-15 min.)
oderate
vere-permanent

0 g./kg.
.3

4 (2/4 died hours after removal)
ght
oderate

5. Phenyl isocyanate
 - a. Single oral lethal dose, 50% in corn oil: 0.94 g./kg.
 - b. Single skin lethal dose: 3.5-4.4 g./kg.
 - c. Single vapor inhalation, deaths/no. exposed: 4/4 (1-2.5 hours)
 - d. Primary skin irritation: moderate
 - e. Eye injury, 50% in corn oil: severe
6. p-Chlorophenyl isocyanate
 - a. Single oral lethal dose, 50% in corn oil: 4.75 g./kg.
 - b. Single skin lethal dose: 4.4-6.0 g./kg.
 - c. Single vapor inhalation, deaths/no. exposed: 4/4 (2-4 hours)
 - d. Primary skin irritation: slight
 - e. Eye injury, 50% in corn oil: severe-permanent
7. p-Phenylene diisocyanate, 25% aqueous
 - a. Single oral lethal dose: >8.0
 - b. Single skin lethal dose: 6.0 g./kg.
 - c. Primary skin irritation: moderate
 - d. Eye injury: moderate
8. Hexamethylene diisocyanate
 - a. Single oral lethal dose: 1.05 g./kg.
 - b. Single skin lethal dose: 1.0-1.25 g./kg.
 - c. Single vapor inhalation, deaths/no. exposed: 0/4
 - d. Primary skin irritation: severe
 - e. Eye injury: severe
9. 1,5 Naphthalene diisocyanate
 - a. Single oral lethal dose, 25% aqueous: >10.0 g./kg.
 - b. Single skin lethal dose: 6.0 g./kg.
 - c. Primary skin irritation: slight
 - d. Eye injury, 50% aqueous: moderate
10. p,p'-Diphenylmethane diisocyanate
 - a. Single oral lethal dose, 25% in corn oil: >31.6 g./kg.
 - b. Single skin lethal dose, 25% in corn oil: 10.0 g./kg.
 - c. Primary skin irritation: none
 - d. Eye injury, 50% solution-suspension in corn oil: slight

- | | |
|--|---|
| 11. Triphenylmethane Triisocyanate | |
| a. Single oral lethal dose,
20% in methylene chloride: | 4.9 g./kg. |
| b. Single skin lethal dose,
20% in methylene chloride: | 10.5 g./kg. |
| c. Single vapor inhalation,
20% in methylene chloride,
deaths/no. exposed: | 4/4 (25-40 min.) death due to
solvent methylene chloride |
| d. Primary irritation,
20% in methylene chloride: | slight |
| e. Eye injury, 20% in methylene
chloride in 50% corn oil: | severe |
| 12. Isocyanate adduct, Mondur CB | |
| a. Single oral lethal dose,
50% suspension in corn oil: | >25 g./kg. |
| b. Single skin lethal dose,
25% suspension in corn oil: | >6.5 g./kg. |
| c. Primary skin irritation: | none |
| d. Eye injury: | only mechanical |

TABLE III ACUTE ANIMAL TOXICITY DATA
Single Vapor

min.) death due to
ethylene chloride

anical

TABLE III ACUTE ANIMAL TOXICITY DATA

Isocyanates	Mobyay Trademark	Single Oral Lethal Dose Rats, g/kg	Single Skin Lethal Dose Rabbits, g/kg	Saturated Vapor-Deaths/No. Exposed Time	Primary Irritation Rabbit-Skin(a)	Eye Injury Rabbits(a)	Single Vapor Inhalation Rats, 6 hrs.	
							Severe	Severe
2,4-Toluene Diisocyanate, CH ₃ (C ₆ H ₄) ₂ (NCO) ₂		4.9-6.7	—	—	Severe	Severe	—	—
Undiluted	Mondur TDS	5.8(b)	>16.0	—	Severe	Severe	—	—
Undiluted, reference 1	Mondur E	0.23	—	—	—	—	—	—
Ethyl Isocyanate, C ₂ H ₅ NCO		—	—	—	—	—	—	—
10% in corn oil		—	—	—	—	—	—	—
p-Butyl isocyanate, CH ₃ (CH ₂) ₃ NCO		0.85	—	6/6 (10-15 min.)	Moderate	Severe-Permanent	—	—
25% in corn oil		—	—	—	—	—	—	—
75% in corn oil		—	6.0-8.0	—	—	—	—	—
Octadecyl isocyanate, CH ₃ (CH ₂) ₁₇ NCO	Mondur O	>30.0	>12.0	0/4(c)	Slight	Moderate	—	—
Phenyl isocyanate, C ₆ H ₅ NCO	Mondur P	—	3.5-4.4	4/4 (1-2.5 hrs.)	Moderate	—	—	—
50% in corn oil		0.94	—	—	—	Severe	—	—
p-Chlorophenyl isocyanate, C ₆ H ₄ ClNCO		4.75	4.4-6.0	4/4 (2-4 hours)	Slight	Severe-Permanent	—	—
50% in corn oil		—	—	—	—	—	—	—
p-Phenylene Diisocyanate, C ₆ H ₄ (NCO) ₂		>8.0	>6.0	—	Moderate	Moderate	—	—
25% aqueous		6.5	—	—	—	—	—	—
25% in corn oil		0.94-1.4	—	—	—	—	—	—
10% in peanut oil		—	—	—	—	—	—	—
Hexamethylene Diisocyanate, OCN(CH ₂) ₆ NCO	Mondur HX	1.05	1.00-1.25	0/4	Severe	Severe	—	—
1,3-Naphthalene Diisocyanate, C ₁₀ H ₆ (NCO) ₂	Multra-thane N5	>10.0	—	—	Slight	—	—	—
25% aqueous		>10.0	—	—	—	—	—	—
25% in corn oil		>10.0	—	—	—	—	—	—
50% in aqueous		—	6.0	—	—	Moderate	—	—
p,p'-Diphenylmethane Diisocyanate OCN(C ₆ H ₄) ₂ CH ₂ (C ₆ H ₄) ₂ NCO	Multra-thane M	>31.6	>10.0	—	None	Slight	—	—
25% in corn oil		—	—	—	—	—	—	—
50% solution-suspension in corn oil		—	—	—	—	—	—	—
Triphenylmethane Triisocyanate, CH(C ₆ H ₅) ₃ NCO	Mondur TM	4.9	10.5	4/4 (25-40 min.)(d)	Slight	Severe	—	—
20% in methylene chloride above in 50% corn oil		—	—	—	—	—	—	—
Isocyanate Adduct (TDI & TMP, NCO/OH 2:1)	Mondur CB	>25.0	>6.5	—	None	Only Mechanical	—	—
50% suspension in corn oil		—	—	—	—	—	—	—
25% suspension in corn oil		—	—	—	—	—	—	—

a) See text
b) Oral L.D.—ref. 1
c) 2/4 died several hours after removal from exposure
d) Death due to anesthetization from solvent, methylene chloride

TDI

NDI

NDI

NDI

NDI

NDI

15

TABLE IV admin for 10 days / observed 14 days
 SUBACUTE ORAL TOXICITY (5 rats)

COMPOUND	DAILY DOSE (grams/kilogram)	RESULT Deaths/no. fed (Day of death)	WEIGHT CHANGE
2,4-Tolylene Diisocyanate	1.5	3/6	—
Phenyl Isocyanate	0.19	1/6 (7)	3% — 7%
Parachlorophenyl Isocyanate 50% in oil	0.95	2/6 (8, 10)	4% — 5%
Triphenylmethane Triisocyanate 20% solu. in methylene chloride	0.98	0/6	8% — 13%
Octadecyl Isocyanate Undiluted	6.1	0/6	3.5% — 5%
Paraphenylicene Diisocyanate 25%	6.1	10/10 (4)	—
1,5-Naphthalene Diisocyanate 25%	6.1	3/10 (12)	8.0% — 0.0%

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10/10 (4)
3.0% — 0.05%
3/10 (12)
6.1
25%
1,5-Naphthalene Diisocyanate
25%

RESPIRATORY EFFECTS OF INHALED ISOCYANATES

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I. INTRODUCTION

Isocyanates comprise a group of highly reactive chemicals used in the manufacture of polyurethanes. The common feature of this class of chemicals is the presence of an $-N=C=O$ group. Because of their chemical reactivity, isocyanates have the ability to combine readily with biological molecules and cause diverse health effects. These effects range from irritation, which occurs following exposure to high isocyanate concentrations, to hypersensitivity reactions, which can be triggered by low isocyanate concentrations, to chronic pulmonary effects. During the past 20 years, more than 250 articles have appeared which have addressed the health effects following isocyanate exposures, and over 10 animal models have been presented to better understand these effects. This review will critically examine the state of knowledge concerning pulmonary effects resulting from both acute and chronic exposures to various isocyanates. It will then compare the observed clinical effects with those noted in animal studies to enable not only evaluation of the animal models, but additionally the likelihood of successful extrapolation from the animal systems to the human situation in order to predict health effects from isocyanate exposure.

In 1978, following an extensive review of the literature, the National Institute for Occupational Safety and Health (NIOSH) recommended¹ that in order to protect the health of workers the occupational exposure to diisocyanates be limited to 5 ppb for up to a 10-hr workshift, 40-hr workweek. The standard was promulgated for all diisocyanates, although at that time very little was known regarding the toxicity of isocyanates other than toluene diisocyanate. This review will address the health effects of various isocyanates and, wherever possible, relate the differences which have been found to isocyanate chemistry.

In critically reviewing the isocyanate literature, several prominent factors emerged. These factors included: accurate assessment of exposure levels, the length of isocyanate exposure, and the interpretation of methods used to measure health effects. When appropriate consideration is afforded to each of these factors, many inconsistencies and misconceptions in the isocyanate literature disappear. For this reason, the role of these factors in determining isocyanate toxicity will be emphasized. It is hoped that, as a result of this critical evaluation of the isocyanate literature, there will develop a better

understanding of the place of isocyanates in the spectrum of industrial chemicals for which health effects have been described.

II. CHEMISTRY OF ISOCYANATES

A. Chemical Reactivity

The reactivity of the highly unsaturated isocyanate group has led to its use in a great variety of applications. In 1980, worldwide use of polyurethane resins exceeded 7 billion lb.¹ Monoisocyanates have found use in insecticides and herbicides, whereas polyfunctional isocyanates, those with two or more isocyanate groups, have been used in the systematic build-up of large polymers with specific properties.²

Isocyanates are highly reactive toward a large number of compounds having active hydrogen, but are also capable of reacting with themselves to form dimers, trimers, and higher polymers. As will be discussed in a subsequent section, it is this capacity for self-polymerization that makes difficult the synthesis of uniform isocyanate reagents for use in the diagnosis of immunologic responses to these chemicals.^{3,4}

Currently, the two isocyanates most widely employed in the production of rigid polyurethane products for consumer use are toluene diisocyanate (TDI) and diphenylmethane-4,4'-diisocyanate (MDI) (Figure 1). TDI is usually formulated from two isomers (2,4 and 2,6) added in varying proportions, but usually in ratios of 65:35 or 80:20, respectively.

Isocyanates vary in their chemical reactivities. The presence of a substituent ortho to the NCO group retards the tendency for dimer formation. Thus, TDI is less prone to dimer formation than is MDI. Frequently, however, industrial catalysts such as tertiary amines or organo-tin compounds are used to promote polymerization of isocyanates.⁵ At high temperatures, the dimerization reaction is reversible with complete dissociation above 200°C. This reversibility has implications for fire situations; toxicologic effects having been reported in firemen following exposure to isocyanates.^{6,7}

The differential reactivity of individual isocyanate groups in different media influences the quantities of the various isomers which will be present in reactive form in the industrial atmospheres. For example, the isomeric composition of airborne TDI was measured in two polyurethane foam plants.⁸ Analysis indicated a large increase in the amount of airborne 2,6-TDI relative to 2,4-TDI when compared with the starting material. The relative increase in the 2,6 isomer was attributed to steric factors which lower its reactivity compared with the 2,4 isomer. The composition of the atmosphere influenced analytical determination of isocyanate concentration as well since some analytical techniques (i.e., the Marcali method)⁹ respond differently to the two isomers.

B. Aliphatic Isocyanates

Aliphatic isocyanates have found industrial importance because of their "light stable" characteristics.¹ Hexamethylene diisocyanate (HDI) (see Figure 1) has a relatively high vapor pressure and therefore may pose a handling problem. However, a biuret mixture derived from HDI (Figure 1) has been successfully used in coating applications.¹ In these formulations, monomeric HDI comprises at most 1% of the isocyanate composition. For this reason, many reports of industrial exposures to HDI are, in fact, exposures predominantly to HDI biuret. Although the assumption could be made that, due to its chemical volatility, monomeric HDI would be the major isocyanate species in the atmosphere during product utilization, in practice the isocyanates are usually sprayed during coating applications. Thus, substantial quantities of the biuret become airborne. Persons exposed under such conditions are exposed predominantly to HDI biuret with little monomer exposure. The health implications of such exposures are considerable. Whereas both isocyanates are irritating, in murine studies, the biuret has

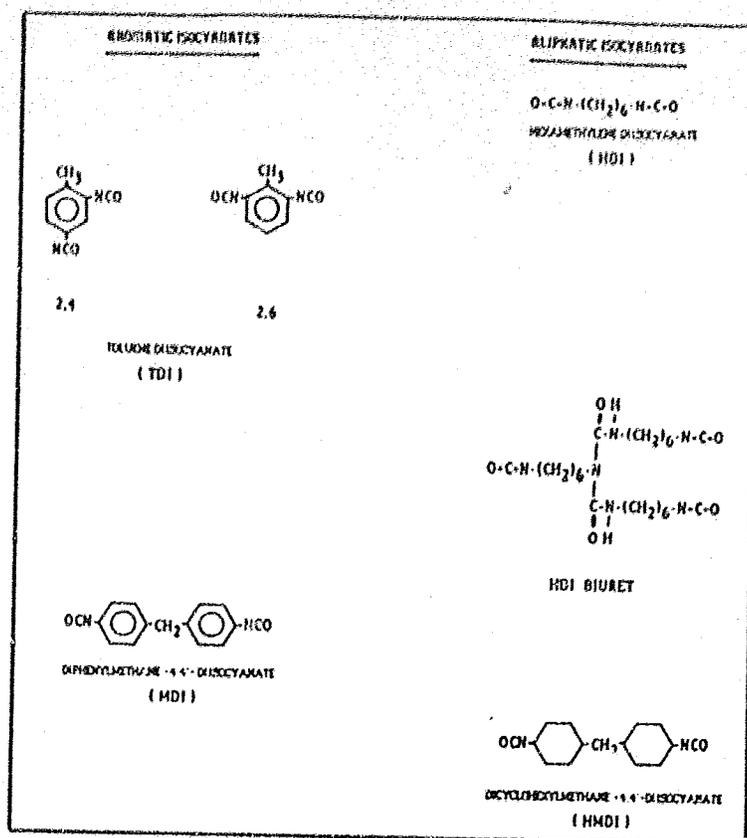


FIGURE 1. Structures of selected aromatic and aliphatic isocyanates.

been shown to produce pulmonary irritation,¹⁰ whereas in the same system, the HDI monomer was a potent sensory or upper respiratory tract irritant.¹¹

Knowledge of the actual isocyanate species prominent during an exposure was found to be important in a case of respiratory sensitization.¹² The various isocyanates differ in their respiratory sensitization potencies.¹² Moreover, in order to detect sensitization, diagnostic antigens should be composed of the isocyanate hapten to which the individual was actually exposed. Recently,¹² a car spray painter was diagnosed with "HDI" asthma when he displayed occupationally related respiratory symptoms. In this case, however, use of the HDI biuret (see Figure 1) in preparing the antigen for RAST testing provided greater ability to detect IgE antibodies in the serum than did the use of an antigen prepared with monomeric HDI.¹² These results imply that the patient may have been exposed to HDI biuret in spray paint application.

Not all isocyanates are respiratory tract sensitizers.¹³ The commercially successful aliphatic diisocyanate, hydrogenated MDI (HMDI) (see Figure 1), serves as an example. This chemical is prepared by hydrogenation, then phosgenation of diphenylmethane diamine to yield a liquid isocyanate.³ The commercial advantages of this isocyanate are its low vapor pressure and its liquid form. Although the chemical is frequently heated during industrial utilization, experience with HMDI has indicated only rare occurrence of respiratory sensitization. However, dermal reactions were frequently noted.^{14,15} Recent animal studies^{16,17} with this isocyanate have confirmed clin-

ical reports. Respiratory sensitization was rarely detected in guinea pigs, whereas dermal sensitization was extensive.

C. Measurement of Isocyanates in Air

Studies relating the respiratory effects of inhaled isocyanates to exposure concentrations must rely upon accurate, sensitive measurement of isocyanate concentrations in the air. For many years, the Marcali method⁹ has been the standard analytical procedure. In the analysis, TDI is absorbed into an aqueous acidic solution resulting in formation of toluene diamines. The amines are then diazotized and coupled with *N*-1-naphthylethylenediamine. The colored reaction products are measured from their absorption at 550 nm. This method is capable of detecting 10 ppb of 2,4-TDI.⁹

A more sensitive method was developed by Keller et al.¹⁸ The method employs reaction of an isocyanate with 4-nitrobenzyl-*N*-*M*-propylamine dissolved in toluene to form a urea. Following reduction of the nitro group, the resulting amine is diazotized with *N*-1-naphthylethylenediamine to yield a colored reaction product. Using thin-layer chromatography for separation, this method can readily detect 10 ppb TDI in a 10-*l* air sample. When using high-performance liquid chromatography, TDI levels of 0.7 ppb \pm 10% can be detected in a 20-*l* air sample.¹⁸ An additional advantage of the latter method, compared with that of Marcali, is the ability of the latter to detect aliphatic, as well as aromatic isocyanates.

Still further, sensitivity in detecting lower concentrations of airborne isocyanates was achieved using a fluorescence-active reagent, *N*-methyl-1-naphthalene methylamine to form urea derivatives of isocyanates.²⁰ Using fluorescence, detection limits were improved by factors of 25 for aromatic isocyanates and by more than 100 for aliphatic isocyanates from those detectable with the nitroreagent. The detection limits for TDI, MDI, and HDI using this procedure were 0.42, 0.49, and 0.14 ppb, respectively, based on 1-*l* air samples.²⁰

III. SYNDROMES ASSOCIATED WITH ISOCYANATE EXPOSURE

Exposure to isocyanates has been associated with neurological, dermal, respiratory, and immunologic effects, as well as isolated cases of hematologic or gastrointestinal symptomatology.¹ It is apparent from many reports that the observed effects were directly related to:

1. The concentration of isocyanate at exposure
2. The length of the exposure
3. The route of exposure

Neurological effects were described in firemen after a single exposure to TDI which lasted for several hours.⁴ The fire occurred in a factory where polyurethane foam was manufactured. There were few abnormal physical signs on neurological examination of the 35 participating fireman, but euphoria, ataxia, and loss of consciousness were apparent in 5 individuals. Longer lasting effects, including headache, poor memory, and difficulty in concentrating, were noted in an additional nine individuals. In some, symptoms lasted for 4 years.

As noted with neurological symptoms, pulmonary effects from exposure to isocyanates include both acute and chronic symptomatology. The pulmonary health hazards associated with isocyanate exposure are predominantly irritation and sensitization of the respiratory tract. Symptoms may occur immediately upon exposure, or they may be delayed and occur several hours following the exposure. The typical responses to isocyanate inhalation are a feeling of chest "tightness", breathlessness, difficulty in

Table 1
CLASSIFICATION OF RESPIRATORY EFFECTS ASSOCIATED
WITH INDUSTRIAL ISOCYANATE EXPOSURE

Response	Exposure Concentration (ppb)	Ref.
Irritation	≥100 TDI	22-26, 56
	30-70 TDI	27-28
	10-25 TDI	82
Elicitation of hypersensitivity reactions	6-20	29-33, 41, 44
Pulmonary function decrement upon low level exposures	20 TDI	45
	15 TDI	115
	5 TDI	42
	3.5 TDI	113
	1.5-3.5 TDI	116
	87 MDI	47

breathing, and a dry cough. Symptoms are most severe in those with highest exposures.²²⁻²⁷ When concentrations are greater than 100 ppb, these symptoms are due to the *primary irritant effect* of isocyanates (Table 1). Thus, reports of ocular irritation, bronchitis, nasal congestion, sore throat, headache, nausea, and vomiting in workers exposed to 100 ppb and greater TDI atmospheres indicated irritation reactions.²²⁻²⁶ On the other hand, irritation was not observed when concentrations were below 30 ppb.²⁸

In a small percentage of exposed persons (estimated to be 5 to 10%), exposure to low concentrations of isocyanates (6 to 20 ppb) may elicit respiratory symptomatology consisting of coughing, wheezing, and dyspnea. Again, these symptoms may occur immediately upon exposure or may be delayed several hours in onset. These responses are due to *sensitization* and may occur only in persons with *previous exposure* to isocyanates. Although these respiratory reactions are elicited by low concentrations of isocyanates, evidence from both clinical²⁹⁻³³ and experimental^{11,24-27} studies has associated the initial *process of sensitization* with a *high* isocyanate exposure. Once sensitized, however, low concentrations may elicit responses. Such evidence will be discussed in Section IV.B.

Another allergic lung disease has very recently been described in persons exposed to isocyanates. Hypersensitivity pneumonitis is usually associated with recurrent exposure to a variety of inhaled organic dusts.³⁸ This syndrome is characterized by fever, malaise, nonproductive cough, leukocytosis, and elevated immunoglobulin levels. It is a restrictive lung disorder in which there is reduced gas transfer. It has been described by several investigators as resulting from exposure to TDI³⁹ and other isocyanates, including HDI⁴⁰ and MDI.⁴¹⁻⁴⁴ In a case described by Malo et al.,⁴⁰ the individual was exposed to a spray of paint containing polymeric HDI and demonstrated shortness of breath, wheezing, malaise, and chills late in the afternoon on working days. Symptoms lasted for several hours and were accompanied by wheezing at night. Inhalation challenge under simulated occupational exposure conditions reproduced the symptoms and thereby identified the source of the active material.

In addition to irritation of the respiratory tract, a third pulmonary effect of isocyanates has been described. Several investigators have described progressive diminution of lung function as a result of regular exposure to TDI at concentrations below 20 ppb. The effect is recognized as an impairment in some indication of pulmonary function frequently measured as a decline in FEV₁. Wegman et al.⁴⁵ reported a loss of FEV₁ in TDI workers who were exposed for more than 2 years to 20 ppb TDI. Pham et al.⁴⁷ reported a decline in vital capacity and in carbon monoxide gas transfer in workers exposed more than 60 months to MDI. Moreover, values were significantly lower in the group having the longest exposure.

Some investigators have reported a relationship between the cumulative TDI exposure of workers and declines in pulmonary function,⁴⁸ whereas others find no respiratory impairment following chronic isocyanate exposure.⁴⁹

The sections which follow critically evaluate each of the categories of respiratory effects associated with isocyanate exposure and, wherever possible, compare clinical descriptions with experimental findings to gain a fuller understanding of the mechanism underlying each toxicologic effect.

IV. EVALUATION OF RESPIRATORY SYNDROMES AND COMPARISON WITH ANIMAL MODELS

A. Acute Irritation

1. Clinical Reports

The ability of isocyanates to cause respiratory tract irritation has been well documented.¹ With exposure to high concentrations, chemical bronchitis may occur with severe bronchospasm. Highest exposures may result in chemical pneumonitis and pulmonary edema.

These effects are concentration dependent. When TDI concentrations in a plant were around 100 ppb, cases of chemical intoxication were frequent, whereas when concentrations were 10 ppb, cases were rare.⁵⁰ Similarly, a high incidence of illness was associated with TDI levels of 30 to 70 ppb, but no cases were observed when concentrations were below 30 ppb.⁴⁸

The irritation effects are not only concentration dependent, but appear to be time dependent as well. In a study with human volunteers, eye irritation was experienced by three of six persons exposed to 50 ppb TDI for 10 min. Exposure to this concentration for 15 min resulted in symptomatology in five of six individuals.⁵¹

The irritation effects of TDI and other isocyanates were studied using an established animal model.⁵² The results, discussed below, indicated that response in the animal system demonstrated the same characteristics as that observed in humans, i.e., concentration and time dependence for response.

2. Experimental Studies

The effects of TDI inhalation were reported by Sangha and Alarie in mice.⁵³ Animals were exposed, using a "heads-only" system, to the concentrations of 7 to 30 ppb TDI. A slow development of sensory irritation was noted with a slow recovery when exposures were for 3 hr. As had been noted clinically, the extent of irritation was not only concentration dependent, but time dependent as well. Using a 10-min exposure to concentrations of 100 to 1000 ppb, animals demonstrated a 10 to 60% decrease in respiratory rate. When the duration of exposure was 180 min, the same effect was obtained with one fifth the TDI concentration (20 to 200 ppb).

A cumulative effect was observed when TDI concentrations were greater than 23 ppb.⁵³ Irritation was more severe with daily exposure at the same concentration and overnight recovery was not complete. However, after several days without exposure, responses to renewed exposure were the same as those seen with naive animals. These studies clearly demonstrated a cumulative response in mice at levels of 23 ppb or higher. The effect was not seen with 1.6 to 18 ppb TDI.

Other aromatic and aliphatic isocyanates were evaluated for irritative ability in the murine system.¹¹ Using TDI, HDI, phenyl isocyanate, hexyl isocyanate, as well as *o*-, *m*-, and *p*-tolyl isocyanate, concentration-response and time-response effects were observed. In addition, overnight recovery was incomplete following exposure to the higher concentrations for long periods of time. Based on these murine findings, a concentration of 5 ppb TDI and HDI was suggested as a safe level of exposure for workers,

whereas 20 ppb tolyl isocyanate isomers and phenyl isocyanate was suggested as safe to protect against irritation.

Comparison of the responses observed in mice with those reported for workers indicated that in both systems the severity of the irritation response to isocyanates was characterized by concentration and time dependence. In the proposed extrapolation of animal data^{11,12} to predict human response, 20 ppb TDI would be recommended as the ceiling value and 6 ppb is suggested as a concentration at which there would be no cumulative effects for TDI. The 1983 Threshold Limit Value (TLV) for TDI was recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) to be 5 ppb with a Short Term Exposure Limit (STEL) of 20 ppb.¹³

3. Recovery from Acute Exposure

Clinical — Several studies have evaluated the extent of recovery following acute isocyanate exposure. Schmidt-Nowara et al.¹⁴ reported the recovery of a truck driver accidentally exposed to TDI vapor. The accident involved TDI being sprayed over the face and body, completely soaking the individual's clothing. Symptoms of dyspnea, coughing, wheezing, and rales, in addition to edematous and tearing eyelids, developed over several hours. Pulmonary function tests indicated reduced FEV₁ and FVC. However, 1 month later, testing indicated that both functions had returned to normal (predicted) values. Gandevia¹⁵ studied 20 workers exposed during normal workdays to isocyanate vapors. He too concluded that although respiratory symptoms may persist for several weeks in patients complete recovery is usually the rule.

Experimental — Recovery from acute exposure was also studied in an animal model system.¹⁷ Guinea pigs were exposed to 1.4 ppm TDI for 3 hr/day on 5 consecutive days. The acute pulmonary toxicity which resulted from exposure was assessed by measurement of pulmonary performance using a CO₂ challenge method previously described in detail elsewhere.¹⁶ In this method, pulmonary damage is indicated by impairment of the animal's ability to increase its respiratory frequency and tidal volume in response to inhalation of air containing 10% CO₂. The respiratory impairment resulting from the 5-day TDI exposure is shown in Figure 2. Animals exposed to 1.4 ppm TDI had reduced pulmonary performance by day five of exposure, which persisted for several weeks. Repeated testing at weekly intervals indicated recovery of function by 3 weeks following the exposure. This result in the animal system was similar to that described above for industrial workers in that recovery occurred within weeks following severe acute TDI exposure.

4. Cholinesterase Determinations

The pulmonary response seen in workers following isocyanate exposure has been observed with both immediate-onset as well as delayed-onset symptomatology.^{11,12,16} Numerous mechanisms have been proposed to explain such reactions, yet there is still considerable controversy concerning the importance of various factors for these reactions. In many instances, animal models have assisted in elucidation of the complex factors affecting the clinical syndromes.

Characteristics of the irritation response such as slow onset and slow recovery suggested²² covalent interaction of diisocyanates with nerve receptors of the respiratory tract. Since inhibition of cholinesterase activity can trigger nerve endings and cause smooth muscle contraction, reaction of isocyanates with cholinesterase could account for some of the symptomatology observed in industrial workers. Both experimental and clinical studies have been conducted to determine if isocyanates react with cholinergic molecules.

A clinical study was performed²³ on 30 TDI workers to examine levels of serum and erythrocyte cholinesterase activity. Erythrocyte enzyme activity was decreased for 70%

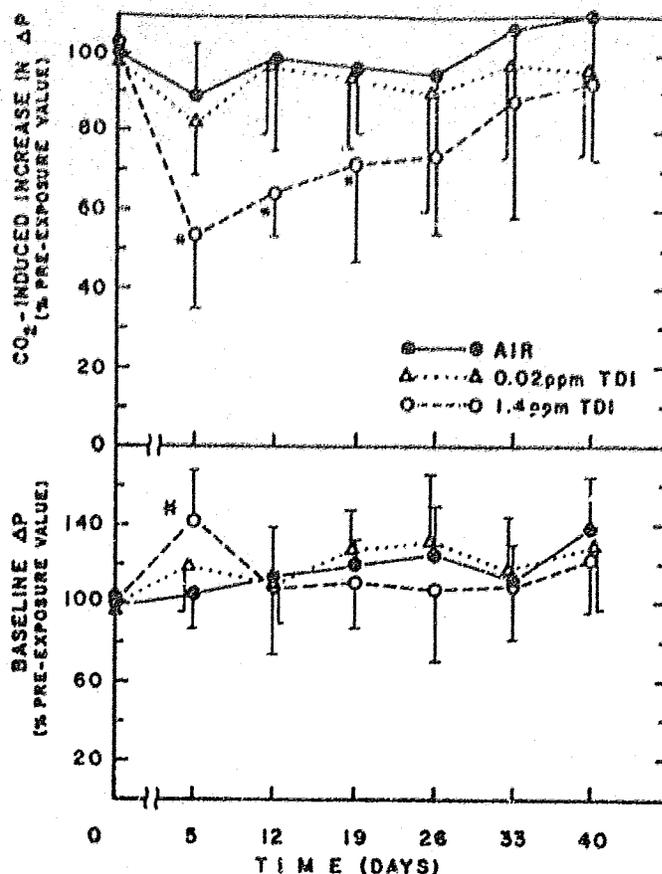


FIGURE 2. Effects in guinea pigs of TDI inhalation (0.02 or 1.4 ppm) on the CO₂-induced increase in ΔP and baseline ΔP . ΔP indicates the pressure change in the whole-body guinea pig plethysmograph. Each point and the vertical bar represents the mean \pm SD of 6 to 8 guinea pigs. * indicates the mean is statistically different from that of the controls. (From Wong, K. L., Karol, M. H., and Alarie, Y., *J. Toxicol. Environ. Health*, 15, 137, 1985. With permission.)

of the workers, while the serum enzyme level appeared to be increased, although not significantly. No pulmonary function abnormality was noted in the workers. This study implied that there might be interaction of TDI with cholinesterase in the industrial setting, but the reduced enzyme activity had no correlation with respiratory symptomatology.

A second study⁴⁴ compared levels of serum and red cell cholinesterase in groups of TDI workers, including 13 with "TDI asthma". Enzyme levels were measured before, immediately after, and 4 hr following TDI challenge exposure. Results were presented as average group responses (rather than individual responses) with groups based upon respiratory response to TDI. The group showing no response to TDI ($n = 5$) averaged a 6% drop in erythrocyte activity after 4 hr; that showing a late response ($n = 5$) averaged a 13% drop, whereas the group which displayed dual responses to TDI ($n = 3$) showed a 16% drop in acetylcholinesterase activity. While these results may be suggestive of a correlation between TDI response and inhibition of enzyme activity, it

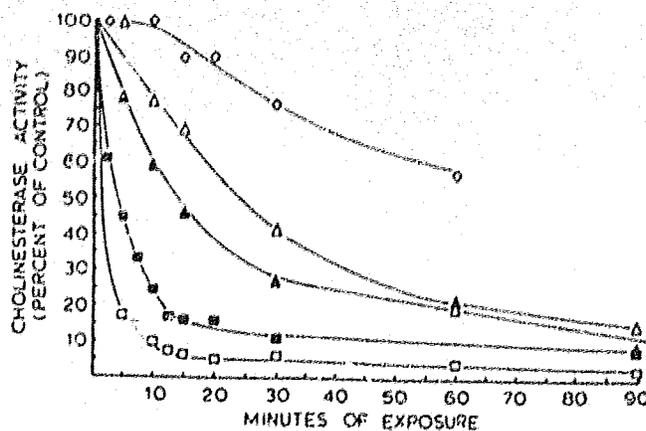


FIGURE 3. Exposure of cholinesterase to isocyanate vapors. Aliquots of 1 ml of horse serum cholinesterase (12.7 units/mg, 1 mg/ml) in 0.02 M potassium phosphate buffer, pH 6.9, were exposed to 1 ppm isocyanate vapors. The curves represent exposures to HDI (open squares), hexyl isocyanate (filled squares), 2,6-TDI (open triangles), 2,4-TDI (filled triangles), and *o*-tolyl isocyanate (diamonds). (From Brown, W. E., Green, A. H., Karol, M. H., and Alarie, Y., *Toxicol. Appl. Pharmacol.*, 63, 45, 1982. With permission.)

is unlikely that the minimal degree of enzyme inhibition detected would have any physiological consequence.^{65,66}

In vitro studies were undertaken of cholinesterase interaction with isocyanates. Brown et al.⁶² investigated the interaction of several isocyanates with mammalian cholinesterases. Of the various aliphatic and aromatic isocyanates tested, HDI was found to interact stoichiometrically with the enzyme such that it was a good competitor of the natural enzyme substrate. Molar ratios of 4:1 (isocyanate to enzyme) completely inhibited serum cholinesterase activity. This interaction took place whether the HDI was added directly (diluted in acetone) to the enzyme solution or whether interaction was between HDI vapor and a solution of the enzyme (Figure 3). Other isocyanates, such as 2,4-TDI, phenyl isocyanate and *o*-tolyl isocyanate, were much less effective in inhibiting enzyme activity with molar ratios of 50:1 or greater required for 50% enzyme inhibition.

Dewair et al.⁶⁷ also investigated the in vitro interaction of several isocyanates with acetylcholinesterase from human erythrocytes. They confirmed that aliphatic isocyanates were more potent inhibitors than aromatic isocyanates. Subsequently, both groups of investigators noted that the in vitro enzyme inhibition was reversible at pH 7.5.^{68,69} The half time of the HDI-enzyme inhibition was 12 hr.⁶⁸ The possibility was suggested⁶⁹ that the reversibility of the inhibition might explain the slow recovery from irritation observed in the murine model described previously.²³

5. In Vivo Studies

To determine if the marked in vitro cholinesterase inhibition by isocyanates had physiologic significance, in vivo studies were undertaken.⁷⁰ Guinea pigs were exposed via a "heads-only" inhalation system to vapors of HDI. Enzyme activity both in the serum and associated with erythrocytes was monitored prior to and directly following exposures. Results indicated no change in enzyme activity as a result of HDI exposure even though animals were exposed for 3 to 6 hr to concentrations ranging from 0.5 to 4.0 ppm HDI (approximate LD₅₀ for guinea pigs). A further indication of the lack of in

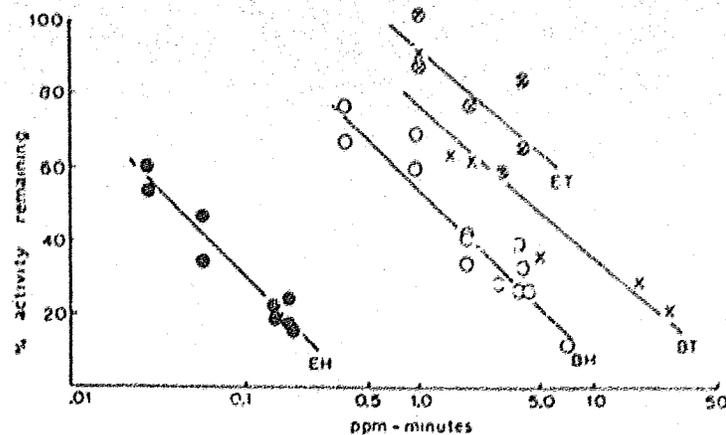


FIGURE 4. Concentration-response relationship of immobilized cholinesterase to vapors of HDI and TDI. Immobilized acetylcholinesterase was exposed to atmospheres of HDI (EH) and TDI (ET), and immobilized butyrylcholinesterase was exposed to atmospheres of HDI (BH) and TDI (BT). PPM-MIN represents the product of the concentration of isocyanate times the minutes of exposure. (From Brown, W. E., Shamoo, A. Y., Hill, B. L., and Karol, M. H., *Toxicol. Appl. Pharmacol.*, 73, 105, 1984. With permission.)

vivo interaction of HDI with these enzymes was evidenced by the failure to detect any difference in the cholinesterase activity of lung lavage between animals exposed for 2 hr to 1.8 ppm HDI and control nonexposed animals.

Although the reaction between HDI and cholinesterase appeared to be without apparent biological significance, recognition of this highly specific stoichiometric reaction did have practical application. This reaction became the basis for design of a simple passive dosimeter for detection of airborne isocyanates.⁷¹ To prepare the dosimeter, butyryl or acetylcholinesterase was absorbed onto polystyrene strips and exposed to atmospheres containing increasing amounts of HDI. Measurement of residual enzyme activity gave an accurate indication of atmospheric HDI concentrations (Figure 4). The sensitivity of the system allowed for detection of the TLV concentration of HDI (5 ppb) within 12 min. The same detection strip was also applied to indicate airborne TDI concentrations.⁷¹ Although less sensitive for TDI, the strip dosimeter detected 700 ppm TDI in 10 min. Since both isocyanates are not likely to be present simultaneously in the same atmosphere, this detection device allowed monitoring of either isocyanate in a range appropriate for the industrial setting.

B. Sensitization

1. Clinical Syndromes

Symptomatology associated with respiratory sensitization closely resembles that described for acute irritation. Both immediate and delayed-onset responses have been noted. Wheeze and chest tightness are almost always present in the immediate-type response. Symptoms associated with the delayed-onset response are cough, mild breathlessness, and a little yellow sputum.⁴¹ As mentioned previously, pneumonia-like symptoms have also been associated with isocyanate sensitivity.¹⁸ Reactions due to sensitization differ from those caused by irritation in that sensitization is *elicited* by low concentrations of isocyanates (1 to 20 ppb) (Table I) and occurs only in persons with *previous exposure* to the isocyanate. Like irritation, it appears to develop as a

result of exposure to high isocyanate concentrations.^{12,19,22,23} Mechanisms proposed for this "occupational asthma" include:

1. Pharmacologic bronchoconstriction
2. Allergic or immunologically mediated bronchoconstriction
3. Hyperreactive airways

Evidence to support each of these mechanisms is presented below.

2. Pharmacologic Mechanism

Several authors have proposed a pharmacologic mechanism to account for the acute bronchoconstriction observed in sensitized individuals upon exposure to isocyanates. In studies with human peripheral leukocytes, Van Ert and Battigelli²⁴ observed that TDI, in concentrations of 10^{-3} to $10^{-4}M$ did not induce histamine release from leukocytes, but did reduce cAMP stimulation produced by catecholamines. In this way, it behaved as a β -blocker. The effect was seen with leukocytes from nonsensitized atopic subjects as well as nonatopic individuals and was observed using exceedingly high concentrations of TDI added directly to cells. As a result, the relevance of these findings to symptomatology resulting from industrial TDI exposure is unclear.

Other investigators have also proposed clinical significance to the pharmacologic effects of TDI. Butcher et al.²⁵ examined the function of the β -receptor and adenylyl cyclase in human lymphocytes. They found that TDI abolished the cAMP increase caused by isoproterenol. The effect was seen in lymphocytes from sensitized as well as nonsensitized individuals. However, a relationship between lymphocyte activity and isocyanate sensitization was suggested by the finding that in one case of TDI sensitization the individual's decreased lymphocyte ability to produce cAMP returned to near normal levels 2 years following loss of his clinical manifestations of TDI sensitivity.²⁶

3. Immunologic Mechanism

The classic asthmatic symptomatology of the immediate-onset pulmonary response^{21,22,29,30} has prompted many investigators to propose an immunologic mechanism for the response. This idea has met with mixed enthusiasm. Some studies have reported detection of IgE antibodies in a high proportion of sensitized individuals,^{31,32,33} whereas others detected antibodies in 15 to 20% of symptomatic cases,^{31,32} and still others were completely unsuccessful in detecting antibody in individuals with immediate asthmatic response to TDI.³⁴ The difficulty undoubtedly arises from the chemical reactivity of diisocyanates. In order to detect IgE antibodies, one must prepare appropriate hapten-conjugate antigens.^{35,36} Use of conjugates made by reacting TDI with human serum albumin has frequently led to failure in detecting antibodies in symptomatic workers^{32,36,37} and in TDI-exposed animals.³⁸ By contrast, antigen preparations using monoisocyanate analogs³⁹ of industrial diisocyanates has led to general acceptance that at least a percentage of the clinical cases of isocyanate sensitization can be attributed to an antibody-mediated immunologic mechanism.^{12,29,44,50,51,52,59,60} Problems associated with preparation of isocyanate-protein antigens are discussed below.

a. Antigens for Diagnostic Use

The isocyanate functional group is highly reactive under physiologic conditions. Reactions may occur with $-OH$, $-SH$, or $-NH_2$ groups on proteins. Thus, the potential exists for reaction at many protein sites and cross-linking of proteins is likely.^{77,81} Additionally, isocyanates may self-polymerize, leading to formation of dimers, trimers, and higher polymers which in turn may react with proteins either at single or multiple sites. Preparation of hapten-conjugates using diisocyanates as haptens thus has the

Table 2
ANIMAL MODELS FOR ISOCYANATE HYPERSENSITIVITY

Agent	Route of exposure	Animal species	Immune response	Respiratory* response	Ref.
Hapten-Protein Conjugates					
TDI-OA	Intravenous	Rabbit	Antibody	—	88
p-TMI-OA	Inhalation	Guinea pig	Cytophilic antibody	Yes	4
HMI-OA	Inhalation	Guinea pig	Cytophilic antibody	Yes	92
IEP-SA, IEM-SA	Inhalation	Guinea pig	Skin sensitivity	Yes	93
TDI-SA	Intraperitoneal (alum)	Mouse	IgE	No	97
Chemicals					
TDI	Inhalation	Guinea pig	Dermal sensitivity	Yes	101
		Monkey	—	No	
p-TMI	Inhalation	Guinea pig	Cytophilic antibody	Yes	34
TDI	Dermal	Guinea pig	Cytophilic antibody	Yes	36
TDI	Inhalation	Guinea Pig	Cytophilic antibody, skin sensitivity	Yes	34, 35
TDI	Intratracheal	Dog	IgA, IgG, IgM skin sensitivity	—	99
HMDI	Inhalation	Guinea pig	Skin sensitivity	No	17
MDI	Intratracheal	Dog	IgA, IgG, IgM skin sensitivity	—	98

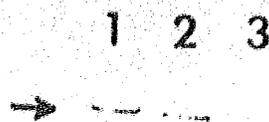
Note: Abbreviations: OA, ovalbumin; SA, serum albumin; TDI, toluene diisocyanate; p-TMI, p-tolyl isocyanate; HMI, hexyl isocyanate; HMDI, dicyclohexylmethane diisocyanate; MDI, diphenylmethane diisocyanate; IEP, isocyanatoethyl propionate; IEM, isocyanatoethyl methacrylate.

* Altered pulmonary response to a low, nonirritating concentration of isocyanate.

potential for producing extremely diverse and complex reaction products. Historically, such products had been used in attempts to detect antibodies and understandably yielded little success.^{12,22,23,87}

In 1978, Karol et al.⁴ reacted p-tolyl monoisocyanate (TMI) with ovalbumin to form a hapten-protein conjugate for immunization of animals. By exposing guinea pigs via inhalation to aerosols of the monoisocyanate-protein conjugate, respiratory sensitization was produced with specificity directed toward the tolyl isocyanate hapten (Table 2). No respiratory reactivity was produced to the ovalbumin portion of the conjugate. Using the same animal system, the aliphatic monoisocyanate, hexyl isocyanate, when reacted with ovalbumin and used for inhalation exposure of animals, also induced exclusively hapten-specific respiratory hypersensitivity²² (Table 2). These two studies initiated the concept of studying respiratory hypersensitivity to isocyanates through use of inhalation exposure with monofunctional isocyanate haptens coupled to protein carriers. Confirmation of the method was reported using serum albumin conjugates of isocyanatoethyl methacrylate and isocyanatoethyl propionate to produce exclusively hapten-specific respiratory sensitivity to inhaled isocyanates without need of protein carriers.^{24,25} These developments will be discussed in Section IV.B.3.c.

The hapten-protein conjugates formed using aliphatic and aromatic monoisocyanates were compared with those produced from diisocyanate haptens.²⁷ Using SDS-polyacrylamide gel electrophoresis, the diisocyanate antigens were found to contain multipolymeric species, as evidenced by multiple bands. On the other hand, conjugates formed from reaction of proteins with monofunctional isocyanate haptens had uniform molecular sizes. In the example shown in Figure 5, much of the diisocyanate



69kD

FIGURE 5. SDS-gel electrophoresis of protein conjugates formed by reaction of guinea pig serum albumin (GSA) with hexyl isocyanate (HMI) and hexamethylene diisocyanate (HDI). Lane 1, HDI-GSA synthesized at pH 9.3; lane 2, HDI-GSA synthesized at pH 7.4; and lane 3, HMI-GSA synthesized at pH 9.3. Fifty μ l of protein solutions (2 mg/ml) were placed in each lane of the 7.5% acrylamide gel.

Table 3
ANTIGENS CAPABLE OF DETECTING
ANTIBODY TO ISOCYANATES

Isocyanate at exposure	Conjugate antigen used for testing	Ref.
Clinical Studies		
TDI	TDI-HSA	79, 80, 83
	<i>p</i> -TMI-HSA	75--83, 89, 90
	<i>o</i> -TMI-HSA	79
HDI	HMI-HSA	12
	HDI-HSA	12, 40
HDI-Biuret	HDI-Biuret-HSA	12
MDI	MDI-HSA	41, 43, 94, 95
	MMI-HSA	44, 83, 95
	<i>p</i> -TMI-HSA	94
Animal Systems		
TDI	TDI-GSA	5, 34, 35, 36
	TDI-DSA	96
	<i>o,p</i> -TMI-GSA	5, 35, 59
HDI	HDI-GSA	12
	HMI-GSA	12
MDI	MDI-GSA	94
	MDI-DSA	98
	<i>p</i> -TMI-GSA	94
CI	CI-GSA	16

Note: Abbreviations: TDI, toluene diisocyanate; HDI, hexamethylene diisocyanate; HMI, hexyl isocyanate; MDI, diphenylmethane diisocyanate; MMI, diphenylmethane monoisocyanate; *o,m,p*-TMI, tolyl isocyanate isomers; CI, cyclohexyl isocyanate; HSA, human serum albumin; GSA, guinea pig serum albumin; DSA, dog serum albumin.

conjugates was sufficiently large to remain excluded from the gel. The greater homogeneity of monoisocyanate antigen preparations was of fundamental importance in developing antigens for use in routine diagnostic assays to detect isocyanate-specific antibodies.

It was recognized that in the workplace individuals are neither exposed to, nor sensitized by, monoisocyanates. Rather, the exposure and thus immunologic stimulus is generated by diisocyanates. It remained to be shown therefore that monoisocyanate-conjugate antigens could detect antibodies produced by exposure to industrial diisocyanates. In animal experiments, conjugates prepared using ortho- and para-tolyl monoisocyanate coupled to guinea pig serum albumin were found successful in detecting antibody to TDI^{5,25} (Table 3). Similarly, in TDI-sensitized workers, IgE antibodies were detected using *o*-, *m*-, and *p*-TMI-serum albumin antigens.²⁶ Best reactions have been obtained using *o*- and *p*-TMI-HSA antigens. Subsequently, hexyl isocyanate antigen was able to detect antibodies produced to HDI or HDI biuret,¹² and *p*-tolyl isocyanate was successful in detecting antibodies to MDI,²⁷ as was diphenylmethane 4-4'-monoisocyanate.²⁸ Thus, the general applicability of using monoisocyanates as haptens for detecting antibodies to corresponding aromatic or aliphatic isocyanates has been firmly established. Recently, monoisocyanate-protein conjugates have been success-

fully applied for skin testing patients with sensitivity to MDI¹¹ or TDI¹¹ and for inhalation challenge.¹⁴

One further problem arises from the high degree of chemical reactivity of isocyanates. In preparing hapten conjugates using isocyanates and serum albumin, it is not unusual to obtain preparations in which the hapten to protein ratio is in excess of 55:1.¹⁴ While such conjugates may appear superficially to be ideal for detecting anti-hapten antibodies, in practice these antigens are inappropriate for such purposes and in fact may fail completely to detect such antibodies.¹⁴ For use in RAST, conjugates containing 10 to 30 hapten groups per mole of protein have been found by numerous investigators to yield greatest success in detecting antibodies to diisocyanates.^{12,19,20,21,22,23}

b. Lymphocyte Reactivity

Whereas there has been frequent association of an IgE-mediated mechanism with the immediate-onset or early symptomatic response to isocyanates, the etiology of the late-phase clinical response to TDI is less certain. Both cell-mediated mechanisms and bronchial hyperreactivity have been implicated as possible mechanisms underlying the late-onset response. Gallagher et al.¹⁹ examined lymphocytes from 15 workers with TDI asthma and 17 nonexposed controls. They found TDI-HSA stimulated lymphocyte activation, as measured by lymphokine (LIF) release, in some of the workers while not in any of the control subjects. However, HDI-HSA also stimulated LIF release, although these individuals had no history of HDI exposure. Of interest was the finding that lymphocyte reactivity was no longer detected when the same workers were removed from exposure for 3 months. The importance of performing immunologic analyses at specified times following exposure has been stressed by others.⁴

The lymphocyte reactivity noted above¹⁹ was associated with both immediate and delayed-onset TDI asthma. Another finding in the study was that, in contrast with ability to detect antibodies, minimally substituted antigens (TDI-HSA conjugates with hapten to protein ratios of 2:1) gave more leukocyte stimulation reaction than did more highly substituted conjugates (13:1 ratio). The authors attributed this finding to in vivo formation of "new antigenic determinants" and recognition of HSA as part of the new determinant. Difficulties with interpretation of this study arise from the frequent minimal level of stimulation obtained, with inconsistencies in the effect of antigen dilution on activity, and with the lack of correlation between lymphocyte activation and another indicator of delayed sensitivity, skin response, in the same individuals using the same antigen. Based on their findings, the authors proposed 4 clinical subpopulations of the 15 sensitized individuals: (1) RAST positive; (2) LIF positive; (3) LIF positive, skin test positive; and (4) negative for all of the above immunologic tests. Correlation of symptoms with the above classifications would be of interest.

c. Animal Models of Respiratory Sensitivity

Recognition of the diverse syndromes associated with isocyanate-induced respiratory diseases prompted development of animal models to best elucidate the mechanisms of the diseases. Various species were employed, as summarized by Karol et al.,²⁷ including mice,²⁷ dogs,^{28,29} rabbits,⁶⁸ guinea pigs,^{4,5,16,17,22,91,94,100,101} and monkeys¹⁰¹ (Table 2). Assuming that isocyanates would function in a similar manner to other immunologic haptens, many studies utilized standard procedures for production of hapten-specific immunologic response. Such methods included use of hapten-protein conjugates,^{68,92,97,100} immunologic adjuvants,^{97,101} and injection^{28,29} or installation^{28,29} of antigen into the respiratory tract. A current example of this approach is provided by Ufkes and Ottenhof¹⁰² in their attempt to produce sensitivity to trinitrophenyl (TNP) hapten. Brown-Norway rats were injected intraperitoneally with TNP-ovalbumin in

the presence of aluminum phosphate adjuvant. Following sensitization, the anaphylaxis response was elicited by intravenous injection with the hapten-protein conjugate. Animal models which utilized such invasive procedures have frequently demonstrated antibody production,^{94,97,99,100} often with hapten-specificity,^{94,97,99} and in some cases exposure to hapten-conjugates via the inhalation route has resulted in hapten-specific respiratory hypersensitivity.^{94,97,99} These models were noteworthy for their demonstration that respiratory sensitivity could have isocyanate-directed specificity. However, one must question the relevance of using such animal models for study of the mechanisms underlying occupationally induced isocyanate respiratory hypersensitivity.

i. Sensitization Using Reactive Haptens

In 1970, TDI in reactive form was noted¹⁰¹ to cause respiratory sensitization in guinea pigs, but not in monkeys. For sensitization, guinea pigs were exposed to concentrations of 0.01 to 5.0 ppm TDI for 6 hr on three occasions. Three weeks later, animals were challenged with 0.02 ppm TDI to assess respiratory sensitization. Guinea pigs reportedly showed an "altered" respiratory pattern to TDI challenge when compared with the response of naive animals and were positive when skin tested with TDI.

Another study⁹⁹ utilized TDI in reactive form to attempt development of an animal model for TDI respiratory disease. Dogs were exposed to a TDI aerosol, using endotracheal administration, at a dosage of 1 mg/kg (Table 2). This single exposure is 2 to 3 times the equivalent of that received by a person working for 2 weeks in an atmosphere of 20 ppb TDI. In the protocol, animals received this dosage every 2 weeks for 4 months. This protocol appears to best simulate occupational exposures to repeated "spills" of TDI. Results of the study indicated that, under such exposure conditions, TDI was immunogenic as assessed by four criteria: (1) antibodies to TDI were detected after 2 to 4 weeks of exposure; (2) immediate skin reactivity appeared within 4 weeks; (3) lymphocyte reactivity was detected, on occasion, during the 41 weeks of experimentation; and (4) respiratory patterns of exposed dogs were notably different from those of naive animals. Regarding the latter point, however, it is difficult to conclude that respiratory "hypersensitivity" reactions were observed because of the high dosage at each exposure. In this study, as in one previously cited,¹⁰¹ respiratory reactions in experimental animals were distinctive both from those observed in naive animals and from reactions in experimental animals prior to the fourth TDI exposure.

Using the inhalation route, respiratory sensitivity was achieved in guinea pigs following exposure to vapors of reactive isocyanates.^{94,95} In the initial report,⁹⁵ sensitization was achieved to TDI and to *p*-TMI using head-only exposure of animals. For TDI, the exposure was 0.25 ppm TDI for 3 hr/day on 5 consecutive days; for *p*-TMI, exposure was for 5 consecutive days to concentrations of 1 to 4 ppm TDI. Hapten-specific cytophilic antibodies and hapten-specific respiratory hypersensitivity was produced to TMI as detected by response of guinea pigs to TMI-serum albumin conjugates. The respiratory response was characterized by immediate-onset increases in respiratory rate with decrease in tidal volume. No responses were elicited following inhalation challenge with carrier proteins alone. By simulating occupational exposure to airborne reactive isocyanates, this study demonstrated that inhalation of reactive isocyanates could result in respiratory sensitivity without need of protocols utilizing adjuvants or injection. These findings have been confirmed by others.¹⁰² Subsequent studies were designed to determine the *conditions* of exposure most likely to result in sensitization. It was revealed that exposure protocol greatly influenced the development of respiratory hypersensitivity.

ii. Sensitization is Concentration Dependent

TDI — The concentration of inhaled isocyanate was found to directly influence the development of hypersensitivity.⁹⁴ Sets of guinea pigs were exposed to increasing con-

Table 4
 PROTOCOLS FOR SENSITIZATION OF GUINEA PIGS TO TDI

Total ppm · hr	Days of exposure (hr/day)	Exposure concentration (ppm)	Antibody titer (X)	Lung sensitivity (% responders)	Dermal sensitivity (% responders)
8.7	70 (6)	0.02	0	0	0
9.1	5 (3)	0.61	42	25	100
9.6	2 (3)	1.60	8	13	—

From Karol, M. H., *Toxicol. Appl. Pharmacol.*, 68, 229, 1983. With permission.

concentrations of TDI. The experimental protocol utilized head-only exposure on 5 consecutive days for 3 hr each day to TDI concentrations ranging from 0.12 to 7.6 ppm (Table 4). Immunologic response was measured in three ways: (1) by production of TDI-specific cytophilic antibody, (2) by elicitation of TDI-specific respiratory hypersensitivity, and (3) by the presence of TDI-specific skin sensitivity.¹⁶ Antibody measurements were made 3 weeks from the start of exposure. The results are shown in Figure 6. Both the titer of anti-TDI antibody and the percentage of guinea pigs which produced antibody were dependent upon the initial concentration of TDI at exposure. For example, exposure to 0.36 ppm resulted in 50% of the animals producing antibody, whereas exposure of a group of animals to 0.93 ppm TDI resulted in antibody production by 100% of the animals. Of equal importance in these studies was the observation of a "no effect" or threshold level for response. No antibodies were detected following exposure to concentrations of 0.12 ppm TDI or lower (Figure 6).

In addition to the antibody response, TDI-specific respiratory responses were elicited. These responses, too, were concentration dependent in that no responses were elicited in any animal which had been exposed to 0.12 ppm TDI, whereas a percentage of those exposed to 0.36 ppm or greater displayed sensitivity upon challenge.

The critical influence of exposure protocol on respiratory sensitization and antibody production was established in the following series of experiments. As indicated in Table 4, three groups of guinea pigs were exposed to the same cumulative amount of TDI vapor (approximately 9 ppm·hr) by varying the airborne concentration, number of days and hours per day of exposure, and intervals between exposure. Results indicated significant differences in response dependent upon the exposure protocol. The protocol most favorable for production of both respiratory sensitivity and antibody titer was 5 consecutive days of exposure to a moderately high concentration (600 ppb) of TDI.

A striking example of the effect of exposure protocol on sensitization was also evident from the study by Doe et al.¹⁰⁴ Inhalation exposure of guinea pigs to 1 ppm TDI for 5 hr resulted in production of suppressor cells which suppressed subsequent development of TDI contact sensitivity. Thus, sensitization procedures undoubtedly influence populations of suppressor, helper, and effector cells with the clinical outcome reflecting a balance of opposing effects.¹⁰⁵ The need for strictly defining exposure conditions and concentrations cannot be overstated.

iii. Respiratory Sensitization Following Dermal Exposure

Numerous clinical reports related development of isocyanate sensitivity to accidental isocyanate exposures during industrial spills or splashes.^{25,31,32,106} Such accidents frequently involve isocyanate contact with the hands, face, and sometimes the whole body. They suggest that a single exposure to a very high concentration of isocyanate involving not only inhalation, but also dermal contact may be an appropriate protocol for production of sensitization.

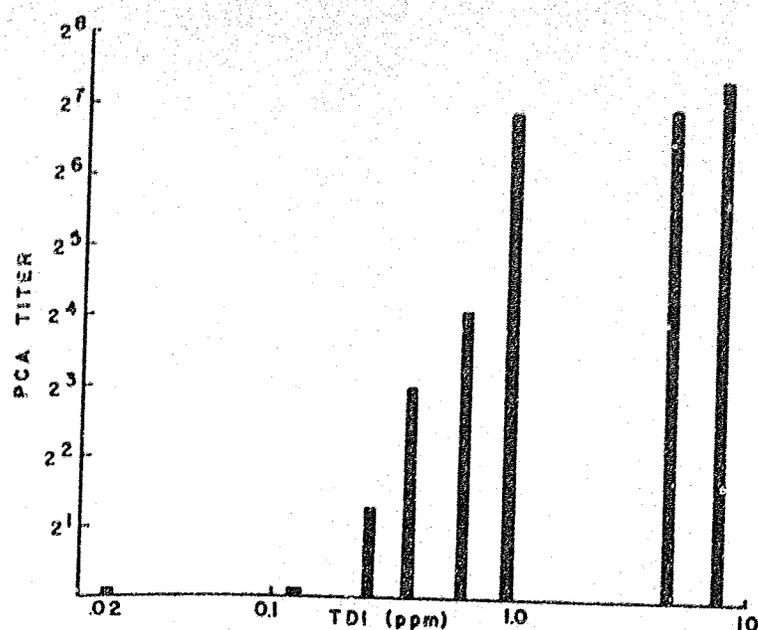


FIGURE 6A. Mean antibody titers to TDI. Values are for sera drawn 22 days following initial TDI inhalation exposure. Titer is the reciprocal of the highest serum dilution which yielded a positive reaction in the PCA assay using TDI-HSA as antigen. For 0.12 to 1.6 ppm, $n = 8$ to 16; for 0.02 ppm TDI, $n = 24$ (From Karol, M. H., *Toxicol. Appl. Pharmacol.*, 68, 229, 1983. With permission.)

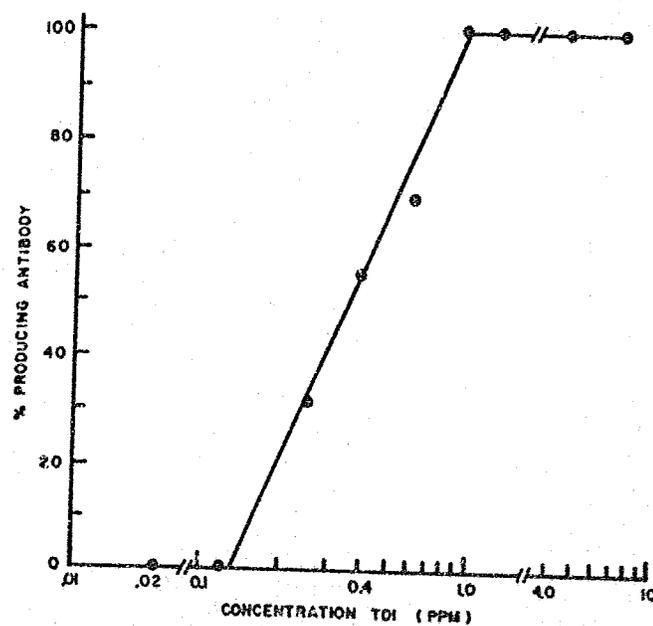


FIGURE 6B. Percentage of animals producing antibody to TDI. Animals were exposed to the indicated concentrations of TDI for 3 hr/day on 5 consecutive days. Regression equation: $Y = 43.61 \log x + 92.22$, $r = 0.949$, $SE = 13.27$. (From Karol, M. H., *Toxicol. Appl. Pharmacol.*, 68, 229, 1983. With permission.)

Dermal contact with reactive haptens is known to result in delayed-onset cutaneous hypersensitivity.¹⁰⁷ For example, application of dinitrochlorobenzene or oxazolone onto the shaved skin of guinea pigs has been known to result in contact sensitivity.¹⁰⁸ To explore possible respiratory sensitivity resulting from dermal contact, guinea pigs were exposed to 50 μl of TDI solutions (1 to 100%) by applying the solution to a shaved, but not abraded, dorsal site.¹⁴ While sites were air drying, animals were held in plastic cages before being returned to their quarters in the animal room. Since TDI has a high vapor pressure, while in the plastic cages animals would be inhaling a small amount of isocyanate. Accordingly, the concentration of TDI in the atmosphere of the cage was measured using a portable MDA tape monitor (MDA Scientific, Inc.). Results indicated an initial peak exposure of 0.4 to 0.5 ppm lasting for several minutes, then dropping to undetectable levels. Comparison of such airborne concentrations with protocols previously shown to be required for sensitization via inhalation (Table 4) indicated that the level of TDI in the air following dermal application was below that necessary for sensitization of naive animals.

For sensitization, TDI solutions were applied in 25- μl amounts to each of two dorsal areas. Solutions were spread over an area of 25 cm in diameter using a glass rod. Seven days later, contact sensitivity was apparent from challenge of animals using 25 μl 0.1% TDI onto a naive depilated dorsal site.¹⁴ The sensitivity was TDI-specific since it could not be elicited upon topical challenge with either HDI or olive oil diluent.

The possible presence of pulmonary hypersensitivity as a consequence of dermal contact with TDI was investigated by bronchial provocation challenge of animals. At 14 to 21 days following exposure, animals were challenged by inhalation of antigen conjugates composed of TDI-protein or TMI-protein or with 0.005 ppm TDI vapor. Out of 12 animals, 4 responded to challenge with an increase in respiratory rate and decrease in tidal volume, typical of a hypersensitivity response. This study thus indicated that a single dermal exposure to TDI resulted not only in contact sensitivity, but in pulmonary sensitivity in a percentage of the animals.

Further evidence for the immunologic stimulus resulting from dermal contact with TDI was obtained from serologic evaluations. Blood was drawn 14 days following dermal application of TDI and evaluated for anti-TDI antibodies. Antibodies were detected in animals which had been exposed to 1% TDI (Table 5). Moreover, results indicated a dose-response relationship between the concentration of TDI solution at exposure and both the number of animals producing antibody and the antibody titer.

In order to apply experimental findings to possible diagnostic use for dermally exposed TDI workers, the duration of the antibody response was investigated. Serial bleedings were taken from animals at weekly, then monthly, intervals for a period of 4 months following the single dermal exposure. Maximum antibody titers were detected 2 to 3 weeks following the exposure. A percentage of these antibodies were of the IgE class. By analogy, identification of the optimum time for detection of anti-TDI antibody following exposure of TDI workers was found to be essential for successful clinical screening of workers using RAST.^{5,29}

4. Recovery from Sensitization

a. Clinical Studies

One of the important questions relating to occupational pulmonary hypersensitivity is the concern over permanence of sensitization. Following removal from isocyanate exposure, will there be return of normal pulmonary function and/or loss of hypersensitivity? Such occurrence would enable eventual return of the individual to the workplace. Few reports have addressed these questions.

Burge et al.¹⁰⁹ reported recovery of pulmonary function in 20 sensitized workers following inhalation challenge using isocyanate fumes. Peak expiratory flow rate was

Table 5
 PASSIVE CUTANEOUS ANAPHYLAXIS TITERS OF
 GUINEA PIGS TOPICALLY SENSITIZED WITH
 INCREASING CONCENTRATIONS OF TDI

Concentration TDI used for sensitization	Number of animals tested	Number positive/ total	Antibody titers*
1%	5	0/5	0, 0, 0, 0, 0
10%	6	4/6	0, 0, 2, 4, 4, 16
25%	4	3/4	0, 32, 32, 128
100%	8	8/8	32, 32, 32, 32, 32, 64, 64, 64
100%, 2 days (total amount, 100 μ l)	4	4/4	64, 64, 64, 64

- * Titer is the reciprocal of the highest serum dilution yielding a reaction of at least 5-mm diameter. Intravenous challenge was performed with 2.5 mg TDI-GSA in 0.5 ml PBS containing 5 mg Evans blue dye. A latent period of 6 hr was allowed between intradermal injection and intravenous challenge.
- * Total amount, 50 μ l.

From Karol, M. H., Hauth, B. A., Riley, E. J., and Magreni, C. M., *Toxicol. Appl. Pharmacol.*, 58, 221, 1981. With permission.

measured at various times following challenge of individuals with 1 to 7 ppb TDI, and then complete removal of individuals from all further isocyanate exposure. Results indicated a slow recovery of pulmonary function beginning 4 to 7 days following removal from the workplace. Recovery was essentially complete 70 days after leaving the workplace.

Loss of sensitization was addressed separately by Karol¹⁹ and Butcher et al.²⁰ in a study of the same individual. The subject was a 32-year-old gentleman who had been employed for 10 years by a TDI manufacturing company. After 1.5 years of employment, he had begun to experience immediate-onset respiratory symptoms after exposure to isocyanates. Bronchial provocation challenge at that time with 6 ppb TDI resulted in a 76% decrease in FEV₁ within 10 min.²⁰ The worker was removed from all further TDI exposure, and the status of his sensitivity followed for several years by provocative inhalation challenges. Respiratory sensitivity to TDI was lost within 11 months (Figure 7). Whereas before removal, the individual had responded within 10 min to challenge with 6 ppb TDI, 11 months later there was no response to challenge with up to 20 ppb TDI (the TLV at the time) for 15 min. Loss of other reactivities was also noted.²⁰ Bronchial hyperreactivity (assessed by response to methacholine challenge) was lost after 17 months, while lymphocytic cAMP responsiveness and RAST titer returned to near normal levels after 2 years.

Karol¹⁹ studied the anti-TDI IgE antibody response of this worker during the period of sensitization and continuing for 6 years following removal from exposure. Serial blood samples were drawn approximately bimonthly for 2 years, then annually. RAST titers are shown in Figure 7. Currently, in the author's laboratory, positive RAST titer is defined by the degree of binding of sera to antigen-coated discs (RAST binding), and inhibition of that binding by unbound TDI antigen (percentage inhibition). Specifically, RAST binding greater than 5% coupled with RAST inhibition greater than 25% is indicative of a positive titer. By this criterion, the worker had essentially lost RAST titer by 18 months following removal from isocyanate exposure. On the other hand, if RAST titer is defined as "positive" when the ratio of cpm bound to TMI-

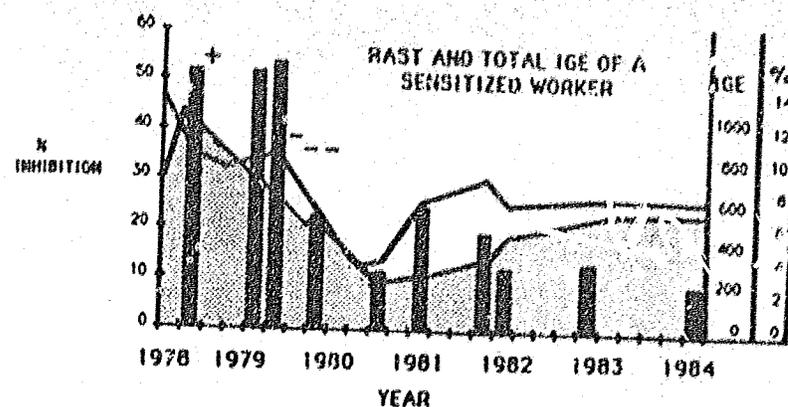


FIGURE 7. RAST and total IgE titers of serial serum samples from an individual who displayed positive respiratory sensitivity (+) in March 1978. Bronchial challenges in 4/79, 9/79, and 2/80 were negative (-). Positive RAST titer: RAST binding of 5% or greater (shaded area, legend far right) with RAST inhibitor greater than 25% (bar graph, left legend). Total IgE content of serum samples is indicated by the line graph (right legend).

HSA discs divided by cpm bound to HSA discs is greater than 2,¹⁵ RAST reactivity of the worker's serum was positive until 2 years postsymptomatology (titer fell from an 11.5 ratio at the time of symptomatic challenge to a ratio of 3.5 at 2 years postexposure). The difference in definition of RAST "positive" titer led the two groups of investigators to draw different conclusions regarding the role of IgE antibody in this worker's respiratory hypersensitivity.

A study was undertaken of the relationship between isocyanate exposure concentration in the workplace, development of antibody response, and time required to lose RAST titer.¹⁶ A survey was performed to detect TLV antibodies in workers of a large TDI manufacturing company (Table 6). Approximately 100 workers in the company were exposed only to the TLV concentration (20 ppb) TDI or lower throughout the 2-year study. As seen in the table, none of these individuals was found to produce antibodies to TDI and, further, there was no clinical indication of sensitivity to TDI in any of these workers.

Results were quite different, however, in workers who, during the study, had experienced accidental exposure to high TDI concentrations. Attempts were made to estimate the concentration of TDI at exposure by noting the severity of the irritative symptomatology at exposure. Using this estimate, exposures were grouped by severity, with the highest exposures being those which caused immediate-onset symptomatology accompanied by pulmonary function decrement. Least severe exposures were those reported by workers which did not cause any respiratory symptomatology either immediately following exposure or within the next 24 hr. Table 6 presents the antibody response for each group. Three of the four workers in the highest exposure group developed specific IgE antibodies following exposure. Antibody titers were apparent within 3 to 4 weeks following exposure. By contrast, individuals with less severe exposures seldom developed an antibody response. These results thus agree with the animal studies discussed above, that high exposures lead to immunologic stimulation and antibody response.

In the above study, antibody titers were followed over time in all individuals who developed an immunologic response. Two general patterns of decline were noted. For "nonatopic" individuals (those with total IgE < 200 IU/ml), decline was rapid with normal RAST values attained by 4 to 6 months following exposure. In atopics, on the

Table 6
 PRODUCTION OF ISOCYANATE-SPECIFIC IgE ANTIBODY
 IN ISOCYANATE WORKERS DURING A 2-YEAR STUDY^a

Exposure concentration ^a	Number of workers	Number with positive RAST titer
Ambient (<20 ppb TDI)	96	0
High (immediate irritation with spirometric change)	4	3
Moderate (immediate irritation, no spirometric change)	9	1
Low (delayed irritation, no spirometric change)	5	0
Very Low (spill but no symptomatology)	11	0

^a Exposure concentration was estimated from the severity of the irritation response at acute exposure.

other hand, RAST titers took between 6 months to several years to return to normal levels, although isocyanate exposure had ceased.

These findings have implications for performing RAST assay for diagnostic purposes. As emphasized previously, RAST should be performed on sera drawn 2 to 4 weeks following symptomatic exposure and antigens used for RAST assay must have received prior evaluation for effectiveness in detecting anti-TDI antibody. With these guidelines, the controversy regarding the prevalence of IgE antibody in cases of TDI exposure would diminish.

b. Significance of IgE Antibody

In the animal study,²⁴ all guinea pigs exposed to 1 ppm or greater TDI for 5 days developed cytophilic anti-TDI antibodies. However, only a percentage of these animals displayed respiratory sensitivity upon inhalation challenge. In the industrial study,²⁵ IgE antibodies were produced following some acute isocyanate exposures. Although such antibodies are associated with immediate hypersensitivity reactions, it cannot be assumed that all individuals possessing such antibodies would have responded to bronchial provocation challenge with TDI. In both studies, the presence of specific IgE antibodies indicated acute exposure. However, animals and workers with elevated titers most frequently displayed bronchial sensitivity. It must be concluded that RAST screening of acutely or routinely exposed workers can offer an early indication of developing isocyanate sensitivity.

5. Hyperreactive Airways

The relationship between "hyperreactivity" and "hypersensitivity" has intrigued many investigators. High concentrations of TDI (0.1 ppm or greater) are known to cause respiratory tract irritation. Chester et al.¹⁰ studied the irritative effects of low concentrations of TDI in 20 symptomatic TDI workers and 10 extrinsic asthmatics with no history of exposure to TDI.

Those symptomatic TDI workers who responded to challenge with 0.02 ppm TDI had methacholine sensitivity, whereas symptomatic TDI workers who did not respond to challenge (n = 11) had less hyperreactivity. Of the responders, three were late onset, one was immediate onset, and five had dual-onset responses. None of the extrinsic asthmatics responded to TDI challenge although they had methacholine sensitivity. Thus, the response to TDI is specific. A similar conclusion was reached by O'Brien et

al.¹⁰ These investigators found that only individuals with a history of isocyanate exposure responded to isocyanate provocative challenge. Control patients with hyperreactive airways did not respond to isocyanate challenge. However, it has been suggested that hyperreactivity may be necessary to elicit the response in sensitized subjects.¹⁰ Repeated testing of two TDI-exposed workers at a time when they had upper respiratory tract infections and methacholine sensitivity produced response to provocative TDI inhalation challenge, whereas previously they did not respond.¹⁰ It can be concluded that TDI does not cause asthma by a nonspecific irritant effect, but, concomitant irritation or hyperreactivity of the airways may produce heightened respiratory tract responsiveness (or recurrent asthmatic reactions)¹¹ in isocyanate-sensitized individuals. On the other hand, Smith et al.¹¹ described an individual with TDI hypersensitivity who responded to bronchial provocation challenge with 16.5 and 30 ppb TDI, but did not have methacholine reactivity.

C. Pulmonary Effects from Low Level Exposure

Numerous studies have been performed in which pulmonary function was measured in workers having chronic exposure to low levels of isocyanate (Table I). Most reports relate to TDI rather than other isocyanate exposure undoubtedly because other isocyanates have only recently come into large scale use.

Gandevia¹² measured FEV₁ of workers during each of three normal working shifts. Monitoring 15 workers, he observed a mean decrease of 0.18 l and related this finding to TDI exposure since the decrease did not occur on days when the process involving liberation of isocyanate was stopped. Recovery of function was not complete overnight because Friday morning values were lower than those on Monday. Based on these findings, the author concluded that adverse respiratory effects resulted from exposure to low concentrations of isocyanates.

Wegman and collaborators¹³ also found an acute pulmonary effect from low concentrations of TDI. Further, the effect followed a dose-response relationship in the 112 workers studied. These studies were performed only on the first day of the working week.

To explore a possible chronic effect of the exposure, 2 years later, the same group was restudied.¹³ Of the 63 workers available, only those exposed to TDI concentrations of 3.5 ppb showed an FEV₁ decline greater than that expected. Of considerable interest was the finding of a significant association between the acute and chronic decrement in FEV₁. Exposures were determined using personal and area monitoring with analysis according to Marcali. Long sampling periods were required because of the low TDI concentrations. However, such procedures eliminate the possibility of observing sporadic peak exposures. The ability of the authors to group workers according to 2-year exposures of 1.5 ppb, 2 to 3 ppb, and 3.5 ppm to conclude a dose (concentration) response effect must be questioned.

Another study¹⁴ failed to identify decreased pulmonary function in workers with chronic isocyanate exposure. Workers averaging 11-years employment in the corporation showed no drop in ventilatory function compared with control subjects. However, symptomatic workers, i.e., those with asthma-like symptoms, chronic emphysema, or chronic bronchitis, did show an FEV₁ value 267 ml lower than that predicted. The conclusion from this study would be that diminished lung function occurred only in sensitized individuals or those with chronic lung disease.

The possibility of developing fibrosis following a long period of exposure to low concentrations of TDI was suggested from a study reported by Pham et al.¹⁵ Bronchitis was more prevalent in the exposed than control group. Long term exposure (more than 5 years) to MDI tended to cause restriction of pulmonary function and decline in carbon monoxide transfer. This suggestion of fibrosis was only seen in the group of men

($n = 27$) exposed for more than 60 months and was not evident in women exposed for 60 months or longer, or in men exposed for 20 to 60 months.

In contrast to the previous reports, Musk et al.⁴⁵ failed to detect any decrement in FEV₁ in groups of workers exposed for 5 years to MDI and TDI. The ambient isocyanate concentrations were 1 ppb, time-weighted average. In fact, the latter FEV₁ values were higher than those predicted for healthy subjects, and there was no change in FEV₁ values after a vacation period. These investigators also looked for an acute pulmonary effect during the workshift and did not observe any. The conclusion from this study was that exposure to extremely low levels of isocyanates was not associated with chronic respiratory symptoms or diminished ventilatory capacity.

Another recent study has drawn a similar conclusion regarding exposure to extremely low levels of TDI. Diem et al.⁴⁶ investigated pulmonary function during a 5-year period in a new TDI manufacturing plant. Respiratory health of 277 workers was investigated prospectively, while exposures to TDI were monitored through the use of personal monitors. Three exposure groups were recognized: high (5 ppb TDI or greater), medium (2 ppb), and low (less than 2 ppb). Results indicated that workers in the high TDI exposure group (cumulative exposure) had a significant decline in pulmonary function when compared to those in the lower exposure category after adjustment had been made for smoking. The high and low groups spent 15 and 2% of their time, respectively, in atmospheres of greater than 5 ppb TDI. The significant decline was 37 ml/year, a small volume compared to that of 152 ml/year found by Peters and Wegman¹⁵ for workers exposed to 15 ppb. The conclusion was drawn⁴⁶ that an association existed between exposure to higher TDI concentrations and greater than expected declines in FEV₁ and FEF₂₅₋₇₅. This association was recognized only for non-smokers, while for previous and current smokers there was no difference between high and low exposure groups.

Further studies continued to indicate an association between exposure concentration and impairment of pulmonary function. FEV₁ declines were related in a dose-response manner to TDI concentrations in the range of 1.5 to 3.5 ppb.^{11*}

Many of the cited studies reported a pulmonary decrement associated with TDI levels in the range of 3 to 5 ppb, but not below 1 ppb. These conclusions are highly dependent upon an accurate assessment of the exposure received by the individual workers. Obtaining such accurate exposure measurements from area monitors would appear impossible. Personal monitoring performed on occasion also assumes uniform exposure during the several years of study. Before accepting that pulmonary impairment may develop from exposure to 5 ppb TDI, the current recommended TLV for TDI (ACGIH, 1983), one must question whether persons in the highest exposure groups have job descriptions which would entail their being exposed on occasion to considerably higher levels of isocyanate, and perhaps these sporadic high exposures are causing the pulmonary impairment.

1. Recovery from Low Level Exposure

As described in Section IV.A.3, several reports relate complete recovery (as assessed by return to predicted lung function ability) following acute TDI exposure. By contrast, disagreement exists concerning recovery from low level isocyanate exposures. Wegman et al.⁴⁶ studied 111 workers exposed to TDI during a workshift. They reported detecting acute declines in FEV₁ during the shift which was correlated with the level of exposure. Levels were in the range of 2 to 13 ppb and FEV₁ losses were 78 ml when exposures were 2 to 3 ppb; 112 ml following workday exposures of 4 ppb; 106 ml at 5 ppm at 180 ml as a result of workshift exposures of 6 to 13 ppb. The uncertainty in measurement of these low exposures using the Marcali methods or area sampling monitors is obvious. Additionally, corrections for effects of diurnal influences, smoking,

and lung volume must be made. Nonetheless, consideration should be given to evaluating the effect of low level exposure on pulmonary function.

To obtain such needed information, continuous monitoring of workers is implied (to detect sporadic high exposures), but unlikely to be achieved. On the other hand, similar information has been obtained from animal studies where exposures were monitored continuously. These results are discussed below.

2. Chronic Effect of Low Dose Exposure

Guinea Pigs -- A study was performed on guinea pigs of the effect of long-term exposure to 20 ppb TDI.¹⁷ Using the CO₂-challenge method¹⁸ to assess pulmonary performance, animals were exposed for 6 hr/day, 5 days/week for 70 days to atmospheres of 20 ppb TDI. Weekly pulmonary performance was measured, and additionally, animals were evaluated for possible development of TDI sensitization.¹⁹ Results indicated neither impairment of pulmonary function as a result of these exposures (Figure 2), nor any sign of sensitization when assessment was made on the basis of antibody production, skin sensitivity, or pulmonary response to 20 ppm inhalation challenge (Table 4). In this instance then, the animal does not agree with the clinical report.

V. CONCLUSIONS

Exposure to isocyanates has been shown to produce a number of adverse health effects, from pulmonary symptoms to neurologic and dermal disorders. Certain isocyanates tend to produce one type of disease, whereas others may produce a different effect. For example, HMDI is strongly associated with dermal sensitization,^{14,15,17} in contrast to TDI which produces predominantly pulmonary reactions.^{21,22} It follows that generalizations and predictions about likely effects of new isocyanates would be inappropriate at this time without prior testing.

A number of pulmonary syndromes have been identified following isocyanate exposure, including sensory and/or pulmonary irritation, dermal and/or pulmonary sensitization, and acute and/or chronic impairment of lung function following low-level exposures. Animal models have been developed to examine each of these effects. Remarkably, all classes of effects, except one response to low-level exposure, have been reproduced in some animal model system.

Acute effects resulting from high concentrations of isocyanate during industrial accidents have been described.^{23,24} These effects are typically associated with concentrations greater than 0.1 ppm. Similar effects of sensory and pulmonary irritation have been produced during exposure of animals to TDI,²⁵ MDI,^{11,17} HMDI,^{11,17} and HDI,¹¹ as well as with several monofunctional isocyanates.¹¹

Respiratory sensitization has been associated with TDI production and utilization. Numerous investigators have recognized an association between development of sensitivity and involvement in an accidental spill or splash. A guinea pig model for sensitization to TDI has been described.^{24,25} The model differs from others in that exposure of animals is via conditions which simulate occupational exposures. Using this model, immunologic response to TDI was shown to follow a concentration-response relationship.²⁴

Inhalation of a second isocyanate, HMDI, has been associated with dermal sensitization.^{14,15} Interestingly, in the guinea pig model, dermal sensitivity resulted from *inhalation* of the chemical.¹⁷ Sensitization was concentration dependent. Higher concentrations at exposure resulted in both greater severity of skin reactions and in a greater proportion of animals becoming sensitized. Just as skin sensitivity may result from inhalation or dermal exposure to HMDI, respiratory sensitivity to TDI was found to

result from either route of exposure.^{24, 25} For both routes, concentration-dependence for TDI sensitization was observed.

Concern is frequently expressed regarding the permanence of respiratory effects from isocyanates. For example, is there complete recovery of pulmonary function following an industrial accident? If the exposure results in sensitization, would the sensitivity be permanent, or, in time, would there be loss of sensitivity? Clinical studies have indicated full recovery of lung function following acute isocyanate exposure.^{26, 27} Similarly, guinea pigs acutely exposed to TDI vapors and demonstrating loss of pulmonary function regained full pulmonary performance following weeks without exposure.²⁷

The above parallels between human and animal responses to isocyanates are impressive. Moreover, for many of the responses, dose-response relationships were existent. These two findings imply that following calibration of each animal system, predictions can be made regarding not only effects following exposures, but also of safe exposure levels to isocyanates resulting in no adverse pulmonary reactions. Calibration of one of these animal models to man has been made by Alarie.²⁸ Using the calibration, the suggested TLV for TDI was 6 ppb. Recently, based on clinical reports, ACGIH re-evaluated the current 20 ppb TLV for TDI and suggested it be reduced to 5 ppb TLV-TWA. In a similar manner, calibration of the other animal systems should enable prediction of safe levels of exposure to protect against respiratory and skin sensitivity.

The human and animal systems differ with respect to effects from continued low-level exposure to TDI. Guinea pigs showed no impairment of pulmonary performance during or following exposure to 20 ppb TDI for 6 hr each day for 70 days.²⁷ Additionally, none of the animals developed any indication of sensitivity to TDI when evaluated for skin sensitivity, respiratory sensitivity, or antibody production.²⁴ In contrast, some investigators have reported diminished lung function in workers after 2-years exposure to 1.5 to 3.5 ppb.⁴⁵ It is possible that the human lung is much more sensitive to a chronic effect than is that of the guinea pig and that such an effect may be produced in guinea pigs at higher exposure levels. More likely, however, is the possibility of excursions from exposures of 1 to 3.5 ppb TDI during the lengthy studies. Detection of low levels of TDI required obtaining the average exposure during a long sampling period. Short exposures to high concentrations would be missed and it has now been demonstrated that such exposure spikes could account for pulmonary effects.

With the increased consumer use of isocyanates during the past 20 years, there has been active interest in the health effects of these chemicals. As a result of efforts by investigators throughout the world, great progress has been made in identifying the effects of isocyanates, in determining exposure concentrations, and in understanding the mechanisms of the responses. Development of appropriate animal models for these effects and recognition of dose-response relationships can be expected to provide the necessary guidance for setting appropriate exposure levels for current and future industrial isocyanates. It is hoped that by using these recently developed tools future cases of isocyanate respiratory disease will be extremely rare.

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