Antibodies to Toluene Diisocyanate in an Environmentally Exposed Population

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Residents living near a polyurethane foam manufacturing facility expressed concern to health officials over chemical emissions from the plant. Environmental monitoring of ambient air near the plant indicated the presence of toluene diisocyanate (TDI), which was used in foam production. Health officials collected blood samples from 113 residents and analyzed the blood sera for antibodies to TDI and related diisocyanates. Ten of the 113 residents (9%) had elevated levels of IgG or IgE antibodies specific for one or more diisocyanates. Exposure histories were taken from antibody-positive individuals to identify possible occupational exposure to TDI or the use of diisocyanate-containing consumer products. Exposure to TDI in ambient air may be responsible for the positive antibody responses detected in some residents of the community. *Key words:* ambient air, antibodies, environmental exposure, toluene diisocyanate (TDI). *Environ Health Perspect* 106:665–666 (1998). [Online 11 September 1998]
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Residents of a community in Randolph County, North Carolina, contacted the Agency for Toxic Substances and Disease Registry (ATSDR) because of health concerns over possible exposures to chemical emissions from a manufacturing plant in their neighborhood. The facility produced polyurethane foam by reacting a resin, typically a polyether such as polyoxypropylenetriol, with toluene diisocyanate (TDI) and water. Small quantities of an emulsifying agent, a polymerization catalyst, and a silicone lubricant were also added. Emissions from the foam-making process were directed to stacks, which vented them to ambient air. Emissions from the process could also escape from air vents in the building. Foam production occurred in batches, which resulted in episodic releases of emissions.

Using a TDI tape meter, ATSDR staff detected TDI in residential ambient air near the facility at concentrations as high as 29 ppb (209 $\mu g/m^3$). The presence of TDI in ambient air was confirmed by an alternate method in which diisocyanates were captured on glycerol-impregnated filters, chemically derivitized, and analyzed using high performance liquid chromatography.

TDI releases were episodic and usually occurred every few days, although releases were sometimes detected as often as twice a day. During TDI releases, air levels were typically elevated for less than 10 min. However, on a few occasions, TDI levels remained elevated for more than 1 hr.

Residents living near the plant expressed concern over possible health effects from breathing chemicals in air emissions from the plant. TDI was of particular concern because TDI can cause respiratory irritation and asthma, which some residents had reported. In response to these concerns, ATSDR, in cooperation with the Randolph County Health Department (RCHD), initiated an exposure investigation. The purpose of this investigation was to conduct biological monitoring of residents to determine if they had been exposed to TDI or other diisocyanates.

To measure occupational exposure to TDI, researchers have measured levels of toluene diamine (TDA) in hydrolyzed plasma and urine (*1*). However, the half-life of TDA in plasma is 6–10 days, so TDA biomonitoring can only detect recent exposure.

Other researchers have measured serum antibodies to TDI and other diisocyanates in occupationally exposed workers (*2–5*). Biomonitoring for antibodies has the advantage that antibodies can be detected even though exposure has not recently occurred. In TDI-sensitized workers, antibody titers decrease after exposure has stopped, but antibodies can still be detected several months after total removal from diisocyanate exposure (*6*).

In this investigation, blood samples were obtained from the residents and tested for antibodies to diisocyanates. The presence of antibodies to these chemicals was used as a biomarker of exposure. As defined by the National Research Council, "A biological marker of exposure is a xenobiotic chemical or its metabolite or the product of an interaction between the chemical and some target cell or biomolecule" (*7*).

Materials and Methods

The RCHD mailed flyers to residents to inform them of the investigation. Residents who lived within one-quarter mile of the plant and those who were experiencing respiratory health problems were particularly encouraged to participate. The minimum age for participation was 2 years.

A total of 113 residents volunteered for testing. This population consisted of 99 adults and 14 children (16 years of age or younger). The gender distribution was 63 females and 50 males. This investigation was conducted as a public health service, so anyone who requested to be tested was accepted; no attempt was made to select a test population that paralleled the demographic makeup of the community.

Before testing, each adult participant signed an informed consent form. Minors and their parents or guardians signed an assent form. A licensed medical technician collected blood samples from 113 participants by venipuncture. The blood samples were collected in 10-ml Vacutainer tubes. At the end of the collection day, the tubes were centrifuged and stored in a refrigerator until they were shipped via overnight mail on ice packs to the laboratory for analysis.

The blood specimens were sent to the University of Cincinnati Diagnostic Allergy Laboratory. The blood sera were analyzed by an enzyme-linked immunosorbent assay (ELISA) for IgG and IgE antibodies to TDI-human serum albumin (HSA) conjugates, hexamethylene diisocyanate (HDI-HSA), and diphenylmethane diisocyanate (MDI-HSA).

IgG antibodies were assayed in triplicate by coating microtiter plates with 100 µl of an antigen solution, consisting of diisocyanate-conjugated human serum albumin. Plates were washed four times with phosphate-buffered saline (PBS)–0.5% Tween 20 (T-PBS) and blocked for 1 hr with 1% T-PBS containing 1% bovine serum albumin (BSA). After washing, 100 µl of test sera was diluted 1:10 and 1:100 in 5% BSA in T-PBS and added to each test plate. Controls included a positive reference serum and a panel of normal sera obtained from laboratory volunteers. After washing, goat anti-human IgG conjugated to alkaline phosphatase and diluted in 1% BSA was added, and the plates were incubated for 1 hr at room temperature. After washing, 100 µl of diluted substrate was added to each well. The substrate consisted of a 1 mg/ml solution of

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p-nitrophenyl phosphate in substrate buffer (10% diethanolamine containing 0.5 mM $MgCl_2$, pH 9.8). The plates were read at 410 nm using an ELISA plate reader.

IgE antibodies were analyzed by a modification of the IgG protocol, substituting ELISA reagents with specificity for the IgE immunoglobulin isotype. Test sera were diluted in 1% BSA instead of 5% BSA. In addition, an extra step was added to the IgG protocol; rabbit anti-goat IgG (H+L) conjugated to alkaline phosphatase was added after the goat anti-human IgE.

Results were analyzed by determining the mean optical density (OD) of the three replicate wells for each serum sample tested at 1:10 and 1:100 dilutions. Samples were classified as positive if they exceeded three standard deviations above the mean OD of seven negative control samples plus the OD of the patient's human serum albumin at the same serum dilution.

Results

Of the 113 participants that were tested, 10 people (9%) had antibodies to one or more of the diisocyanates. Nine participants had IgG antibodies to TDI, and one participant had IgE antibodies to TDI. Four participants had antibodies that reacted with more than one diisocyanate. The results of the antibody tests are summarized in Table 1.

Individuals with positive antibody tests were interviewed to identify possible sources of exposure to diisocyanates. One of the 10 positive individuals reported having occupational exposure to TDI or other diisocyanates. In addition, two individuals reported using polyurethane varnishes, a possible source of diisocyanates, in their home. None of the other seven positive individuals reported exposure to polyurethane varnishes, concrete sealers, or other known sources of diisocyanates (*2,8*).

Table 1. Participants with antibodies to diisocyanates[a]

	TDI	HDI	MDI
1[b]	IgG (1:100)	–	IgE (1:100)
2	IgG	–	–
3	IgG (1:100) IgE	IgG	–
4[c]	IgG	–	–
5	IgG	–	–
6	IgG (1:100)	–	–
7	IgG	–	IgG
8	IgG (1:100)	–	IgG
9[c]	–	IgE	–
10	IgG	–	–
Total	9	2	3

Abbreviations: TDI, toluene diisocyanate; HDI, hexamethylene diisocyanate; MDI, diphenylmethane diisocyanate.
[a]The sera dilutions were 1:10 except where indicated.
[b]Occupational exposure.
[c]*Possible* exposure to diisocyanate-containing consumer products.

Discussion

Occupational exposure to TDI and other diisocyanates can cause irritation of the eyes, upper respiratory tract, and skin. It has been estimated that 5–10% of workers exposed to diisocyanates will develop occupational asthma (*2*). In some instances, exposure to TDI results in sensitization, which is defined as a tendency to be susceptible to TDI at concentrations much below those that affect most persons. The exposure level of TDI that causes sensitization is not well characterized, but it can occur at levels below the Occupational Safety and Health Administration (OSHA) short-term exposure level of 20 ppb (*9*). In one study, sensitization reportedly occurred at exposure levels as low as 2 ppb (*3*).

It has been estimated that 10–30% of symptomatic workers develop IgE antibodies to diisocyanates (*2*). In one study of 1,780 workers who were exposed to diisocyanates in the workplace, IgE antibodies to diisocyanates were detected in 13.6% of symptomatic workers and in 8.4% of all workers (*4*). Symptomatic workers were those who had experienced bronchial asthma, chronic bronchitis, rhinitis, or conjunctivitis. In a representative subgroup of this same population, IgG antibodies were somewhat more prevalent, being detected in 24% of symptomatic workers and 17% of asymptomatic workers.

There are few published data on the incidence of antibodies to diisocyanates in the general public. In one study, none of 157 control subjects had IgG antibodies to HDI (*10*). In another study, no IgE antibodies to TDI, HDI, or MDI were detected in 40 unexposed referents from a blood donor pool (*11*). These studies provide evidence that antibodies to diisocyanates are seldom detected in the general public.

In the present investigation, antibodies to diisocyanates were detected in 9% of the people living near the plant. This percentage is lower than what has been reported in occupationally exposed populations. However, this finding is not unexpected because TDI exposures from residential air were likely lower than TDI exposures from an indoor occupational environment. Seven of the positive individuals reported no known exposure to diisocyanates. The presence of TDI antibodies in these individuals could have resulted from exposure to TDI in ambient air near the facility.

Several of the participants had positive antibody reactions to more than one diisocyanate. Similar cross-reactivity of diisocyanate antibodies has been previously observed in occupationally exposed workers (*4,10*). In one study, about 60% of positive sera cross-reacted to varying degrees with one or more diisocyanate-HSA conjugates (*4*).

Occupational exposure to TDI and other diisocyanates can lead to asthma and hypersensitivity pneumonitis (*2,10*). However, the presence of TDI antibodies does not necessarily lead to clinical disease. Occupational studies have demonstrated that some workers have both antibodies to TDI and exposure to TDI, yet they remain asymptomatic. Conversely, occupational asthma occurs in some TDI workers in the absence of TDI antibodies. Therefore, the presence or absence of TDI antibodies is a poor predictor of clinical disease. In this investigation, antibodies to diisocyanates were used as a biomarker of exposure. No attempt was made to correlate the presence of antibodies with respiratory disease.

Some of the participants in this investigation reported health problems that they attributed to emissions from the plant. Therefore, individuals who tested positive for diisocyanate antibodies, as well as individuals who were experiencing symptoms of respiratory disease, were encouraged to seek further clinical evaluation. The North Carolina Department of Health and Human Services made arrangements for qualified individuals to receive further evaluation at a university medical center.

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