Inhalation Toxicity of 1,6-Hexamethylene Diisocyanate Homopolymer (HDI-IC) Aerosol: Results of Single Inhalation Exposure Studies

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The early acute pulmonary response of female Wistar rats exposed nose-only to a mixture of 1,6-hexamethylene diisocyanate homopolymer (HDI-IC) aerosol was examined. This study was designed to investigate the time course of the relationship between acute pulmonary irritation and ensuing disturbances of the air/blood barrier in rats exposed to concentrations of 3.9, 15.9, 54.3, or 118.1 mg HDI-IC/m³. The duration of exposure was 6 h, followed by serial sacrifices 0 h, 3 h, 1 day, 3 days, and 7 days postexposure. Concentrations were selected based on the results of a 4-h acute inhalation study in rats (LC₅₀ = 462 mg/m³). Bronchoalveolar lavage (BAL) fluid was analyzed for markers indicative of injury of the bronchoalveolar region, including phospholipids as proxy of altered surfactant homeostasis. Glutathione (GSH) was determined in BAL fluid and lung tissue. BAL cells with increased intracellular phospholipids were observed on day 1 and especially day 3, with some residual increase on day 7. Increased intracellular phospholipids and activity of acid phosphatases appear to suggest that phagocytized phospholipids may transiently affect lysosomal function. Following exposure to 15.9 mg/m³, changes returned almost entirely to the level of the air-exposed control on day 7. Especially at higher exposure concentrations, lung weights and total number of cells in BAL were still statistically significantly elevated at this time point. Experimental evidence suggests that markers indicative of a dysfunction of the air/blood barrier, such as angiotensin-converting enzyme, total protein, and phospholipids engulfed by alveolar macrophages, were most sensitive to probe such type of changes. Although GSH in BAL was increased following exposure, there was an apparent depletion of tissue GSH immediately after cessation of exposure. In summary, this study suggests that respirable HDI-IC aerosol appears to cause a transient dysfunction of the air/blood barrier indicated by an increased extravasation of plasma constituents. Despite the remarkable extent of effects observed, most changes were reversible within a postexposure period as short as 7 days. First evidence of increased leakage of pulmonary epithelial barrier was observed at 3.9 mg/m³. With respect to changes of early markers of pulmonary epithelial barrier dysfunction, ∼ 3 mg HDI-IC/m³ was considered to be the threshold concentration for acute pulmonary irritation.

Key Words: isocyanate aerosol inhalation; pulmonary edema; aerosol of HDI-homopolymer; HDI-isocyanurate; surfactant; polyurethane coating.

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Hexane, 1,6-diisocyanate (HDI), the homopolymer used in this study, is a viscous liquid of no appreciable vapor pressure (<10⁻³ kPa at 20°C). Chemically, it is a mixture of isocyanurate-type HDI-homologues with n = 3, 5, 7, 9, . . . It contains approximately 50% of the trimeric HDI-homologue (n = 3, Fig. 1). This mixture is abbreviated HDI-IC.

Polyurethane coatings containing HDI-homopolymer–based activators have several outstanding technical features such as durability, color stability, resistance to abrasion and chemicals, and a high resistance to temperature or weather extremes. These characteristics are already achieved by curing at room temperature and make these coatings increasingly popular, especially for exterior paints. The typical polyurethane paint system has 2 components: polyol with pigments, solvents, and additives; and a second component containing the polymeric isocyanate (hardener) in an appropriate solvent. The major forms of HDI polymers currently in use are those of the biuret type (HDI-BT) and those of the isocyanurate type (HDI-IC).

The focus of this paper is on HDI-IC only. Potential adverse health effects are related to the use pattern, i.e., brushing and spray painting. Based on pulmonary irritation studies with HDI polyisocyanates (Ferguson et al., 1987; Valentini et al., 1983; Weyel et al., 1982), a threshold limit value-time weighted average (TLV-TWA) for HDI polyisocyanates of 0.5 mg/m³, with 1.0 mg/m³ as maximum concentration permitted, was proposed (Greenberg and Foureman, 1995; Janko et al., 1992).

The use pattern of this product makes it possible that workers are inhaling this material in aerosol form. However, due to the aerosolization of a complex system of chemically reactive agents, reported health effects may not necessarily be causally related to HDI polyisocyanates alone. Moreover, in this context, it is important to recognize that the content of the more volatile HDI monomer (vapor pressure ∼ 1.4 10⁻³ kPa at 25°C) has decreased over the past decades, i.e., results obtained from earlier studies may have been confounded by the purging or evaporation of HDI monomer from the nonvolatile HDI-homopolymer liquid. Appreciable amounts of HDI has been shown to be associated with the aerosol fraction of HDI polyisocyanates (Rando and Poovey, 1999). Alexandersson et al. (1987) found evidence of small airway disease in auto spray painters, but no sensitization was found. On the other hand,
Vandenplas et al. (1993) described positive asthmatic reactions in sensitized subjects after exposure to HDI homopolymers.

The objective of this study is to analyze the concentration dependence and time dependence on changes in the bronchoalveolar lavage fluid (BALF) of rats as a result of single inhalation exposure of 6 h to aerosolized HDI-IC during a postexposure period of 1 week. BALF was analyzed for end points indicating noncytotoxic and/or cytotoxic noxious effects on the bronchial epithelial barrier function, including an impairment of the vascular endothelium. An increased extravasation of plasma constituents was addressed by analysis of the angiotensin-converting enzyme (ACE) and total protein. A possible involvement of pulmonary surfactant has indirectly been addressed by the determination of phospholipids in BALF and BAL cells (BALC). Additional end points were considered to address the function of type II pneumocytes (alkaline phosphatase), cell injury and lysis (lactate dehydrogenase), and lysosomal instability (acid phosphatase). All end points were determined directly after cessation of a single 6-h exposure period and following postexposure periods of 3 h and 1, 3, and 7 days to determine the onset and progression of effects related to direct cellular injury and/or interactions with pulmonary surfactant and ensuing inflammatory or compensatory response.

MATERIALS AND METHODS

Test material. Hexane, 1,6-diisocyanato-, homopolymer (CAS-No: 028182-81-2) used in this study is a yellowish, translucent, viscous liquid, abbreviated as HDI-IC. The NCO content of the test specimens was 22% (w/v); the viscosity was 3500 mPa·s. It contains approximately 50% of the isocyanurate-type HDI homologue shown in Figure 1. The balance of the ingredients are higher isocyanurate-type HDI-homologues. The content of monomeric 1,6-hexamethylene diisocyanate (HDI) is 0.1%. The trade name of this mixture is Desmodur® N 3300 (Desmodur® is a trade name of Bayer AG, Leverkusen, Germany). HDI-IC was stored at room temperature and was handled under an inert gas atmosphere (dry nitrogen). HDI was also from Bayer AG, Leverkusen, Germany (Desmodur® H).

Animals, diet, and housing conditions. Specific-pathogen-free female Wistar rats of the strain Hsd Cpb:WU (SPF) were purchased from Harlan Winkelmann GmbH, Borchen, Germany. The choice of strain and gender was based on experience obtained from previous studies (Pauluhn et al., 1999; Pauluhn, 2000). At the commencement of the study the rats were approximately 2 months old. Animals were quarantined for at least 5 days prior to being placed on study. Animals were placed in polycarbonate cages (one per cage) containing bedding material (low-dust wood shavings) and were provided Altromin 1324 feed and water ad libitum except during exposure. The light cycle was automatically controlled in the animal holding room to provide 12 h of fluorescent light and 12 h of darkness each 24 h. Temperature and relative humidity were continually monitored, with daily means in the range of 22°C and 40–60%, respectively. All experiments and procedures described were performed in compliance with Good Laboratory Practice (GLP) requirements (OECD, 1983), taking into account the European Union (EU) animal welfare regulations (European Community Directive 86/609, 1986).

Experimental design. Three studies are addressed in this paper: determination of the acute median lethal toxic potency (LC50) of aerosolized HDI-IC in relation to evaporated HDI on rats according to the OECD No. 403 (Organization for Economic Cooperation and Development, 1981) and OPPTS 870.1300 testing protocols (single 4-h exposure; United States Environmental Protection Agency, 1998), and concentration dependence and time course of end points in bronchoalveolar lavage following single 6-h exposure of rats to aerosolized HDI-IC.

Acute median lethal toxic potency (LC50). At the end of the acclimatization period, rats were randomly assigned to the respective exposure group, each consisting of 5 male and 5 female rats per group. In the HDI-IC study, some exposures were made using 10 rats per group and gender. The rats were exposed nose-only inhalation to the HDI vapor or the HDI-IC aerosol in a single exposure of 4 h on day 0 followed by postexposure periods of 14 (HDI-IC) days or 4 weeks (HDI) due to the long duration of signs. Body weights were recorded before exposure, on days 3 and 7, and weekly thereafter. Clinical signs were observed twice daily. All animals were sacrificed at the time of death or at the end of the postexposure period.

Concentration response and time course of bronchoalveolar lavage. At the end of the acclimatization period, the rats were randomly assigned to the exposure groups, each consisting of 30 female rats. The rats were exposed nose-only to actual breathing zone concentrations of 0 (conditioned dry air), 3.9 ± 0.5, 15.9 ± 0.6, 54.3 ± 1.0, and 118.1 ± 8.4 mg HDI-IC/m3 air (5–7 samples per exposure; ± represents the standard deviation, SD) in a single exposure of 6 h. This duration of exposure allows a direct comparison with results from repeated exposure 3- and 13-week inhalation studies with this test agent. Dosimetrically adjusted, these concentrations were considered to be equivalent to 6, 24, 81, and 177 mg/m3 of a 4-h exposure period, i.e., the highest concentration slightly exceeds the LC50 of 163 mg/m3 (Fig. 2; for definition of LC50 see “Statistical Analyses”). Clinical observations were made daily. Body weights were recorded before exposure and prior to each serial exposure.

![FIG. 1. Representation of trimeric hexamethylene diisocyanate (HDI) in its generic form as HDI-isocyanurate (HDI-IC).](image-url)
sacrifice. After complete exsanguination, the excised lungs of the animals were weighed, then lavaged as described below. Lavage samples were taken directly after cessation of exposure (0 h) and following postexposure time periods of 3 h, 1, 3, and 7 days.

**Exposure Technique and Generation and Characterization of Test Atmospheres**

**HDI-IC aerosol.** Single inhalation exposures were for 4 h (LC\textsubscript{50} study) or 6 h (time-course study) in a multiport, dynamic, directed-flow, nose-only system similar to that described previously (Pauluhn et al., 1999; Pauluhn, 2000). In the LC\textsubscript{50} study, the reservoir containing HDI-IC was heated to approximately 80°C to decrease the viscosity of the test substance. In the time course study, the viscosity of HDI-IC was reduced by addition of 10% dry acetone (v/v). The inhalation chamber used minimized rebreathing of atmosphere. In the more recent time course study, HDI-IC was metered (digitally controlled Hamilton Microlab M pump) into a modified Schlick-nozzle Type 970, form-S 3 (Schlick GmbH, Coburg, Germany) using conditioned (dry, oil-free), compressed air (15 l/min, dispersion pressure approximately 600 kPa). Following dispersion, the targeted concentrations were achieved by subsequent extraction/dilution cascades and maintaining the total inhalation chamber air flow rate of 30 l/min. This provided an air flow rate per exposure port of approximately 0.75 l/min. The nozzle was maintained at \(\approx 35°C\) using a water jacket connected to a digitally controlled thermostat. The stability of the aerosol generation and exposure system was measured continuously by using a RAM-1 or RAS-2 real-time aerosol photometer (MIE, Bedford, Massachusetts). Flow meters were calibrated using soap bubble meters (Gilibar, Ströhlein Instruments, Kaarst, Germany). Humidity and temperature were monitored electronically at the exposure location and the respective values were less than \(\approx 5\%\) and approximately 24°C. The humidity of exposure atmospheres was kept as low as possible to prevent hydrolysis of the isocyanate in the inhalation chamber. Untoward effects as a result of the relatively low humidity values of the test atmosphere were not observed, which is consistent with other low-humidity studies (Pauluhn and Mohr, 1999).

All exposure atmospheres were characterized using the modified nitroreagent derivatization technique of Dunlap et al. (1976). Details have been described previously (Pauluhn et al., 1995; 1999). Additionally, filter analyses (Sartorius glass fiber filters) were taken. Chamber air was sampled from the vicinity of the breathing zone of the rats at least one sample per hour for filter analyses or one sample per exposure for nitro-reagent analyses. For particle-size analyses, a low-pressure critical orifice AERAS stainless steel cascade impactor was used (HAUKE, 4810 Gmunden, Austria). The mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were calculated as described previously (Pauluhn, 1994). In the LC\textsubscript{50} study the MMAD was in the range of 2.4–2.9 \(\mu\)m (GSD \(\approx 1.7\)); in the time-course study the MMAD was 1.7–2.0 \(\mu\)m (GSD \(\approx 1.5\)). The total gravimetric mass concentration measured by cascade impactor analyses, filter, and/or nitro-reagent analyses provided virtually identical results. Therefore, no distinction of results obtained by the various techniques was made. Because of the greater number of samples available, concentrations refer to gravimetric analysis. Further details of the exposure methodology and its validation have been published elsewhere (Pauluhn, 1994; Pauluhn et al., 1999).

**HDI vapor.** Dry conditioned air was bubbled through the HDI liquid (ca. 90 ml) contained in a glass bubbler (kept in a water bath at 45°C). This atmosphere was subsequently diluted with dry, conditioned air to attain the targeted concentration. The stability of the test atmosphere was monitored continuously using a total hydrocarbon analyzer equipped with a flame ionization detector (Compur, Munich, Germany). Five groups of rats were nose-only exposed, as described for HDI-IC, to the following actual concentrations of results obtained by the various techniques was made. Because of the greater number of samples available, concentrations refer to gravimetric analysis. Further details of the exposure methodology and its validation have been published elsewhere (Pauluhn, 1994; Pauluhn et al., 1999).

**Statistical analyses.** Data were analyzed by a one-way analysis of variance followed by a Tukey-Kramer post hoc test. For all tests the criterion for statistical significance was set at \(p < 0.05\). Asterisks in figures denote statistically significant differences: * indicates \(p < 0.05\) and ** indicates \(p < 0.01\). Each group consisted of 6 rats per serial sacrifice. For each sacrifice, relative data were calculated by division of group means and standard deviations of treatment groups by the mean of rats of the control group sacrificed at the same time point. This was made to allow a better appreciation of the time- and
concentration-related changes. Data in figures were expressed relative to the data of the respective control group. The median lethal concentration (LC$_{50}$) and its confidence interval were calculated according to Rosiello et al. (1977). The regression parameters of the linear probability mortality–log concentration relationship were used to calculate the LC$_{01}$. The 1% response (LC$_{01}$) was chosen because it is considered to give values reasonably close to experimentally observed LC$_{01}$ values (AEGL, 1999).

RESULTS

Acute Median Lethal Toxic Potency (LC$_{50}$)

Appreciable gender-specific susceptibilities were not observed. Therefore, for the calculation of LC$_{50}$s, the mortalities of male and female rats were combined. A graphic representation of the results of this study is provided in Figure 2. The 4-h LC$_{50}$ of HDI vapor was found to be 124 mg/m$^3$, with 110 and 140 mg/m$^3$ as the 95% confidence limits (slope: 4.1; Fig. 2). The extrapolated LC$_{01}$ was 70 mg/m$^3$. Signs were characterized by rales, labored respiration, and severe respiratory distress, including serous discharge from nose, blepharospasm, lacrimation, cyanosis, pallor, hypothermia, and loss of body weight. Rats exposed to concentrations in the range of the LC$_{50}$ experienced clinical signs throughout the 4-week postexposure period. Deaths occurred in all groups within the first and sixth postobservation days. On the other hand, the 4-h LC$_{50}$ of respirable HDI-IC aerosol was found to be 462 mg/m$^3$, with 404 and 529 mg/m$^3$ as the 95% confidence limits (slope: 2.2; Fig. 2). The extrapolated LC$_{01}$ was 163 mg/m$^3$. During exposure many rats showed labored respiration and respiratory distress, including salivation, blepharospasm, and loss of body weight. In most instances, signs resolved completely within the first 4 postexposure days. Deaths occurred in all groups on the day of exposure and first postexposure day, and in some cases up to the fourth postexposure day. Grayish, somewhat distended lungs, edema, hydrothorax, a few pulmonary hemorrhages, and nasal discharge were seen in the higher dosed rats.

Time Course of Pulmonary Response of Rats Exposed for 6 Hours to HDI-IC

Rats exposed for 6 h to 54.3 mg/m$^3$ and above elaborated concentration-dependent signs, most of them related to respiratory tract irritation such as irregular and labored breathing pattern, bradypnea, and nostrils with red encrustations. Rats exposed to 54.3 mg/m$^3$ displayed signs up to the second, and in the 118.1 mg/m$^3$ exposure group, up to the fifth postexposure day. Necropsy findings of rats exposed to 118.1 mg/m$^3$ provided evidence of macroscopic alterations such as distended lungs, edema, hydrothorax, consolidation, and pulmonary hemorrhages. On postexposure day 7, lung-associated lymph nodes appeared to be enlarged. One out of 30 rats exposed to this concentration succumbed the night after exposure. Necropsy findings of rats exposed to 54.3 mg/m$^3$ were less pronounced and were related mainly to focal pulmonary hemorrhages and less-collapsed lungs. A marked, but transient decrease of body weights was observed in rats exposed to 54.3 mg/m$^3$ and above (data not shown). Wet lung weights were statistically significantly increased in rats exposed to 15.9 mg/m$^3$ and above, with maximum effects 3 h and 1 day postexposure (Fig. 3). Rats exposed to 3.9 mg/m$^3$ did not experience any statistically significant increase of lung weights.

Data from determinations in the fluid and cells from bronchoalveolar lavage of female rats exposed for 6 h to respirable HDI-IC aerosol. At each serial sacrifice 0 h, 3 h, 1, 3, and 7 days after exposure, 6 rats were examined. Data represent group means and SD and are expressed relative to the air control group (100%). Asterisks denote statistical significance compared to control group (* p < 0.05, ** p < 0.01).

FIG. 3. Wet lung weights and total cell count in bronchoalveolar lavage of female rats exposed for 6 h to respirable HDI-IC aerosol. At each serial sacrifice 0 h, 3 h, 1, 3, and 7 days after exposure, 6 rats were examined. Data represent group means and SD and are expressed relative to the air control group (100%). Asterisks denote statistical significance compared to control group (* p < 0.05, ** p < 0.01).
118.1 mg/m³ group directly after cessation of exposure (10% lower than the average; data not shown). Serial sacrifices of rats exposed for 6 h to 15.9 mg HDI-IC/m³ and above revealed that most endpoints examined in bronchoalveolar lavage showed a remarkable time-related increase from 3 h after cessation of exposure to postexposure day 1 and, in some cases, also postexposure day 3. Changes considered to be related to pulmonary irritation returned almost completely to the level of the control group on day 7. Those changes apparently related to tissue restoration or compensatory response, e.g., tissue GSH or the increased lysosomal activity of BALT were still statistically significantly increased on day 7.

The relative comparison of effects suggests that the most sensitive marker indicative of a dysfunction of the air-blood barrier was characterized by a marked concentration-dependent increase of ACE activity and total protein in BALF, which peaked in parallel on postexposure day 1 (Fig. 4). The activity of LDH in BALF was statistically significantly increased in groups exposed to 54.3 mg HDI-IC/m³ and above (Fig. 4). At these high exposure levels, the time course of changes of protein and ACE coincided, whereas that of LDH was different following exposure to 118.1 mg/m³. The coexisting statistically significant increase of intracellular phospholipids in relation to that of intracellular acid phosphatase suggests that elevated amounts of phospholipids, possibly originating from surfactant, were phagocytosed by alveolar macrophages (Fig. 5). The somewhat upregulated lysosomal activity appears to be in line with elevated catabolism of phagocytosed phospholipids. The uptake of intracellular phospholipids appears to occur in a concentration-related, somewhat protracted fashion, i.e., intracellular phospholipids were less elevated on the day of exposure and markedly elevated on the first and third postexposure days. Despite this coincidence, the acid phosphatase in BALT did not appear to increase proportionally with the engulfment of phospholipids (day 3), and the subsequent restoration of lysosomal function appeared to be contingent upon the extent of cellular loading. The concentration phospholipids in BALF showed a similar time course of changes; however, the relative magnitude of changes was less pronounced (peak response on day 3 approximately 5 times the control value) and less concentration dependent (data not shown). Alkaline phosphatase activity in BALF displayed maximum effect on day 1 (approximately 3 times the control) without showing any concentration-dependent increase between the 15.9 and 118.1 mg/m³ exposure groups (data not shown).

The total cell count was elevated in rats exposed to 54.3 mg/m³ and above during postexposure days 1 and 3 (Fig. 3). In rats exposed to 15.9 mg/m³ and above, cell numbers from HDI-IC exposure groups were still statistically significantly elevated on day 7. Data shown in Figure 6 illustrate that the time course of the increased concentrations of GSH in BALF resembled that of total protein and ACE. On the other hand, in lung tissue GSH was decreased immediately after cessation of exposure followed by an apparent rapid replenishment and a more plateau-like response thereafter. Though statistically significantly increased, the extent and time-course of γ-GT activity in BALF cannot be causally associated with any change of GSH (Fig. 6).

**DISCUSSION**

The 4-h LC₅₀ of respirable HDI-IC aerosol was found to be markedly higher than that of HDI vapor. Thus, it appears that the LC₅₀ of respirable HDI-IC aerosol is closer to the LC₅₀ of other aerosolized isocyanates, such as polymeric MDI (4,4'-
diphenylmethane diisocyanate), than to HDI vapor. The LC₅₀ of respirable polymeric MDI has been reported to be 490 mg/m³, with 376 and 638 mg/m³ as the 95% confidence limits (Reuzel et al., 1994). Thus, the comparison of data suggest that lethality was most likely related to both the relative irritant potency as well as the lung region predominantly involved. In this context, HDI vapor appears to be a markedly more potent respiratory tract airway irritant and acute high-level exposures were associated with longer-lasting signs of respiratory distress and a more delayed onset of mortality. HDI-IC aerosol, in turn, appears to penetrate the lower respiratory tract, with the alveolar region as major target site. Overall, this interpretation of findings is not at variance with previously published data obtained in mice (Weyel et al., 1982).

It could be speculated that the approximately 4 times higher acute toxic lethal potency of HDI vapor is related to the greater number of reactive –NCO groups per molecule (HDI: molecular mass, 168; content of –NCO: 50%; HDI-IC: molecular mass of isocyanurate₃ = 504; content of –NCO: 25%). However, experimental evidence obtained with other volatile isocyanates supports the view that the pathomechanism causing predominant damage within the respiratory tract is more de-

FIG. 5. Concentration of phospholipids and acid phosphatase in bronchoalveolar lavage cells (BALC) and acid phosphatase in bronchoalveolar lavage fluid (BALF) of female rats exposed for 6 h to respirable HDI-IC aerosol. At each serial sacrifice 0 h, 3 h, 1, 3, and 7 days after exposure, 6 rats were examined. Data represent group means and SD and are expressed relative to the air control group (= 100%). Asterisks denote statistical significance compared to control group (*p < 0.05, **p < 0.01).

FIG. 6. Concentration of glutathione in lung tissue and glutathione and γ-glutamyltranspeptidase (γ-GT) in bronchoalveolar lavage fluid (BALF) of female rats exposed for 6 h to respirable HDI-IC aerosol. At each serial sacrifice 0 h, 3 h, 1, 3, and 7 days after exposure, 6 rats were examined. Data represent group means and std and are expressed relative to the air control group (= 100%). Asterisks denote statistical significance compared to control group (*p < 0.05, **p < 0.01).
pendent on the location of highest deposition. Especially for volatile isocyanates, it is believed that water solubility and chemical reactivity appear to be more decisive for the outcome of study than the mere content of reactive isocyanate groups per molecule. As reported previously, the ratios of 4-h LC50 in rats of n-iso-propyl isocyanate was 1:~3, whereas that of n-iso-tertiary-butyl isocyanate was 1:~4:~30, demonstrating that changes in reactivity have marked impact on acute inhalation toxicity (Pauluhn, 1988). Moreover, volatile isocyanates with a high acute lethal toxic potency showed a biphasic type of mortality, whereas intermediate to lower potency ones demonstrated a more monophasic, i.e., early type of mortality due to pulmonary irritation.

The particle size of the aerosolized HDI-IC was adequate to penetrate the lower respiratory tract of rats. Therefore, the acute 6-h study with subsequently performed serial sacrifices for bronchoalveolar lavage focused on the investigation of the time course of acute lung injury occurring in the bronchoalveolar region of the lung. Based on the results of this single 6-h exposure study, the most salient changes observed were related to a transient disturbance of the air/blood barrier function. After exposure up to 15.9 mg/m³, this dysfunction was characterized by an ephemeral permeability of total protein and ACE. Appreciably increased activities of the marker of cytotoxicity LDH could only be detected following exposure to high concentrations of aerosol, i.e., 54.3 mg/m³ and above. The apparent sequence of effects occurring on the first and third postexposure days appears to be consistent with affected surfactant homeostasis. The concentration dependence of protein and LDH in BALF and phospholipids in BALC obtained on day 1 is illustrated in Figure 7. Among other possibilities, alterations in surface tension on the alveolar surface may have contributed to increased transudation of fluid and solutes from the capillaries (Albert et al., 1979; Nieman, 1985). Qualitatively similar findings were described to occur with polymeric MDI aerosol after acute inhalation exposure, and experimental evidence suggests that partially decreased sulphydryl levels by pharmacological intervention made the lung more susceptible to the edemagenic potency of respirable MDI aerosol (Pauluhn, 2000). Likewise, the measurements of GSH in lung tissue in this study also appear to suggest that pulmonary levels of GSH may be a modulating factor of susceptibility.

With respect to total protein in BALF, a linear log-concentration–log-effect relationship existed despite the wide range of HDI-IC concentrations examined (Fig. 8). A somewhat minor and transient increase of this end point was already observed after exposure to 3.9 mg HDI-IC/m³. As illustrated in Figure 8, such linear relationship could not be established for LDH in BALF, i.e., the increase of this end point appears to be contingent upon several mechanisms. The one operative at low exposure concentrations may be due to increased extravasation of LDH from pulmonary capillaries, whereas at higher concentrations LDH may also be derived from lysed cells. Nonetheless, the increase of total protein in BALF unequivocally precedes that of LDH. Taking this into account, the concentration-effect relationship of protein in BALF can aptly be used to calculate the acute irritant threshold concentration. Based on the variability observed in controls (mean + 2 SD equals 100–125%; Fig. 8), the single 6-h exposure no-observed-adverse-effect level (NOAEL) is within the range of 2.6–3.5 mg/m³, or approximately 3 mg HDI-IC/m³. Thus, these data provide supportive evidence for the proposed threshold limit value–time-weighted average (TLV-TWA) for HDI polyisocyanates of 0.5 mg/m³, with 1.0 mg/m³ as maximum concentration permitted (PEL).

In summary, it can be concluded that the specific and possibly also selective electrophilic reactivity of this aerosolized homopolymer of the isocyanurate type contributes to the blood/air barrier-perturbing properties of this class of chemicals. Especially at noncytotoxic concentrations, these properties are considered to be causative for the disturbed fluid dynamics of the lung. Moreover, it emerges that pulmonary dysfunction...
occurs only at exposure concentrations that appear to exceed the buffering capacity of the pulmonary lining fluids to scavenge chemically reactive electrophilic agents. This means that by titrating out endogenous agents contained in the lining fluids in the alveolar region, such as nonprotein sulfhydryl groups, the lung depletes its own level of protection. If the delivery rate of isocyanate exceeds the dynamic of replenishment of such endogenous scavengers, toxic insult is likely to occur. Thus, the rate of aerosol delivery in relation to the turnover of these endogenous scavengers, toxic insult is likely to occur. Thus, the rate of aerosol delivery in relation to the turnover of these factors is a critical, yet ill-defined variable. This overall assumption appears to be supported further by the results of 13-week subchronic inhalation studies in rats, conducted in accordance with current testing guidelines, with either polymeric MDI-aerosol (Reuzel et al., 1994) or HDI-IC aerosol (Pauluhn, unpublished) which unanimously demonstrate that the NOAELs from single 6-h and repeated exposure 13-week inhalation studies were virtually identical.

ACKNOWLEDGMENTS


REFERENCES


AEGGL (1999). Standing Operating Procedures of the National Advisory Com-


FIG. 8. Concentration-effect relationship of total protein and LDH in BALF of rats exposed for 6 h to respirable HDI-IC aerosol and sacrificed on postexposure day 1. All data are normalized to the mean relative to control (＝ 100%).


