



# **TOXICOLOGICAL REVIEW**

**of**

## **METHYLENE DIPHENYL DIISOCYANATE (MDI)**

(CAS No. 101-68-8 and 9016-87-9)

**In Support of Summary Information on the  
Integrated Risk Information System (IRIS)**

February 1998

U.S. ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, DC

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## **DISCLAIMER**

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## FOREWORD

The purpose of this review is to provide scientific support and rationale for the hazard identification and dose-response assessments for both cancer and noncancer effects (the inhalation reference concentration [RfC]), from chronic exposure to monomeric methylene diphenyl diisocyanate (MDI) and polymeric MDI (PMDI). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of either MDI or PMDI.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response (U.S. EPA, 1995a). Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the individual assessments and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 202-566-1676.

## CONTRIBUTORS AND REVIEWERS

### **Chemical Manager/Author**

Mark M. Greenberg  
National Center for Environmental Assessment  
Office of Research and Development  
U.S. Environmental Protection Agency  
Research Triangle Park, NC 27707

### **Reviewers**

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA (U.S. EPA, 1994a). Subsequent to external review and incorporation of comments, this assessment has undergone an Agencywide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development, Office of Air and Radiation, Office of Prevention, Pesticides, and Toxic Substances, Office of Solid Waste and Emergency Response, Office of Water, Office of Policy Planning and Evaluation and the Regional Offices.

### ***Internal EPA Reviewers***

James W. Holder, Ph.D.  
Toxicologist  
National Center for Environmental Assessment  
U.S. Environmental Protection Agency  
Washington, DC

Cheryl Scott  
Epidemiologist  
National Center for Environmental Assessment  
U.S. Environmental Protection Agency  
Washington, DC

Eric D. Clegg, Ph.D.  
Toxicologist  
National Center for Environmental Assessment  
U.S. Environmental Protection Agency  
Washington, DC

## External Peer Reviewers

Charles H. Hobbs, DVM  
Director of Toxicology  
Lovelace Biomedical and Environmental Research Institute  
Albuquerque, NM

Thomas Ledoux, Ph.D.  
Division of Science and Research  
New Jersey Department of Environmental Protection  
Trenton, NJ

William E. Brown, Ph.D.  
Professor and Head  
Department of Biological Sciences  
Carnegie Mellon University  
Pittsburgh, PA

Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix B.

## 1. Introduction

This document presents the derivation of the noncancer concentration-response assessment for inhalation exposure, the inhalation reference concentration (RfC), and the cancer hazard assessment. There are no data with which to evaluate toxicological effects via the oral route; thus, a derivation of the oral reference dose has not been presented.

The RfC is meant to provide information on long-term toxic effects other than carcinogenicity. The RfC is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. The RfC is expressed in mg/m<sup>3</sup>. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects).

The carcinogenicity hazard assessment reflects a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen as well as the conditions under which any carcinogenic effects may be manifested. The information available is insufficient to derive a quantitative risk estimate for MDI.

Development of these hazard identifications and the concentration-response assessment for MDI has followed the general guidelines for risk assessments as set forth by the National Research Council (1983). Other EPA guidelines that were used in the development of this assessment include the following: the Risk Assessment Guidelines (U.S. EPA, 1987), the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), the (new) Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), (proposed) Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994b), (proposed) Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1995b), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994c), Recommendations for and Documentation of Biological Values for Use in Risk Assessments (U.S. EPA, 1988), and the Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995c).

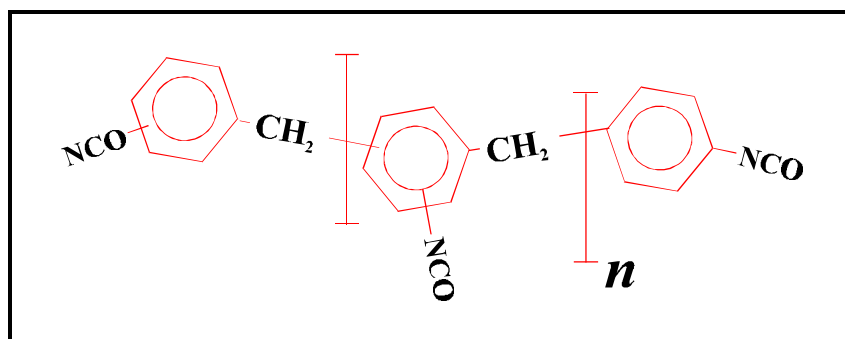
The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE,

CANCERLINE, MEDLINE, and MEDLINE backfiles. Recently published information was tracked via Current Contents.

Any pertinent information submitted by the public to the Integrated Risk Information System (IRIS) submission desk also was considered in the development of this document

## 2. Chemical and Physical Information Relative to Assessments

Methylene diphenyl diisocyanate (MDI) in commercial form typically exists as a mixture of 4,4'-MDI (monomer) and certain oligomers of MDI (Encyclopedia of Chemical Technology, 1983). It has a CASRN of 9016-87-9. The content of monomeric MDI generally is between 45% and 65% on a w/w basis (Mobay, 1983). The mixture is designated as polymeric MDI (PMDI). It contains about 50% monomeric MDI and 50% trimeric species and higher molecular weight oligomers (Ulrich, 1983); this composition, which is very similar to that used in the workplace, renders the material semisolid and suitable for aerosol generation. Monomeric MDI (CASRN 101-68-8) is formed as a byproduct of PMDI synthesis and is rarely separated from the mixture except in special-use applications. The generic structure of PMDI is shown in Figure 1.



**Figure 1. Representation of polymeric MDI in generic form**

The exact composition of monomeric MDI in the mixture likely varies with the manufacturer. Any change in the monomeric composition is expected to be compensated by an increase or decrease in oligomer content. Only monomeric MDI (herein referred to as MDI) is listed as a hazardous pollutant under section 112g of the Clean Air Act Amendments of 1990. Both MDI and PMDI are reactive with water, with resultant production of amines, oligoureas, and carbon dioxide. For this reason, PMDI is always stored under an inert gas such as nitrogen until use. In practice, PMDI is stored at room temperature.

Monomeric MDI is a solid at room temperature; the PMDI mixture is a viscous liquid. The vapor pressure of both the monomer and PMDI is extremely low, about  $2 \times 10^{-6}$  kPa at 20°C (CMA, 1994). Both MDI and PMDI exist in workplace air primarily as aerosols generated during polyurethane foam formation (Dharmarajan and Weill, 1978; Dharmarajan, 1979). In field studies involving PMDI and conventional airless spraying equipment, these investigators found that, in contrast to impingers without filters, more than 78% of the material collected on Teflon filters with stainless steel backup pads upstream of the impingers was either urethane or MDI urea, since MDI was not detected in the leached material. The size distribution of the particles, determined with a seven-stage cascade impactor, had a mass median aerodynamic diameter (MMAD) of 11.0  $\mu\text{m}$  with a sigma g of 3.6. When pure MDI was heated under laboratory conditions, a 0.5- $\mu\text{m}$  pore-size Teflon filter blocked more than 87% of the MDI entering the impinger. It was recommended that concentrations of MDI should be expressed as  $\text{mg}/\text{m}^3$  and not ppm units.

The strengths and limitations of impingers, bubblers, and filters with respect to collection and detection of both MDI aerosols and vapors have been discussed by Streicher et al. (1994); none of these approaches has been found to be acceptable in all exposure environments. Streicher and colleagues suggested that an impinger or bubbler containing a solution of derivatizing reagent upstream of a reagent-impregnated filter would be suitable. The sampling and detection considerations, as discussed by Streicher and colleagues, are of importance not only with respect to occupational exposure environments, but to chamber exposure of laboratory animals as well. Spanne et al. (1996) have found the use of dibutylamine as a derivatizing agent reduces interferences from other substances in a complex isocyanate exposure mixture, results in a UV spectra determined by the nature of the isocyanates rather than that of the derivatizing reagent, and reduces the instability associated with dilute isocyanate solutions because isocyanate reactions with dibutylamine are fast.

The extent to which MDI, either in monomeric or polymeric form, disperses in air beyond the point of release and exposes general populations is not known. Like other members of the isocyanate family of compounds, MDI is highly reactive with a variety of biological functional groups, such as hydroxyl, amine, and sulfhydryl groups (Brown, 1987).

### **3. Toxicokinetics Relevant to Assessments**

Little is known of the distribution and fate of PMDI/MDI in the human body. Animal inhalation studies have shown that PMDI exposure results in significant deposition both in the nasal region and in the alveolar region of the lungs (Reuzel et al., 1990; 1994a,b) In an unpublished pharmacokinetic study (Saclay, 1977), nose-only exposure of male Sprague-Dawley rats (12) to an aerosol (particle sizes less than 5  $\mu\text{m}$ ) of radiolabeled monomeric MDI for 15 min resulted in distribution of radioactivity, predominantly to the lungs and a variety of extrapulmonary sites, principally muscle, liver, kidneys, and the digestive tract. Labeling of the digestive tract was considered to be a result of transference of labeled material from the lungs.

After 4 days, 70% of the absorbed dose was eliminated, with fecal elimination predominant (57%). The experiment was terminated at the end of the fourth day.

In both humans (Schutze et al., 1995; Sepai et al., 1995a) and laboratory animals (Sepai et al., 1995b) exposed to PMDI/MDI, 4,4'-methylenedianiline (MDA), a metabolite/reaction product of MDI that is sometimes used in the production of polyurethane foam (Cocker et al., 1988), has been found in urine prior to acid hydrolysis and as an adduct conjugated with hemoglobin. MDA also has been detected in acid-hydrolyzed urine of workers exposed to PMDI/MDI (Skarping and Dalene, 1995a) and thermal degradation products of MDI elastomers (Skarping et al., 1995b). When pregnant Wistar rats were exposed on day 20 post-conception to 20 mg monomeric MDI/m<sup>3</sup> for 6 h, MDA was detected in maternal blood, placenta, fetal blood, and amniotic fluid after acid hydrolysis (Bartsch et al., 1996). However, in studies of workers occupationally exposed to either PMDI/MDI or MDA, free MDA was detected in urine prior to acid hydrolysis (Schutze et al., 1995). MDA is a known animal carcinogen and has resulted in alveolar/bronchiolar adenomas and malignant lymphomas in female mice administered the compound in drinking water (Lamb et al., 1986).

The extent to which the results of the Lamb et al. (1986) study relate to inhalation exposure of humans to MDI/PMDI and the formation of MDA remains to be determined. It is not clear if MDA originates as a reaction product after PMDI/MDI uptake, is present in the exposure atmosphere along with PMDI/MDI, or is produced upon thermal degradation of materials made with MDI.

Further evidence in support of translocation of isocyanate material to remote sites is the finding by Maitre et al. (1993) that toluene diamine, a metabolite of toluene diisocyanate (TDI), is present in acid-hydrolyzed urine of TDI-exposed individuals. It has also been postulated that thiolytic acid esters, formed when isocyanates react with thiols in the body, may reverse with regeneration of the isocyanate functional group (Brown, 1987). This potential mechanism may allow for systemic delivery of the isocyanate in reactive form. Because measurable radioactivity was found in extrathoracic tissues and body fluids of guinea pigs exposed to ring-labeled TDI for one hour to levels as low as 4 ppb (Kennedy et al., 1989), some form of MDI may distribute to extrarespiratory sites as well.

## **4. Hazard Identification**

### **4.1. Studies in Humans - Epidemiology, Case Reports, Clinical Controls**

Exposure to isocyanates is a leading cause of occupational asthma worldwide (Mapp et al., 1988). The database associating TDI exposure with occupational asthma is extensive, although quantitative concentration-response relationships are lacking. The database for PMDI/MDI is less extensive but nonetheless compelling. There are a number of case reports and

occupational studies that describe sensitivity to PMDI and ascribe hypersensitivity pneumonitis and occupational asthma, but exposure levels are unknown (Zammit-Tabona et al., 1983; Konzen et al., 1966; Tanser et al., 1973; Zeiss et al., 1980; Malo and Zeiss, 1982; O'Brien et al., 1979; Bascom et al., 1985; Mapp et al., 1985; Lob and Boillat, 1981; Baur et al., 1984; Vandenplas et al., 1993). High exposure concentrations, such as might occur during a spill (Brooks, 1982), likely are a risk factor in human sensitization. Support for this view is found in a cross-sectional study (Bernstein et al., 1993). These investigators evaluated MDI-induced sensitization in 243 PMDI/MDI foam workers in a 3-year-old facility in which air levels were monitored continuously by area monitors for 24 h per day, during which time the air levels never exceeded 5 ppm. The average duration of employment was 18.2 mo. Only three cases of occupational asthma were identified, one of which was attributable to a spill.

Genetic predisposition as a function of inter-individual genetic differences in the expression of HLA Class II molecules on antigen-presenting cells has been suggested to play a role in sensitization (Bignon et al., 1994; Balboni et al., 1996). These investigators, using different populations of confirmed TDI asthmatics, found differences from controls in the DQB1 alleles that were interpreted to indicate that some individuals are predisposed to TDI-induced asthma. On the other hand, other groups of investigators (Bernstein et al., 1997; Rihs et al., 1997), with different study populations, found no such differences. All investigators used acceptable molecular biological techniques. Thus, further confirmation is required before any consensus can be reached regarding genetic predisposition as a risk factor in isocyanate-induced asthma.

Immunoglobulin E and G antibodies specific to MDI have been demonstrated in occupationally exposed workers with diagnosis of occupational asthma (Cartier et al., 1989; Liss et al., 1988; Pezzini et al., 1984; Tse et al., 1985; Zeiss et al., 1980). These antibodies are considered biomarkers for exposure only, because the presence of specific antibodies occurs in only a small percentage of individuals with asthmatic symptoms (Baur et al., 1994). MDI-specific immunoglobulin G (IgG) is a more typical finding than immunoglobulin E (IgE), the latter commonly associated with TDI exposure. Occasionally, individuals with sensitivity to one isocyanate exhibit cross-reactivity and subsequent asthmatic symptoms when exposed to another (Malo et al., 1983). MDI exposure of humans has been demonstrated to activate lymphocytes (Bentley et al., 1992; Raulf et al., 1992).

The available human data concerning occupational exposure to PMDI/MDI, coupled with lack of knowledge about mechanism of action and the possible role of genetic predisposition, are insufficient to identify exposure levels and scenarios responsible for isocyanate-induced sensitization. For this reason, PMDI/MDI-induced asthma could not be selected as an endpoint for RfC derivation.

Apart from asthma, a few occupational studies have examined exposure-response relationships with respect to pulmonary function decline and exposure to PMDI. However, each of the studies described below has a variety of limitations that preclude identification of exposure-response relationships for nonoccupationally exposed populations.

Musk et al. (1982) followed 107 workers prospectively for 5 years from 2 plants in a polyurethane manufacturing facility in which both TDI and MDI (presumed to be PMDI) were present in the air. [Note: In a later publication, Musk et al. (1985) reported that only 94, not 107, workers were available for reexamination]. Health data were collected during 2 surveys 5 years apart. The cohort was segregated into workers exposed to MDI only (n=25), TDI only (n=17), MDI and TDI (n=6), and no exposure (n=42). Criteria for selection of study participants were not stated. Health status for bronchitis, dyspnea, and smoking status were evaluated using standard questionnaires. It is stated in the discussion that this group did not include any subjects with symptoms suggesting hypersensitivity to isocyanates, but the criteria for this assertion were not provided. Other demographic data were not provided. Spirometric tests were conducted according to applicable criteria of the American Thoracic Society (ATS). Forced expiratory volume in 1-sec (FEV1) values were obtained from the average of 3 best values of 5 spirometric maneuvers. Effects on FEV1 and forced vital capacity (FVC) were stated to be the same, so only FEV1 data were presented. Spirometry was performed on the study subjects as they arrived to work on a Monday morning after a weekend of no exposure and again in the afternoon of that same day. Measurements were also taken on the Monday morning following a 2-week vacation and again in the afternoon of that same day.

For the comparison of changes in FEV1 over the 5-year study period, 94 of the original 107 subjects were tested. There was no evidence of bias resulting from the selective retirement of subjects from the cohort, as evidenced by the similarity of the mean FEV1 value of the original cohort with that of those who remained in the study. Much of the attrition could be attributed to contraction of the operation.

Exposure data were collected over the last 4 years of the 5-year study period, during which a total of 2,043 measurements were taken for TDI. Samplers were hand-held for 20 to 60 min in the breathing zone of workers, and analysis was done by the Marcali method (an impinger flow rate of 0.1 cu. foot/3 min is assumed). The exposure category for each subject was determined from both these measurements and occupational history. MDI, present in the atmosphere only in the last 2 years of the study, was sampled in a total of 470 measurements. Ninety percent of all measurements of TDI taken over the four years prior to the follow-up study contained less than 0.005 ppm in plant 1 and 0.004 ppm in plant 2. MDI levels ranged from <0.0003 - <0.004 ppm. [Note: true values are likely greatly underestimated, as MDI exists as an

aerosol and not as a vapor and the low impinger flow rate will not collect 100% of the MDI]. The geometric means of TDI and MDI concentrations, respectively, in the 2 plants were reported as 0.001 ppm and 0.0003 ppm in one and 0.0015 ppm and 0.0006 ppm in the other.

There were no statistically significant differences in the total 5-year decrement in FEV1 among those workers with no exposure (0.11 L), those exposed to TDI alone (0.13 L), those exposed to MDI alone (0.03 L), or those exposed to TDI and MDI (0.06 L). Regression analysis showed that the only decrement detected was due to smoking. The data on FEV1 (with similar ranges of n) also showed no effect in either daily changes (morning to afternoon FEV1) or in pre- and postvacation FEV1 changes. The mean annual rate of decrease in FEV1 was 0.02 L, a decrease expected from aging alone. Reanalysis of these data (Musk et al., 1985) using ATS standards for spirometry promulgated in 1979 confirmed these results and conclusions. It was reported that there was no evidence of bias resulting from attrition due to closure of one facility; the mean FEV1 value of those 152 lost to the study was similar to those 107 who remained. Musk et al. (1985) also stated that the annual FEV1 declines were similar to cross-sectional age regression coefficients of predicted normal populations. However, age-regression coefficients from cross-sectional populations have been described as poor benchmarks for the evaluation of longitudinal spirometric data because cross-sectional populations, unlike longitudinally studied populations, are sensitive to “cohort effects” (Glindmeyer et al., 1982). These effects, such as illness, smoking, and occupational exposures, may have affected lung function in the cross-sectional cohort at an earlier age, but not the longitudinal population in question.

Because of the relatively low number of individuals exposed to isocyanates (48) and to MDI alone (25) in this study, the uncertainty over the actual level of MDI exposure, and the short study duration (5 years), this study offers only limited assurance that MDI is not without effect on pulmonary function.

Sulotto et al. (1990) examined the effect of low-level (reported as less than 1 ppb) MDI (presumed to be PMDI) exposure on lung function parameters (e.g., FEV1) in 27 polyurethane foam workers over a workshift and workweek. All workers were asymptomatic for asthma and were age-matched to nonexposed controls from the same factory. There was no selection of participants from within the exposed group. The duration of employment was similar between exposed (14 years) and controls (16 years). During a portion of their employment individuals had also been exposed to TDI. The exposed group had more smokers (12/27) than controls (5/27). There was no significant difference in spirometric data between groups over the workshift or workweek. Spirometric testing was consistent with ATS criteria.

Pham et al. (1988) prospectively studied a group of workers from two polyurethane manufacturing facilities in 1976 and 1981. In 1976, the first year of the study, isocyanate levels (mainly PMDI/MDI) were described as less than 0.02 ppm. At some point between 1976 and 1981, the last year of the study, a new ventilation system was installed, resulting in levels below

0.005 ppm by 1981. There was no further discussion of environmental characterization in the report, and sampling and analytical methodologies were not discussed. Thus, the lack of exposure characterization is a serious limitation of the study that precludes its usefulness in identifying exposure-response relationships. A modified respiratory symptom questionnaire based on the British Medical Research Council's version was administered, with additional questions on atopy and occupational history. Flow-volume curves and single-breath CO diffusion (DLCO) tests were performed, but no criteria for standardization were described. According to their job category in 1976, workers were classified into three groups: unexposed (62 men and 21 women); indirectly exposed (61 men and 56 women), and directly exposed (91 men and 27 women). Those indirectly exposed are assumed to represent those handling cured foam products but not involved in the foam-making process. No differences in age, height, or weight by gender were present between the groups in 1976. The proportion of smokers in each group was the same for females, but slightly higher (stated as insignificant) in males for groups 2 and 3. The study in 1976 found a significantly increased prevalence (18.5%,  $p<0.05$ ) of asthma in women in exposure group 3 and of chronic bronchitis in both males and females in groups 2 (males 18%,  $p<0.01$ ; females 19.6%,  $p<0.001$ ) and 3 (males 11%,  $p<0.05$ ; females 29.6%,  $p<0.001$ ). Criteria for classification of asthma and chronic bronchitis were not described. Pulmonary function testing indicated significant impairments (based on comparison to predicted values from cross-sectional populations) in men only for vital capacity (VC) in group 2 and for VC, FEV1, and DLCO in group 3. Exposure duration was broken down into categories of <20 mo, 20-60 mo, and >60 mo for further analysis of the pulmonary function and diffusion testing. Only VC was reduced in the 20-60 mo exposure category ( $p<0.05$ ). In the >60 mo category, VC ( $p<0.01$ ), FEV1 ( $p<0.05$ ), and DLCO ( $p<0.01$ ) were less than predicted values.

At follow-up in 1981, only half of the initial cohort remained (114 males, 45 females). Losses were highest (64%) among exposed females. Reasons for attrition were not described and comparisons between the lost group and the remaining cohort were not made. Only the results of males in 1981 were presented because of the low number of remaining females. For the longitudinal analysis, exposure categories were classified as: (A) unexposed at both studies (n=45); (B) indirectly exposed at both studies (n=24); (C) directly exposed at both studies (n=30); and (D) exposed in 1976 but removed from contact with MDI in 1981 (n=15). Pulmonary function test and DLCO values were reported either as percent of predicted or change in predicted values from study 1 (1976) to follow-up (1981). The 5-year longitudinal changes in FEV1 were given for each of the four exposure groups and were stated not to be significantly different between the nonexposed group and each of the three exposed groups. Pulmonary function indices were significantly lower in group C (directly exposed) when compared with predicted values. However, it has been noted previously that cross-sectional declines in predictive values are insensitive in detecting chronic changes in longitudinal studies (Glindmeyer et al., 1982). The lack of exposure characterization, high rate of attrition, and inclusion of asthmatics in the cohorts preclude any quantitative assessment of MDI exposure on lung function.

Several studies of polyurethane foam workers examined the role of exposure to isocyanates in relation to cancer incidence and mortality. In these studies, PMDI/MDI was one of a variety of substances in the work environment. Thus, these studies are inadequate for categorizing the carcinogenic potential of MDI. In addition, the cohorts described are relatively young and additional follow-up would be needed before any conclusions could be drawn.

A retrospective mortality and cancer morbidity study was conducted to investigate associations between health risk and exposures from polyurethane foam production, particularly exposures to diisocyanates including MDI (Sorahan and Pope, 1993). The study population was taken from 11 factories in England and Wales that had begun manufacturing foam before 1980. Toluene diisocyanate was the principal isocyanate used; MDI represented about 5% of the amount of TDI. Prior to 1970 only TDI was used.

The cohort consisted of 8,288 workers, of whom 2,465 were women, employed for at least 6 mo and having a portion of this employment between 1958 and 1979. Job histories and descriptions were obtained from personnel records. All jobs were classified into one of four exposure levels: high, low, minimal/zero, and unclassifiable. Higher diisocyanate exposure was defined as above 4 ppb (8-h TWA) or excursions above 10 ppb on most days. Lower exposure was defined as between 1.5 and 4 ppb (8-h TWA) or occasional excursions above 5 ppb. Job history data for each worker were matched to job exposure classification to assign person-years of follow-up to three time-dependent exposure levels. The minimum length of follow-up was 9 years. Only 2% of the cohort was lost to follow-up.

Death certificates were obtained for 803 of the 823 known deaths and were coded according to the 9th revision of the International Classification of Diseases. The National Health Service Central register provided information on 277 incident cancers from 1971 through 1988, the year in which the study ended.

Cause-specific SMRs, concentrating on cancer causes, were calculated by applying gender-specific external rates for England and Wales. Separate SMRs for certain cancers were also presented by gender, by years since first hire, and by relationship to cigarette smoking. The SMR for all cancers was 88 (95% CI=84-100), based on 221 observed and 251.4 expected deaths. For females, a statistically significant excess of pancreatic (SMR=271) and lung (SMR=176) cancer was calculated. However, no trend was seen with time since first hire for either of these cancers. When cancers related to smoking were grouped separately from those unrelated to smoking, the SMRs for women in the entry cohort for cancers related to smoking were 263 and were 67 for cancers unrelated to smoking. Available smoking information as of 1981 on a subset of the study population women revealed that 58% of them were smokers, contrasted with 37% of women in England and Wales in 1980.

The relationship between diisocyanate exposure and mortality from all causes of death, respiratory diseases, lung cancer, and pancreatic cancer was evaluated via a multiplicative relative risk model. The internal referent group was person-years at the reference level of all covariates. Exposure was not a statistically significant addition to the model for any of the four causes. There was some suggestion of increased risk at the higher exposure level for lung cancer with a relative risk of 126 (95% CI=39-409) and respiratory diseases(95% CI=54-573). None of the females who died had been classified as receiving either higher or lower diisocyanate exposure during any of their employment history. The authors concluded that excesses in female cancer were probably due to cigarette smoking and other factors unrelated to diisocyanate exposure. Although this well-designed study did not find an association between exposure to diisocyanates and cancer, the cohort was young and follow-up was relatively short.

A retrospective cohort and mortality and cancer incidence study was conducted to determine whether occupational exposure to TDI and PMDI/MDI is associated with excess cancer deaths or incidences or excess deaths from obstructive lung diseases (Hagmar et al., 1993a). The study involved workers employed at any of nine Swedish polyurethane foam manufacturing plants. The plants commenced operations between 1958 and 1973. From 1965 to 1987 personal and area sampling for isocyanates was performed an average total of 15 days on an average of 9 occasions. The TWA for MDI was typically below 0.010 mg/m<sup>3</sup>. The highest level observed was 0.16 mg/m<sup>3</sup>.

The cohort consisted of 4,154 workers employed for at least one year before 1987 and having data complete enough for analysis. Average length of follow-up was 10.6 years. Only 6% of workers in follow-up years through 1987 were attributed to the 1958-1970 period because the cohort was young and only 187 deaths occurred. Death certificates were coded according to the 8th revision of the International Classification of Diseases. The National Swedish Tumor Register reported 72 cases of cancer incidence. Each worker's job history was combined with exposure data and workers were assigned to one of three exposure categories: no exposure, low/intermittent exposure, and apparent exposure. Person-years were also separated into those occurring during the 10 years after first exposure and those occurring later. Cause-specific age/calendar year/gender-specific mortality and incidence rates were obtained from Statistics Sweden and were used to calculate SMRs and the Standardized Incidence Ratios (SIRs).

With 130 observed deaths the overall SMR for all causes of death was 78 (95% CI=66-93). Malignant neoplasms (33 observed, 43 expected) and nonmalignant respiratory diseases (4 observed, 7.3 expected) had SMRs of 77 (95% CI=53-109) and 55 (95% CI=15-141), respectively. The overall SIR for malignant neoplasms (72 observed, 89.4 expected) was 81 (95% CI=63-102), with nonsignificant excess of 166 (95% CI=61-361) for rectal cancer and 153 (95% CI=42-391) for non-Hodgkin's lymphoma. SIRs for these two cancers appeared higher after, rather than during, the first 10 years from first exposure, with SIR after the first 10 years of 192 (95% CI=55-492) for rectal cancer and 280 (95% CI=76-716) for non-Hodgkin's lymphoma.

Time since exposure did not affect SIRs for lung cancer or all malignant neoplasms. Leukemia (3 observed, 2.4 expected) exhibited an overall SIR of 127 (95% CI=26-370). All other causes of cancer among the 72 incident cases had SIRs less than 1.

To further investigate observations in this cohort, Hagmar et al. (1993b) conducted a nested case-control study of 7,023 members of the previous cohort employed for at least one day during 1958-1987. The study also was initiated to minimize exposure misclassification. Cases were 114 of 119 incident cancers, identified from the National Swedish Tumor Register, having job history data complete enough for exposure evaluation. Information from tumors diagnosed from 1959 to 1987 was coded according to the 7th revision of the International Classification of Diseases. Altogether, there were 313 controls, with each case originally matched to three controls with respect to plant, gender, and year of birth. Five cases and 11 controls were dropped because it was impossible to locate their job history information. Exposure to MDI began between 1964 and 1982, depending on the plant. In addition to personnel records, the basis for exposure assessment was expanded to include information obtained from a selected group of persons with long employment and good personal knowledge of employees and processes. Types of exposure distinguished were MDI only, TDI only, or MDI and TDI combined. Exposure was clarified as high, intermediate, or low. For high or intermediate exposure categories, 18 cases and 65 controls were exposed to MDI only, 9 cases and 16 controls were exposed to MDI/TDI, and 19 cases and 62 controls had TDI exposure only. Exposure levels to MDI were normally below the detection limit of the analytical method ( $<0.01 \text{ mg/cm}^3$ ) and nearly all were below  $0.1 \text{ mg/m}^3$ .

Results were presented for all cancers combined. No distinction was made in the results between exposure to MDI and TDI. Odds ratios (OR) for diisocyanate exposure with and without a 10-year lag were presented with 90% confidence intervals. Using conditional logistic regression, ORs were calculated separately for (1) any exposure, (2) exposure at intermediate or high levels, (3) high exposure, (4) high exposure for up to 5 years, and (5) high exposure for more than 5 years. For the last two analyses, ORs were produced only for all cancers combined. Potential confounders, with the exception of smoking and alcohol use, were controlled by the matching design of the study.

At the 10% level of significance, no statistically significant association was found between all-cause cancer and diisocyanate exposure using any of the five exposure measures or for non-Hodgkin's lymphoma and rectal cancer (five cases). The OR for prostatic cancer appeared elevated with high exposure (OR=2.7). However, risk was not enhanced in analyses examining a 10-year latency period. The OR between colon cancer and any exposure and intermediate/high exposure appeared elevated (OR=2.2 and 3.3, respectively). However, there was no odd distribution of colon cancer cases by subsite. All colon cancer cases had less than a 10-year latency. The apparent association of exposure with prostate and colon cancer may be spurious, as there is no plausible biological connection between these cancers and diisocyanates.

## 4.2 Prechronic, Chronic Studies and Cancer Bioassays in Animals

In a range-finding toxicity study, SPF-Wistar rats (15/sex/group) were exposed to PMDI aerosol at 0.35, 1.4, or 7.2 mg/m<sup>3</sup>, 6 h/day, 5 days/week for 13 weeks (Reuzel et al., 1985a, 1994a). Polymeric MDI was in the form of a dark brown liquid that contained 52% monomeric MDI. The trade name of the mixture used was Desmodur 44 V 20. The PMDI content in test atmospheres was monitored by light scattering, gravimetry, photometry, and high-performance liquid chromatography (HPLC). The content of monomeric MDI in the test material was 52%. Particle sizing, accomplished with cascade impactors, indicated >95% of the particles had an aerodynamic diameter <5 µm. However, sigma g values were not given at each of the concentration ranges. Microscopic evaluation of liver, kidneys, and respiratory tract (nasal cavity, trachea, larynx, and lungs; n = 15) did not reveal any exposure-related lesions. The only exposure-related observation was an increase in yellowish-colored alveolar macrophages in all animals of the high-exposure group, with sporadic signs of a minimal focal inflammatory reaction in the alveolar septa. Urinalysis, hematology, clinical chemistry, and liver enzymes indicated no change in comparison with control animals. It was concluded that no clear adverse effects were observed.

In order to determine appropriate concentrations for a chronic study, a second 13-week, range-finding subchronic toxicity study was performed using specific-pathogen-free (SPF) Wistar rats (30/sex/exposure level) exposed to PMDI aerosol (Reuzel et al., 1985b, 1994a). Rats were exposed to 4, 8, or 12 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 13 weeks. The aerosol was generated by a nebulizer modified to eliminate nonrespirable particles. The MMAD of the throughput, as determined by cascade impaction, was <5 µm, which was representative of 95% of the particles. However, particle size distribution was not reported. Test atmospheres were monitored by gravimetry, photometry, light scattering, and HPLC. Clinical symptoms, ophthalmology, body and organ weights, hematology, biochemical alterations, urine, gross, and histopathologic examination in 10 animals per group at the end of exposure and 4 weeks after the final exposure were evaluated for toxicity.

Lung lavage fluid was analyzed for cell number, cell viability, and survival based on trypan blue exclusion and phagocytic activity in 5 animals per group exposed to 0, 4, and 8 mg/m<sup>3</sup>. In the animals examined immediately after the exposure, severe respiratory distress was noted in animals exposed to 12 mg/m<sup>3</sup>, while similar, although less severe, signs were also seen in the 8 mg/m<sup>3</sup> group. Mortality occurred only in the 12 mg/m<sup>3</sup> groups (15/60 animals) and only during the first 7 weeks of the study. An exposure-related increase in relative lung weight was noted at 8 and 12 mg/m<sup>3</sup> in both sexes. Creatinine values in blood plasma increased in an exposure-related manner in both the 8 and 12 mg/m<sup>3</sup> groups. Degenerative lesions occurred in the olfactory epithelium of the nasal cavity of both sexes at 12 mg/m<sup>3</sup>. Hyperplasia of the respiratory epithelium was found in females, but not males, of the 8 and 12 mg/m<sup>3</sup> groups. There was a significant increase in accumulation of macrophages, observed histologically, in the lungs and

mediastinal lymph nodes of all exposed animals compared with controls. The increase and severity of accumulation at the level of the alveolar septa achieved statistical significance in males at 4 mg/m<sup>3</sup> and females exposed to 8 mg/m<sup>3</sup>. There were no histopathological lesions of other organs examined. No effects were observed on number of lavagable cells, cell viability, or cell survival. In vitro measurements indicated that the phagocytic activity (data not reported) of lavaged alveolar macrophage was reduced in females exposed to 8 mg/m<sup>3</sup>, suggesting to the investigators that lung clearance might be impaired. The lack of effect on lavageable cell number is not consistent with the histological observation of increased accumulation of macrophages, and suggests incomplete recovery of macrophages in the exposed animals. This study demonstrated adverse effects in the lungs and nasal cavity at levels of 4 mg/m<sup>3</sup> and above. However, because of a lack of data on aerosol sizes, a quantitative LOEL(HEC) cannot be derived. These results are, however, consistent with the findings of the chronic study that subsequently was performed (Reuzel et al., 1990, 1994b).

In a 2-year cancer bioassay, SPF-bred Wistar rats of the Cpb:WU strain (60/sex/exposure level) were exposed whole-body to aerosols of PMDI for 6 hours/day, 5 days/week, for 24 mo (Reuzel et al., 1994b). The nominal chronic exposure levels were 0, 0.2, 1.0, and 6.0 mg/m<sup>3</sup>. An additional satellite group of 10/sex/exposure level was exposed similarly and used for histopathology at 12 mo. The selection of 6 mg/m<sup>3</sup> as the high dose was based on a steep dose-response curve identified in the 13-week study (Reuzel et al., 1994a). In the latter study, severe respiratory effects, including mortality, were observed at 12.3 mg/m<sup>3</sup> whereas similar, but much less severe, clinical effects and increased lung weights were seen at the next lowest dose, 8.4 mg/m<sup>3</sup>. These observations, coupled with significant increases in absolute and relative lung weights in the chronic study, are consistent with 6.0 mg/m<sup>3</sup> in the chronic study, approaching a maximum tolerated dose.

The MDI test material (eight different batches) had an average molecular weight of about 400. It was reported that the composition of each batch of the material was analyzed by the supplier (Bayer A.G.) prior to use and, in the case of the first two and last batches, also after three months of storage. The content of monomeric MDI, determined by the supplier, ranged from 44.8% to 50.2%. The remainder, excluding trace impurities, was described as “polymeric” MDI with no further explanation. Test material was kept at room temperature under “common laboratory conditions” prior to use. This is interpreted to mean that the test material was stored under nitrogen or argon, as MDI reacts with water to form carbon dioxide.

Test atmospheres were generated by atomizing the MDI material into droplets using compressed air in a nebulizer fitted with a baffle to remove larger particles from the air stream. The smaller droplets were passed through a cyclone to obtain an aerosol of which 95% of the particles were smaller than 5 µm. Beta attenuation primarily was used, along with intermittent gravimetric determinations, to determine the concentration of the polymeric MDI in the exposure atmosphere. HPLC was used as a secondary standard during the first 3 mo of exposure.

However, neither the content of monomeric MDI in the test atmosphere nor other constituents, such as MDA, were determined. Thus, uncertainty as to the amount of monomeric MDI to which the animals were exposed is a limiting feature of this study. The actual mean values were within  $\pm$  10% of nominal values. Thus, the nominal values will be used in the following discussion. The duration-adjusted exposure levels are 0, 0.036, 0.180, and 1.100 mg/m<sup>3</sup>. The MMAD and geometric standard deviation (in parentheses) corresponding to the exposure levels were 0, 0.68  $\mu$ m (2.93), 0.70  $\mu$ m (2.46), and 0.74  $\mu$ m (2.31), respectively.

The animals were observed for clinical signs twice a day during exposure. Hematological analysis, clinical chemistry, and urinalysis were performed on 10 animals/sex/exposure level after 1 year of exposure. Gross observations and organ weights were obtained on all animals. Histopathological observations were made on the lungs, mediastinal lymph nodes, and nasal cavity (6 levels) of all animals. Histological observations in the main group were made on controls and animals exposed to 6 mg/m<sup>3</sup> and on low- and mid-concentration decedents for 43 tissues. Examples of the tissues examined included testes, lung, nasal cavity, heart, kidney, pancreas, liver, lymph nodes, mammary glands, stomach, brain, etc. The tissues selected for histopathology are representative of types of tissues that would normally be included in, for example, a protocol of the National Toxicology Program.

There was no exposure-related increase in mortality, clinical signs, clinical chemistry, or body weight. Upon necropsy of the satellite group at 1 year, exposure-related changes were seen in the nasal cavity, lungs, and mediastinal lymph nodes. Males and females at 6 mg/m<sup>3</sup> showed a significant increase in relative lung weight. Males only at 6 mg/m<sup>3</sup> exhibited statistically significant minimal-to-moderate disarrangement of the olfactory epithelium (0/10, 0/10, 3/10, and 6/10 in the 0, 0.2, 1.0, and 6.0 mg/m<sup>3</sup> groups, respectively). The incidence of minimal-to-moderate basal cell hyperplasia was not statistically significant at any exposure level (males, 1/10, 0/10, 2/10, and 2/10; females, 0/10, 0/10, 0/10, and 2/10). Accumulation of macrophages with yellow pigment was seen in the lungs of both sexes at 1 and 6 mg/m<sup>3</sup> (males, 0/10, 0/10, 4/10, and 10/10; females, 0/10, 0/10, 3/10, and 9/10). At the site of accumulation, Type II alveolar cells were predominant. Localized fibrosis was significant in males exposed to 6 mg/m<sup>3</sup> and in females at 1 and 6 mg/m<sup>3</sup>. Alveolar duct epithelialization was increased significantly in males and females exposed to 6 mg/m<sup>3</sup> and interstitial pneumonitis was significant in males at 1 and 6 mg/m<sup>3</sup>.

At the 24-mo necropsy of the main group, histologic evidence of damage involved the same tissues as at 12 mo, although severity had increased. Basal cell hyperplasia was evident in the olfactory epithelium of males (14/60, 13/60, 26/60, and 32/60 at 0, 0.02, 1.0, and 6.0 mg/m<sup>3</sup>, respectively) and females (4/60, 8/60, 8/60, and 49/59 at 0, 0.2, 1.0, and 6.0 mg/m<sup>3</sup>, respectively). Statistical significance was achieved with males only in the mid-concentration group and with both sexes at in the high-concentration groups. It was often accompanied by Bowman's gland hyperplasia, which was significant in males at 1 and 6 mg/m<sup>3</sup>. Olfactory epithelial degeneration was elevated significantly in males and females at 6 mg/m<sup>3</sup>, incidence at 1 mg/m<sup>3</sup> was elevated in

males but was not statistically significant. Given the apparent sensitivity of the male to basal cell hyperplasia and the expression of an elevated incidence of both basal cell hyperplasia and olfactory degeneration at both the mid and high concentrations, it is not possible to discern basal cell hyperplasia, on the basis of either the interim or final sacrifice histopathological results, as a compensatory response. Thus, it is prudent to regard basal cell hyperplasia as an adverse response. Because it reached statistical significance at a lower exposure level (male rats only) than olfactory degeneration, the former was chosen as the critical effect.

In the lung there was an increase in the accumulation of pigment-laden macrophage in alveolar duct lumina (incidence in males: 0/60, 3/60, 21/60, and 60/60; females: 0/59, 1/60, 23/60, and 59/59) and in localized fibrotic changes (males: 1/60, 0/60, 9/60, and 44/60; females: 0/59, 0/60, 4/60, and 48/60). Both sexes at 6 mg/m<sup>3</sup> exhibited statistically significant increases in mean absolute lung weight, although relative lung weight was increased in males only. Localized alveolar duct epithelialization was increased significantly in both males and females exposed to 1 and 6 mg/m<sup>3</sup>, and localized alveolar bronchiolization (adenomatous hyperplasia) was significant in both sexes at 6 mg/m<sup>3</sup>. In animals exposed to 1 mg/m<sup>3</sup>, there were minimal foci of Type II pneumocyte hyperplasia, which was more extensive in the high-exposure group. An exposure-related accumulation of yellow pigment in the mediastinal lymph nodes was noted in both males and females. The accumulation of macrophages and the localization of tissue damage in the area of macrophage accumulation suggest that the thoracic effect is due primarily to toxicity to the macrophage (with secondary tissue damage), although some of the effects were described as being distributed evenly throughout the lungs. There were no histological effects in any of the other organ systems examined. The information obtained in this chronic study identifies a NOAEL of 0.2 mg/m<sup>3</sup> (duration-adjusted concentration = 0.036 mg/m<sup>3</sup>) and a LOAEL of 1.0 mg/m<sup>3</sup> (duration-adjusted concentration = 0.18 mg/m<sup>3</sup>) for respiratory tract effects in both the pulmonary and extrathoracic regions.

Rather than use the identified NOAEL for calculation of the RfC, a benchmark concentration (BMC) approach was employed, using all the duration-adjusted data for both basal cell hyperplasia and degeneration of the olfactory epithelium. The BMC at the 10% level of risk (BMC<sub>10</sub>) was calculated (see Appendix A) as the lower bound on the maximum-likelihood estimate. The 10% level of risk was chosen as a result of the findings by Allen et al. (1994) that quantal representation of endpoints from developmental toxicity experiments led to the best match between NOAELs and benchmark concentrations or doses when a 10% level or greater of response was used to define the BMC.

Although there were no compound-related nasal tumors, solitary pulmonary adenomas, described as rare in this strain, were observed in males (6/60) and females (2/59) exposed to 6 mg/m<sup>3</sup> compared to controls (0/120). The adenomas, not thought to be the cause of death, were only a few millimeters in size and were located adjacent to areas in which hemorrhage, macrophage accumulations, and fibroblastic reactions were observed. Only one pulmonary

adenocarcinoma (approximately 10 mm in size) was observed in one male exposed to 6 mg/m<sup>3</sup>. Although this study provides clear evidence of a tumorigenic response to treatment, the significance of only one pulmonary adenocarcinoma is insufficient to distinguish PMDI as an animal carcinogen.

Another chronic toxicity/carcinogenicity study (unpublished) was also conducted in female Wistar rats only, strain CrI:[WI]BR, (80/exposure group) exposed to monomeric MDI in aerosol form to nominal levels of 0.23, 0.70, and 2.05 mg/m<sup>3</sup> for 17 h/day, 5 days/week for 24 mo (Hoymann et al., 1995). The selection of exposure levels was based on results of a previous 90-day study. The highest concentration can be considered a maximum tolerated dose inasmuch as the body weight gain for this group after 17 mo of exposure was 11% less than that of controls.

The nominal levels of the chamber atmosphere were determined photometrically, after calibration using gravimetric values. However, HPLC analysis of the chamber atmosphere indicated that the amounts (0.097, 0.549, and 1.75 mg/m<sup>3</sup>, respectively) of monomeric MDI were considerably less than the photometrically determined levels, particularly at the lowest concentration. According to the manufacturer's specifications, the starting material consisted of 99.5% pure monomeric MDI. It had been heated and mixed with compressed air and passed through a nebulizer to generate the aerosols to which the animals were exposed. The MMAD was determined to be about 1 µm in each of the three exposed groups. A sigma g was not provided; however, the same aerosol generation protocol was used in another study (Buschmann et al., 1996) in which the sigma g was 1.37. Hoymann and colleagues surmised that a portion of the MDI generated with water vapor in the chamber to form reaction products such as urea. This was offered as a likely reason the chemically determined amount of monomeric MDI was less than the gravimetric value, which presumably included reaction products. This observation is consistent with the findings of Dharmarajan (1979). A limitation of the chemical analyses of the exposure atmosphere generated by Hoymann and colleagues is that MDA content was not measured.

Because gravimetric values from the Reuzel et al. (1994b) study had been used (chemical determination of monomeric MDI was not made), the nominal values in the Hoymann et al. study are used throughout the following discussion for comparison purposes. Differences in MDI test material and chemical analyses used by Reuzel et al. (1994b) and Hoymann et al. (1995) preclude assessment of test results on any basis other than gravimetric values. The duration-adjusted values for the Hoymann et al. (1995) study are 0.12, 0.35, and 1.04 mg/m<sup>3</sup>, respectively. In comparison to the duration-adjusted values from the Reuzel et al. (1994b) study, the lowest two exposure values are about four- and twofold higher, respectively.

In addition to animals exposed for 24 mo, 20 animals per exposure group were examined histopathologically at 12 mo. Smaller numbers of animals were assessed at various time points for

lung function and for examination of bronchoalveolar lavage (BAL) fluid for cell counts and protein and enzyme determinations.

Treatment-related and statistically significant pulmonary lesions found in animals exposed for two years included: (1) concentration-related increase in focal/multifocal alveolar and bronchioalveolar hyperplasia of the lungs, (2) concentration-related increase in incidence of focal/multifocal interstitial fibrosis of lungs, (3) concentration-related multifocal accumulation of particle-laden and pigmented macrophages. Alveolar cell hyperplasia, considered preneoplastic, exhibited a concentration-response trend with incidence reaching significance in the high-exposure group (7/80 compared to 0/80 in controls).

The histopathologic effects in the lungs correlated with pulmonary function effects, particularly in the high-exposure group. There were significant differences from controls in forced expiratory flow at 25% (FEF25) and 50% (FEF50) after 6 mo and in CO diffusion after 12 and 17 mo. Bronchoalveolar lavage indicated total protein was significantly elevated after 20 mo in the mid-exposure group; elevation at earlier time points occurred in the high-exposure animals. There was also a concentration-dependent increase in  $\gamma$ -glutamyl transpeptidase activity that was statistically significant in the mid- and high-concentration groups. All groups evidenced significantly increased relative lung weights at all time periods. This latter observation, coupled with significant increases in hydroxyproline and total protein in BAL fluid, clearly indicates adverse effects on the lungs and that a maximum tolerated dose was achieved.

In contrast to the greater incidence of animals with pulmonary adenomas in the high-exposure group of the Reuzel et al. (1994b) study, only 1 of 80 animals (high-exposure group only) exhibited a single bronchio-alveolar adenoma. Tumor data were presented only for the high-exposure group. Although there was a total of 144 benign tumors in this group, the number in controls was even higher (159). There was no significant difference in the incidence of animals with malignant tumors between the controls and the high exposure-group. The incidence of mammary gland tumors was similar between the high-exposure group and controls. Noncancer histopathologic results for tissues/organs other than the lungs were presented for the high-exposure group only. This lack of data for other exposure groups represents a study deficiency. Goblet cell hyperplasia of the nose and focal/multifocal mucosal inflammatory cell infiltration were significantly elevated above controls for the high-exposure group. No other significant nasal effects were reported. There also were no significant differences in mortality or body weight between exposed and control animals.

In the satellite group examined at 12 mo, there were no statistically significant effects on nasal endpoints, but there was a statistically significant dose-dependent increase in interstitial fibrosis, pigment, and particle-laden macrophage in the lung. This is consistent with the 2-year findings for this endpoint. Increases in alveolar and bronchioalveolar hyperplasia were not statistically significant at 12 mo. With respect to tumor incidence, only the high-exposure group



























































