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ORGAN TOXICITY AND MECHANISMS

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Chronic pulmonary effects of respirable methylene diphenyl diisocyanate (MDI) aerosol in rats: combination of findings from two bioassays

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Abstract Two independent bioassays are available which have examined the potential carcinogenicity of monomeric and polymeric methylene diphenyl diisocyanate (MDI) following long-term inhalation exposure in rats. These studies are not directly comparable, however, due to differences in design and conduct of the in-life phase, and differences in nomenclature used for some of the histopathological findings. This paper presents a definitive overview ofthe pulmonary toxicity of MDI developed following a thorough review of both investigations. As part of this process, the test materials and the designs of the studies were compared, and an in-depth review of lung lesions was conducted by an independent reviewing pathologist.This included the re-examination of the original lung slides, supported by an analysis of the exposure regimens, the results of which were used to develop an accurate profile of the doses received by the animals in the two studies. Histopathological findings were then combined with this information to give an overall dose-response curve for both studies as a whole. The range of total inhalation exposures to MDI was calculated as 559, 1972, 2881, 6001, 17,575 and 17,728 mgh/m^3. Major pulmonary effects included increased lung weights together with bronchiolo-alveolar adenomas and hyperplasia, and interstitial fibrosis which occurred consistently in both studies, indicating a very similar qualitative response of the lungs to polymeric and monomeric MDI. The quantitative response of the lung was clearly dose-related in each study, and when the studies were considered as a whole a reasonable overall dose-response relationship was apparent for major lung lesions. Lung tumours (in low incidences) only occurred at the highest dose level in both studies (17,575 and 17,728 mgh/m^3). For inflammatory and other non-neoplastic pulmonary changes, the lowest dose examined (559 mgh/m^3) was regarded as a no-observed-adverse-effect-level for both polymeric and monomeric MDI. It was concluded that the results of the two studies could be combined to serve as a basis for human risk assessment of MDI.

Keywords Methylene diphenyl diisocyanate (MDI) · Inhalation · Combined long-term rat bioassays · Pulmonary effects

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Introduction

Methylene diphenyl diisocyanate (4,4'-diphenylmethane diisocyanate; MDI) is a major industrial chemical, mainly used for the production of polyurethanes. It is available commercially in two forms, monomeric MDI and polymeric MDI (Fig. 1). Monomeric MDI, also known as pure MDI, comprises 4,4'-diphenylmethane diisocyanate with small percentages of 2,4'-diphenylmethane diisocyanate and 2,2'-diphenylmethane diisocyanate. Monomeric MDI is produced from polymeric MDI by crystallization and/or distillation. Polymeric MDI comprises a number of oligomers, primarily monomeric MDI (50%) along with a number of higher homologues (Fig. 1). While the composition of polymeric MDI may show slight variation between manufacturers (reflecting proprietary processes), on a w/w basis the monomer is generally present at 50%, with the remainder of the substance comprising oligomers (26% three-ring oligomer; 13% four-ring; 7% five-ring; $<5\%$ six-ring or higher).

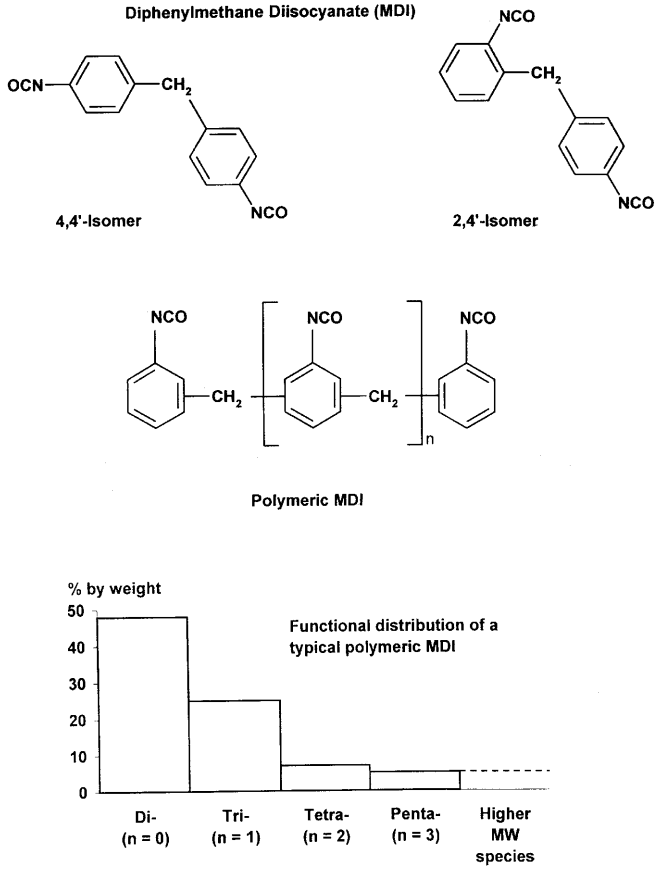


Fig. 1 MDI monomer structure and functional distribution of polymeric MDI

The toxicology of MDI has been investigated quite extensively (summarized in IUCLID 1994), including acute, subacute and subchronic inhalation toxicity investigations (Reuzel et al. 1994b; Hoymann et al. 1995), a teratogenicity study (Buschmann et al. 1996) and two chronic (24-month) inhalation toxicity and carcinogenicity studies in rats. The two chronic studies were fully independent of one another. One study used polymeric MDI and was carried out by TNO Nutrition and Food Research, Zeist at the request of the International Isocyanate Institute (Reuzel et al. 1990, 1994a). The other study used monomeric MDI, was initiated by the Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit/Umwelt-bundesamt, Berlin and was performed by the Fraunhofer Institute of Toxicology and Aerosol Research, Hannover (Hoymann et al. 1995).

In view of their similar chemical and physicochemical properties, monomeric and polymeric MDI are expected to share a similar fate in the moist environment of the airways. Formation of polyureas seems probable, basedon model studies (Yakabe et al. 1999). With both types of MDI, the -NCO-groups that are not involved in hydrolysis and polymerization to polyurea can bind covalently to biomolecules on the surface (e.g. surfactant components, proteins and lipoproteins). Model reaction studies have shown that NH_2-, OH- and SH-groups of amino acids can react with-NCO-groups (Day et al. 1997; Mraz and Bouskova 1998, 1999). Overall, in view of the chemical reactivity and molecular weight (MW) range of both types of MDI and the biomacromolecules available in the respiratory tract, only slight differences in pattern and disposition of reaction products are expected between monomeric and polymeric MDI.

Differences in design between the two studies are listed in Table 1. Despite these differences, the lungs appeared to be the target organ in both investigations and showed very similar lesions, including increased weight, accumulation of particle-laden alveolar macrophages, mononuclear cell infiltrates, interstitial fibrosis, hyperplasia of bronchiolo-alveolar epithelium, and bronchiolo-alveolar adenomas. One pulmonary adenocarcinoma was found; it occurred in a male rat from the high-concentration group (6.03 mg/m^3) exposed to polymeric MDI (Reuzel et al. 1990, 1994a).

A pathogenesis for the MDI-associated lung effects, including tumours, has been discussed in detail by Reuzel et al. (1994a), who suggested that a non-genotoxic mechanism could account for these findings, and that exposure to polymeric MDI at levels which do not result in recurrent lung tissue injurywould not produce lung

Table 1 Conspicuous differences in experimental conditions between the TNO and the Fraunhofer study

Item	TNO study			Fraunhofer study		
Test material						
Nature	Polymeric MDI[a]			Monomeric MDI		
Colour/physical state	Dark-brown liquid			White-yellow flakes		
Particle size (MMAD)[b]	0.68–0.74 μm			1.03–1.06 μm		
Rats						
Strain	Cpb: WU, Wistar random			Wistar, Crl:[WI]BR		
Sex	Males and females			Females		
Number/sex/group	60			80		
Exposure						
Duration (h/day)	6			18		
Concentrations (mg/m^3)	0.19[c]	0.98[d]	6.03[e]	0.23[c]	0.70[d]	2.05[e]
Doses (mgh/m^3)	559	2881	17,728	1972	6001	17,575

[a] Polymeric MDI contains about 50% monomeric MDI
[b] Mass median aerodynamic diameter
[c] Low-dose group
[d] Mid-dose group
[e] High-dose group

tumours. However, while recognizing that chronic inflammation, cell injury and regenerative hyperplasia might play a role, Hoymann et al. (1995) and Vock et al. (1996) suggested that 4,4'-methylene dianiline (MDA, a breakdown product of MDI which is carcinogenic in animals), may play a role in tumour induction. This hypothesis appears to be supported by reports that MDA is present in the urine from rats exposed chronically to monomeric MDI (Hoymann et al. 1995; Sepai et al. 1995). However, since the absolute amount of amine detected appears to vary with the hydrolysis method used for sample preparation (Sepai et al. 1995), it is possible that chemicalconversion of MDI (or MDI-based adducts) to MDA may have occurred during work-up of the urine samples. Further information on the fate of MDI in the rat after inhalation and the possible role of MDA formation can be expected when results from an ongoing metabolism study are available (research project of the International Isocyanate Institute, 1999).

Information on the two long-term bioassays is central to any evaluation of the potential health hazards of MDI. However, in view of differences in design and reporting, some additional analysis was considered necessary to present a single, scientifically coherent overview suitable for product safety, regulatory and risk assessment purposes. In order to progress this integration, the test materials and design of the studies were thoroughly compared. The nomenclature and grading schemes used to describe histopathological changes in lung tissue were then harmonised in a joint effort between pathologists involved in the original study (C.F.K. and H.E.) and an independent reviewing pathologist not involved in the original reading of the slides (B.K). Central to this was re-examination of the lung slides by the reviewing pathologist. The information obtained was used to establish for the studies a common dose-response curve for the main histopathological end-points in the lungs.

This paper describes the comparison and interrelation of the two long-term studies, focusing on the lung findings and the determination of a common dose-response relationship.

Materials and methods

Original studies

Details of the materials and methods used in both long-term studies have been described by Reuzel et al. (1990, 1994a, 1994b), Hoymann et al. (1995, 1998) and Ernst et al. (1998). Only aspects critical to a proper comparison of the pulmonary changes found in both studies will be repeated here (see also Table 1).

Test materials

Figure 1 shows the chemical structure of the various molecular species in monomeric and polymeric MDI. As can be seen, "polymericMDI" consists mainly of molecules with a MW between 270 and 560, while "monomeric MDI" or "pure MDI" is just the smallest species in the mixture with MW 270. The calculation presented in Fig. 1 shows that both test materials have a comparable molar ratio, assuming a 1:1 binding between each MDI species and biomacromolecules as the predominant binding ratio. Overall, it seems likely that both test materials will exhibit a similar chemical reactivity in the respiratory tract.

TNO study. Polymeric methylene diphenyl diisocyanate (polymeric 4,4′-diphenylmethane diisocyanate; polymeric MDI; Bayer A.G., Leverkusen) was used for the study performed by TNO Nutrition and Food Research (further designated as TNO study). The test material was a liquid throughout the normal working and environmental conditions, with a low vapour pressure ($< 10^{-5}$ mbar at 25°C) and a low saturated vapour concentration (about 0.05–0.10 mg/m^3 at room temperature).

Fraunhofer study. Monomeric 4,4′-methylene diphenyl diisocyanate (monomeric diphenylmethane-4,4′-diisocyanate; monomeric MDI; Bayer A.G., Leverkusen) was used for the study carried out by the Fraunhofer Institute of Toxicology and Aerosol Research (further designated as Fraunhofer study). The purity was >99.5%. Monomeric MDI is a solid waxy material at room temperature which liquefies at around 38°C. It has a low vapour pressure of 4×10^{-6} mbar at 20°C and a saturated vapour concentration of ca. 0.1 mg/m^3 at room temperature.

Generation and characterization of test atmospheres

Atmosphere generation and analysis are described in the original study reports (Reuzel et al. 1990, 1994a; Hoymann et al. 1995). Brief details are as follows.

TNO study. Test atmospheres were generated by spraying polymeric MDI liquid into droplets using a nebulizer, which was operated by compressed air at a pressure of approximately 2.5 bar. A baffle combined with a cyclone was used to achieve the required particle size in the aerosol (95% <5 μm; mass median aerodynamic diameter ca. 0.7 μm). The aerosol was then passed through a manifold pipe system to the inlet of the inhalation chambers, where it was diluted with air from the main air supply of the inhalation chambers. By varying the operating air pressure the amount of aerosol could be adjusted to the desired concentration of test material within the chamber.

A variety of analytical methods was used to determine the particle size and the aerosol concentration in the test atmosphere. Gravimetry was the primary standard for assessing aerosol concentration. Beta attenuation was used in parallel with gravimetry during the first 3 months of the study. In view of the proven reliability of beta attenuation during this period gravimetric determinations were carried out only once every 2 weeks thereafter. During the first 3 months of the study, occasional HPLC analyses were carried out for chemical analysis and as a secondary standard for the total mass of polymeric MDI in the various test atmospheres. The highest exposure concentration was continuously monitored by means of an aerosol light scattering photometer. Occasionally, photometry was also applied to the lower concentration levels. The particle size distribution in each of the test atmospheres was determined at weekly intervals, using a ten-stage Berkeley quartz crystal microbalance cascade impactor.

Fraunhofer study. The solid test material was liquefied in a stainless steel container kept in a water bath at 60°C and subsequently nebulized, using a nebulizer operated with compressed air. The mist, consisting of large, mainly non-inhalable particles, was then led into an evaporation tube where it was evaporated at 150°C. The residence time in the evaporation chamber was about 2 s. The vapour was diluted with cold air and thus, immediately recondensed, resulting in an aerosol with a mass median aerodynamic diameter (MMAD) of ca. 1.0 μm. This aerosol was then passed through an aerosol distributor via thermically insulated stainless steel pipes to the inlet of the respective inhalation chambers, where it was further diluted with air to match the desired concentration within each chamber.

A variety of analytical methods was used to determine the particle size and the aerosol concentration in the test atmosphere. Gravimetry served as a calibration standard for assessing aerosol concentration. For continuous monitoring of the aerosol concentration, aerosol light scattering photometers were used. The particle size distribution in each of the test atmospheres was determined, using an eight-stage cascade impactor (type Berner LPI 30, 0.06). HPLC, after derivatisationwith 4-nitro-*N*-propylbenzylamine, was used for chemical analysis. The photometers were calibrated weekly, using the filter samples. The 15-min average values of the photometer signals served as raw data for dose calibration.

Animals, exposure conditions and necropsy

TNO study. Four groups of 60 male and 60 female Wistar rats (Cpb:WU, Wistar random), 6 weeks old, were exposed by inhalation in a chamber to 0, 0.19 (±0.05), 0.98 (±0.11) or 6.03 (±0.54) mg/m^3 polymeric MDI aerosol for 6 h a day, 5 days a week for up to 24 months. The MMAD and the geometric standard deviation (GSD; in parentheses) of the test material in the atmospheres during the total exposure period were 0.68 (2.93), 0.70 (2.46), and 0.74 (2.31) μm for the low-, mid-, and high-concentration groups, respectively. On avarage, the aerodynamic diameter was <4.2 μm for at least 93.5% of the particles.

Any rats that died intercurrently or which were killed due to moribund condition, along with all rats randomly killed according to schedule in weeks105 and 106, were necropsied, and examined for gross pathological changes. The lungs were fixed by intratracheal infusion with aqueous neutral phosphate-buffered 4% formaldehyde solution under 10 cm water pressure and, after fixation and dehydration, embedded in Paraplast, sectioned at 5 μm, and stained with haematoxylin and eosin. From each lung lobe, three medial sections were made; additional recuts were made from selected lesions. See Reuzel et al. (1990, 1994b) for further details.

Fraunhofer study. Four groups of 80 female Wistar rats (Crl:[Wi]BR), about 10 weeks old, were exposed by inhalation in chambers to 0, 0.23 (±0.06), 0.70 (±0.17), or 2.05 (±0.37) mg/m^3 monomeric MDI for 18 h a day, 5 days a week for at most 24 months. The average MMAD and the GSD (in parentheses) of the test aerosol in the atmospheres during the total exposure period were 1.03, (0.17), 1.03 (0.18), and 1.06 (0.23) μm for the low-, mid- and high-concentration groups, respectively. The GSD of the MDI aerosol generated by the condensation apparatus was small (<1.5) for the three concentration groups. For the low-concentration group, about 40% of monomeric MDI was in the vapour phase.

All rats that died intercurrently or were killed in moribund condition between months 12 and 24 of exposure, and all rats killed according to study plan after 24 months, were necropsied, and examined for gross pathological changes. The lungs were fixed by intratracheal infusion of a 10% neutral formalin solution under 20 cm water pressure, and after fixation and dehydration, embedded in Paraplast, sectioned at 4 μm, and stained with haematoxylin and eosin-phloxin. From each lung lobe, one section was made; recuts were made from selected lesions. See Hoymann et al. (1995) for further details.

Present comparative investigation

The comparative evaluation and interrelation of the two studies was performed with respect to the following issues.

Interrelation of test materials and characterization of test atmospheres

The chemistry and biochemistry of the two test materials were subjected to a detailed evaluation, focusing on the similarities and dissimilarities between monomeric and polymeric MDI. Comparison of the test atmospheres included determination of mass size distribution of the two aerosols and calculations of deposition pattern of the aerosol in the respiratory tract. Moreover, for the various test atmospheres the partition of the test materials between gas and particulate phase was assessed.

Interrelation of exposure concentrations and dose calculations

The exposure duration per day of exposure was about 6 h in the TNO study and about 18 h in the Fraunhofer study. To compare properly the exposure to MDI in both studies, and to enable the development of one overall dose-response relationship based on the data from both studies, MDI doses were calculated, using the physicochemical data of the test atmospheres, respiratory physiology parameters and the deposition pattern of MDI aerosol in the respiratory tract.

Animals

In both studies Wistar rats were used. As the Fraunhofer study only used female rats, the re-evaluation of the lung findings was confined to this sex from both studies. It should be noted, however, that results from the TNO study showed a similar pattern of pathological findings for male animals also.

Comparison and interrelation of lung histopathology

For the purpose of comparative evaluation, the relevant data from both studies and the evaluation of the reviewing pathologist were entered into a pathology computer program (acopat[c], BASF Department of Toxicology). This virtual "new study" contains the following information: dose groups (see Table 1), animal number, start and end of exposure, date of necropsy, necropsy status, diagnosis of the study pathologists and diagnosis of the reviewing pathologist.

In order to merge the data, the reviewing pathologist and the study pathologists had to agree upon a common nomenclature and the grading of lung lesions. To attain consensusthe reviewing pathologist studied the whole range of lung lesions observed in both studies, and suggested a harmonized nomenclature and definitions for the various gradings of lesions where appropriate. After consensus was reached, the actual re-reading of the lung slides by the reviewing pathologist was carried out.

In the course of the re-examination of lung slides, selected lung samples from the TNO study were recut and stained with Masson-Goldner-Elastica for collagen or Perl's Prussian Blue for iron (haemosiderin).

Results

Interrelation of test atmosphere characteristics

In both the TNO and the Fraunhofer study, only one generation system was used, generating a basic concentration of test material which was then diluted to achieve the desired concentrations for the various dose groups. The mass size distribution of the aerosols is shown in Fig. 2.

Although the size distributions of the two test aerosols differed, no major difference in theamount of aerosol deposited in the pulmonary, bronchial and nasal region of rats is predicted. This can be concluded from the size dependence of the regional deposition pattern shown in Fig. 2. These data are taken from experiments performed with monodisperse aerosols (Raabe et al. 1988). It can be seen that the size dependence for the pulmonary and bronchial deposition is a flat function

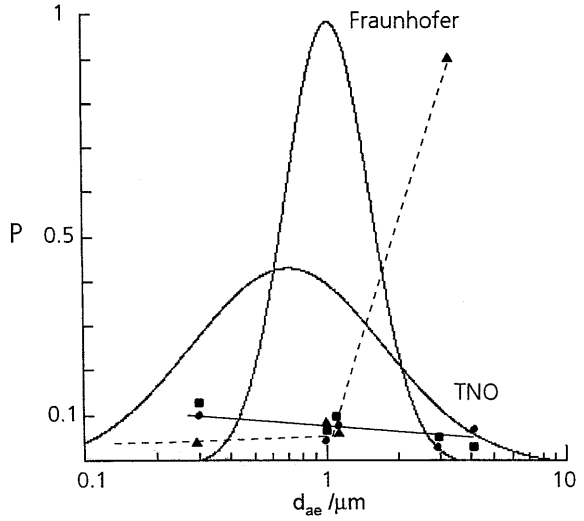


Fig. 2 Mass size distribution of the two test aerosols and regional deposition probability (*rectangles* pulmonary deposition, *circles* bronchial deposition, *triangles* nasal deposition; d_{ae} aerodynamic diameter; *P* deposition probability)

over almost the entire size range covered by the two mass distributions.This means that in both cases the percentage of inhaled aerosol deposited in the bronchial and pulmonary region is approximately the same for both distributions, i.e. ca. 10% each. The nasal deposition curve shows a steep increase from 7 to 85% in the size range between 1 and 3 µm. The percentage of aerosol deposited in the nasal region is given by the ratio of the areas determined by the product of the dashed line and the distribution curves to the total area under the size distribution curves. The resultsof the calculations are ca. 17 and ca. 20% nasal deposition of the inhaled aerosol for the Fraunhofer and TNO study, respectively.

Chemical analysis (HPLC) of the test atmospheres carried out in both studies revealed that at the lowest exposure concentration a certain percentage of monomeric MDI was present as vapour. Although this may have some effect on the reaction rate, it is not expected that this will result in a different molar ratio. In the two studies, different exposure concentrations of thetest substance were used (Table 1). Because of the low vapour pressure a certain amount of the aerosol is expected to evaporate until equilibrium between droplet and vapour phase is achieved. The vapour pressure (total monomeric) of monomeric and polymeric MDI at 30°C have been reported to be 2×10^{-5} and 1×10^{-5} mmHg, respectively. Extrapolation to 25°C gives a saturation concentration of approximately $\rho_s = 100$ and $\rho_s = 50$ µg/m^3 for monomeric and polymeric MDI, respectively. For polymeric MDI, Raoult's law has been used to calculate the vapour pressure from measured data for monomeric MDI (taken from IUCLID database 1994). The time to reach the saturated state depends on the mass concentration, c_m, and the average particle size, d_{ave}, of the aerosol. Starting from the pure aerosol, the saturation ratio, $S = \rho/\rho_s$, of the gaseous phase, i.e. the ratio between the actual vapour concentration, ρ, and the saturation concentration, ρ_s, is determined by the following kinetic equation:

$$\frac{dS}{dt} = -\frac{1}{\tau}(S-1),\ S(t=0) = 0 \tag{1}$$

where the characteristic time, τ is given by (Hinds 1982):

$$\tau = \frac{\rho_p d_{ave}^2}{12\ c_m D_v} \tag{2}$$

Here, ρ_p, is the material density of the aerosol particles (2.5 g/cm^3), and D_v is the diffusion coefficient of the vapour molecules in air (of the order of 0.1 cm^2/s). Using an average particle diameter of $d_{ave} = 1$ µm and a mass concentration of $c_m = 0.2$ mg/m^3 (which corresponds to the lowest exposure concentration in the studies) one obtains $\tau = 0.6$ s. Relative to the residence time of the exposure atmosphere in the inhalation chambers (several minutes) the equilibrium between gas phase and particulate phase is achieved almost instantaneously. The gas phase might eventually react with the humidity in the atmosphere and, thus would not contribute to the overall dose.

Interrelation of doses

Using the physicochemical data of the test atmospheres and the deposition pattern, dose calculations were performed. The results of the calculations are shown in Table 2. The total exposure time is the total time the animals were exposed to the test atmospheres. The differences between the Fraunhofer and the TNO study result from different daily exposure durations, viz. 6 hin the TNO study and 18 h in the Fraunhofer study. This is partly compensated for by the use of different exposure concentrations. The high concentration in the TNO study (6.03 mg/m^3) was about 3 times the high concentration in the Fraunhofer study (2.05 mg/m^3). The inhaled dose was calculated from the mass concentration, the minute volume, MW and the exposure time to:

$$D_{inhaled} = \int dt\ c_m(t) MV(t) \tag{3}$$

The minute volume given in units of ml/min is the amount of air exchanged per minute by the respiratory system. It depends on the animal species and the body weight, g(t). For laboratory animals, two correlations between body weight and minute volume are given in the literature (Guyton 1947; Stahl 1967):

$$MV\ (t) = 379\left(\frac{g(t)}{1000}\right)^{0.8},\ MV\ (t) = 2.1\ g(t)^{0.75} \tag{4}$$

Since the exposure concentration is constant, the inhaled dose is given by the average minute volume times the exposure concentration. The average minute volume was calculated, using the above formulas and a polynomial fit of the experimentally determined body weight

Table 2 Dose calculations

Dose level	TNO study			Fraunhofer study		
	Low	Mid	High	Low	Mid	High
Exposure time, t (h)	2940			8573		
Average minute volume, MV (ml/min)	139			202		
Deposition probability, w_{dep} (pulmonary = bronchial) (%)	10			10		
Average body weight, BW (kg)	0.281			0.449		
Average lung weight, LW (g)	1.89	1.89	2.17	1.42	1.70	1.99
Average lung weight of controls (g)	1.85	1.85	1.85	1.37	1.37	1.37
Exposure concentration, c (mg/m^3)	0.19	0.98	6.03	0.23	0.70	2.05
Cumulative dose administered (mgh/m^3)	559	2881	17,728	1972	6001	17,575
Inhaled dose (total), ct MV (mg)	4.7	24.0	147.9	23.9	72.7	213.0
Inhaled dose (aerosol), $D_{aerosol}$ = (c-cd) t MV (mg)	3.4	22.8	146.6	13.5	62.3	202.6
Retained dose, $D_{ret} = c_{aerosol}\, w_{dep}$ t MV (mg)	0.34	2.28	14.66	1.35	6.23	20.26
Retained dose per body weight (mg/kg)	1.22	8.12	52.18	3.01	13.88	45.13
Retained dose per lung weight (mg/kg)	0.18	1.21	6.76	0.95	3.67	10.18
Retained dose of NCO per lung weight (μmol/g)	0.51	3.38	18.92	3.52	13.57	37.67
Retained dose per control lung weight (mg/g)	0.19	1.23	7.93	0.99	4.55	14.79
Retained dose of NCO per control lung weight (μmol/g)	0.52	3.45	22.19	3.65	16.84	54.72

curves (Fig. 3). From the inhaled dose of total material, the inhaled dose of MDI aerosol was calculated by subtracting the saturation vapour concentration from the total concentration:

$$D_{\text{inhaled}}^{\text{aerosol}} = (c_m - \rho_s)\, MV\, t \tag{5}$$

For the vapour saturation concentration, ρ_s, values of 100 and 50 $\mu g/m^3$ were chosen for the Fraunhofer and the TNO study, respectively (see also the previous section). The calculated quantity $c_m - \rho_s$ corresponds approximately to the concentration determined by HPLC analysis of filter samples. From the inhaled dose, the retained dose was calculated by multiplication with the deposition probability. For the bronchial and pulmonary regions, a constant value of $W_{dep} = 10\%$ was used:

$$D_{\text{retained}}^{\text{aerosol}} = D_{\text{inhaled}}^{\text{aerosol}}\, W_{\text{dep}} \tag{6}$$

Different ways to normalize the dose are presented in Table 2: normalization with respect to lung weight and normalization to the average body weight as well as normalization to thelung weight of the control animals. The lung weight for every dose group was measured after 12 and 20/24 months of exposure. The average of these two values was used as the lung weight in Table 2. For the interrelation of the histopathology, the concentration-time product (mgh/m^3) was used as basis (see Table 1).

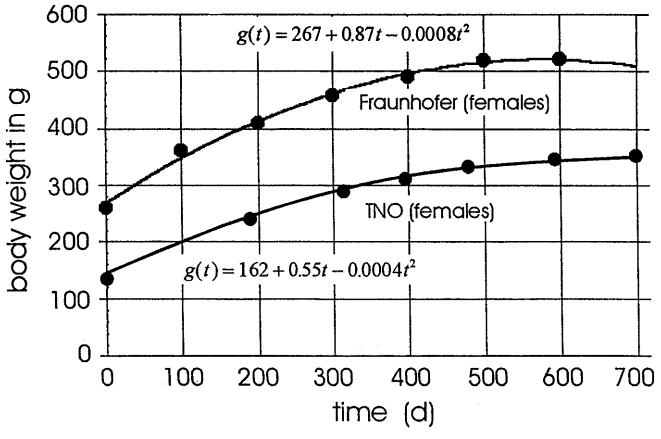


Fig. 3 Development of the body weights in the course of the inhalation studies

Interrelation of body weight, mortality and lung weight

Body weights were unaffected by exposure to MDI in the TNO study. In the Fraunhofer study, a slight, though statistically significant reduction in body weight was found in the mid-and high-concentration groups from 4.5 months onward, and in all exposed groups from 17 months onwards (6.7, 7.9 and 11.3% in the low-, mid- and high-exposure group, respectively). The authors considered the lower initial average body weights in the mid- (2.4%) and high-exposure (2.2%) group partially responsible for these differences. Moreover, in satellite groups killed after 12 months, no such dose-related differences in body weight were observed, suggesting the changes in body weight found in the main groups were not related to MDI exposure.

While in the TNO study mortality at 2 years amounted to 30, 28, 20 and 16% for the control, low-, mid- and high-concentration groups, respectively, mortality in the Fraunhofer study was unusually high in all groups (94–97%), including the control group. The low survival of the rats in the Fraunhofer study was related to an unusually high frequency of spontaneous tumours of the pituitary and mammary glands. On average, the rats in the TNO study survived 169 days longer than those in the Fraunhofer study (Table 3).

In both studies, lung weights were clearly increased in the top-dose group (Table 4). In theFraunhofer study, but not in the TNO study, lung weights were also rela-

tively greater in the mid-dose group. This finding is not unexpected because the mid-dose animals in the Fraunhofer study received a cumulative MDI dose (6001 mgh/m^3) more than twice as high as that administered to the mid-dose animals in the TNO study (2881 mgh/m^3), indicating a positive dose-effect relationship for this parameter. Indeed this finding suggests that the effect of MDI on the lung weight is primarily dose- rather than concentration-dependent because the exposure concentration for the mid-dose group was on average 40% higher in the TNO study (0.98 mg/m^3) than in the Fraunhofer study (0.70 mg/m^3).

Interrelation of lung histopathology

Nomenclature and grading of lung findings

The diagnostic terms used in the two studies were listed, mutually compared and designated as ''identical'' (term used in both studies for the same lesion), ''synonymous'' (two different terms used for the same finding in either study), and ''individual'' (term used for a finding only encountered in one of the two studies). Thereafter, the reviewing pathologist examined a large number of lung slides of both studies and discussed the interimresults with the study pathologists. Only a few terms appeared to be ''identical''. The majority of the terms were ''synonymous'', while four terms were ''individual''. The outcome was harmonization of the diagnostic terms (Table 5). Overall, this involved re-examination of about 5000 slides. The diagnostic criteria agreed upon were somewhat stricter than those originally used by the study pathologists, leading to somewhat higher incidences for certain findings in the present comparative investigation. Moreover, incidental hyperplasia of bronchial epithelium was only diagnosed during the review. Sub-classifications were introduced whenever necessary for a precise differentiation of findings (see also Table 5). The harmonized terminology of tumours and bronchiolo-alveolar hyperplasia, alveolar and bronchiolar type followed nomenclature developed by the World Health Organisation (IARC 1992) and the classification of the International Federationof Societies of Toxicologic Pathologists (Schwartz et al. 1994).

To ensure consistent grading, additional definitions of severity were developed for four different microscopic lung findings (Table 6). Thegrading was primarily determined by histologicalfeatures, completed by size criteria (e.g. bronchiolo-alveolar hyperplasia, alveolar type), or in the absence of suitable histological features, by size only (e.g. bronchiolo-alveolar hyperplasia, bronchiolar type).

Table 3 Average survival of female rats exposed to MDI

TNO study			Fraunhofer study			Differences in survival between the two studies (days)
Exposure concentration (mg/m^3)	Dose (mgh/m^3)	Survival (days)	Exposure concentration (mg/m^3)	Dose (mgh/m^3)	Survival (days)	
0	0	685	0	0	525	160
0.19	559	696	0.23	1972	552	144
0.98	2881	709	0.70	6001	520	189
6.03	17,728	700	2.05	17,575	518	182
Average		698	Average		529	169

Table 4 Mean absolute lung weights and lung-to-body weight ratios of female rats exposed to MDI[a]

Exposure concentration (mg/m^3)	Dose (mgh/m^3)	Exposure duration: 12 months[b]		Exposure duration: 20/24 months[c]	
		Absolute (g)	Relative (g/kg)	Absolute (g)	Relative (g/kg)
TNO study					
0	0	1.67 ± 0.07	5.66 ± 0.29	2.10 ± 0.06	6.14 ± 0.28
0.19	559	1.66 ± 0.05	5.63 ± 0.15	2.09 ± 0.04	5.87 ± 0.09
0.98	2881	1.69 ± 0.06	5.61 ± 0.21	2.06 ± 0.04	5.68 ± 0.11
6.03	17,728	1.93 ± 0.09*	6.51 ± 0.22*	2.41 ± 0.05**	6.68 ± 0.17
Fraunhofer study					
0	0	1.35 ± 0.11	2.91 ± 0.38	1.39 ± 0.10	3.45 ± 0.90
0.23	1972	1.47 ± 0.13	2.77 ± 0.42	1.36 ± 0.09	3.07 ± 0.24
0.70	6001	1.58 ± 0.10*	3.34 ± 0.35	1.80 ± 0.18	3.96 ± 0.70
2.05	17,575	1.80 ± 0.24***	3.64 ± 0.58**	2.18 ± 0.45**	5.86 ± 1.08**

[a] Values marked with asterisks differ significantly (Dunnett's test) from those of the corresponding control group (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

[b] Lung weights obtained from rats in satellite groups killed after an exposure period of 12 months. Number of lungs weighed per group was 10 in the TNO study and 9–10 in the Fraunhofer study

[c] Number of lungs weighed per group varied from 41 to 50 in the TNO study and from 2 to 8 in the Fraunhofer study. The lung weights were obtained from rats killed after an exposure period of 20 months (Fraunhofer study) or 24 months (TNO study)

Table 5 Harmonized terms[a] and corresponding terms used in the TNO and Fraunhofer studies

Harmonized terms	Terms used in TNO study[b]	Terms used in Fraunhofer study[c]
Adenoma, bronchiolo-alveolar	Adenoma	Bronchiolo-alveolar adenoma
Hyperplasia, bronchiolo-alveolar	Localized alveolar bronchiolization/ alveolar duct epithelialization	Bronchiolar/alveolar hyperplasia
Alveolar type	–[d]	Alveolar cell hyperplasia
Bronchiolar type	–	Alveolar bronchiolization
Mixed type	–	–
Flat type	–	–
Hyperplasia, bronchial epithelium[e]	–	–
Fibrosis	Localized fibrosis	Interstitial fibrosis
Interstitial	–	–
Scar/malformation	–	–
Particle-laden macrophages	Accumulation of macrophages with yellow pigment	Accumulation of particle-laden macrophages
Alveolar	–	–
Interstitial	–	–
Macrophage accumulation	Accumulation of alveolar macrophages	Alveolar histiocytosis
Osseous metaplasia	–	Osseous metaplasia
Calcified deposits	Mineralized deposits in the bronchial and alveolar region	–
Granuloma, cholesterol	Granulomata	Cholesterol granuloma
Granuloma, focal	Granulomata	–
Granuloma, foreign-body	Granulomata	Foreign-body granuloma
Giants cells, multinucleated	–	Giant cells, multinucleated
Haemosiderin storage	Interstitial brown pigment accumulation	Brown-pigmented macrophages
Alveolar	Heart failure cells	–
Interstitial	–	–
Mononuclear cell infiltration	Lymphoid aggregates	Interstitial mononuclear cell infiltration
Perivascular/peribronchiolar	Interstitial pneumonitis	–
Interstitial	–	–
Haemorrhage	Haemorrhage	Haemorrhage
Aspiration pneumonia	–	Aspiration pneumonia
Abscess	Abscess	–
Pleural fibrosis	–	Pleural fibrosis
Inflammatory cells	Perivascular polymorphonuclear leukocytic infiltration/purulent pneumonia	Inflammatory cell infiltration
Congestion	Hyperaemia	–
Alveolar oedema	Alveolar oedema	Oedema
Pleuritis	–	Pleurisy
Lymphoma/histiocytic sarcoma	Lymphoma/histiocytic sarcoma	Lymphoma/histiocytic sarcoma
Metastasis	Metastasis	Metastasis

[a] Terms agreed upon by reviewing and study pathologists
[b] Terms used by Reuzel et al. (1994a) and/or by Reuzel et al. (1990)
[c] Terms used by Hoymann et al. (1995)
[d] Term not used/lesion not present
[e] Diagnosis introduced in the present study, no corresponding diagnosis in the original studies

Definitions of the major type of lung lesions

Bronchiolo-alveolar adenomas were defined as lesions in which the alveolar architecture was not preserved, and which contained glandular structures, papillary projections and/or solid sheets of epithelial cells (Fig. 4). The stromal component was slightly more conspicuous than that in hyperplasia.

Bronchiolo-alveolar hyperplasia was subdivided into four types: alveolar, bronchiolar, mixed and flat type (Figs. 5, 6, 7, 8). When 70% or more of the cells of the lesion were either cuboidal and ciliated or flattened to cuboidal and unciliated, the diagnosis was bronchiolar or alveolar type, respectively. The term mixed type was used when a lesion was not predominantly of bronchiolar or alveolar type. The term flat type was introduced for a lesion that appeared to have a different quality: as the other types, it was composed of an increased number of epithelial cells, but they were flattened and elongated, indicative of a differentiation towards a type I pneumocyte phenotype. Grades 4 and 5 alveolar type hyperplasia were interpreted as preneoplastic changes.

Interstitial fibrosis (Fig. 9) was seen to occur first in the interstitium of the alveolar ducts, extending into the septa and peribronchiolar tissue in more advanced cases. Correlating with these findings, in the Fraunhofer study lung function measurements revealed a dose-dependent obstructive-restrictive malfunctionwith diffusion disorder, beginning earlier than 6 months of exposure and increasing at 12 months, but not much more at 17 months (Hoymann et al. 1998).

Particle-laden macrophages (Figs. 10, 11) were observed predominantly in the alveoli close to alveolar ducts or subpleurally in mild cases, but with increasing

Table 6 Definitions of gradings for a selection of microscopic findings

Grade	Description
Hyperplasia, bronchiolo-alveolar, alveolar type	
1	Minimal; flat epithelium; hardly discernible Very small; few up to approximately 15 alveoli
2	Slight; low cellularity Small; 1/4 to 1/2 the size of 100-fold magnification field
3	Moderate; higher cellularity; piling up of cells may be present Medium size; 1/2 to full size of 100-fold magnification field
4	Severe; more often cuboidal; some irregularities in the epithelium Large; full size of 100-fold magnification field
5	Very severe Very large; difficult to differentiate from tumour (borderline cases)
Hyperplasia, bronchiolo-alveolar, bronchiolar type	
1	Minimal; very small; (parts of the) alveolar duct or up to 5 alveoli
2	Slight; small; (parts of the) alveolar duct or up 6–10 alveoli
3	Moderate; medium size; alveolar duct and 11–20 alveoli
4	Severe (not detected in the study – not defined)
5	Massive (not detected in the study – not defined)
Particle-laden macrophages, alveolar/interstitial	
1	Minimal; number of macrophages not obviously elevated; content of test material generally low
2	Slight; number of macrophages slightly elevated; content of test material easily visible
3	Moderate; number of macrophages obviously elevated; macrophages filled with test material
4	Severe; high number of macrophages filled with test material
5	Very severe; massive accumulation of macrophages filled with test material
Fibrosis, interstitial	
1	Very slight; very few alveolar septa thickened (multi)focal Limited to few alveolar structures, e.g. singular ridges in alveolar duct region
2	Slight; some septa slightly thickened Multifocal to diffuse lesion often found in more than one lobe
3	Moderate; mostly diffuse or remarkable thickening of septa Several to many septa involved
4	Severe; diffuse or septa severely thickened Large areas involved
5	Massive diffuse collagen deposits throughout the lung (not detected in the study)

dose, particle-laden macrophages were also observed in fibrotic areas. In the TNO study, aggregate formation was frequently seen,and, although sometimes heavily laden with truly yellow particles, the macrophages in the alveolar space mostly kept their shape and integrity (Fig. 10). In the Fraunhofer study, the macrophages were large and foamy, especially in the top-dose animals (Fig. 11). In contrast to those in the TNO study, the macrophages in the Fraunhofer study contained strikingly low numbers of particles, which were white to light yellow and not immediately apparent. Some of the macrophages showed signs of degeneration such as foamy vacuolation or disintegration of the cell membrane, and cell fusion was apparent. It is likely that this process is responsible for the increased occurrence of multinucleated giant cells in the top-dose group (17,575 mgh/m^3).

Foci of macrophages without recognizable particles were referred to as macrophage accumulations, which were seen both in control and MDI exposed animals in both studies. Compared to the macrophages in rats from the TNO study, those in rats from the Fraunhofer study were relatively large and had a foamy appearance with frequent signs of degeneration.

In the TNO study, mineralized spherical structures were found in the alveolar and bronchiolar lumen, which were referred to as mineralized deposits. The Perl's Prussian blue stain originally performed for the detection of haemosiderin, revealed that these calcified deposits partly reacted positively for iron. In the Fraunhofer study, focal mineralization in the alveolar and bronchiolarlumen occurred in two different forms: osseous metaplasia and calcified deposits.

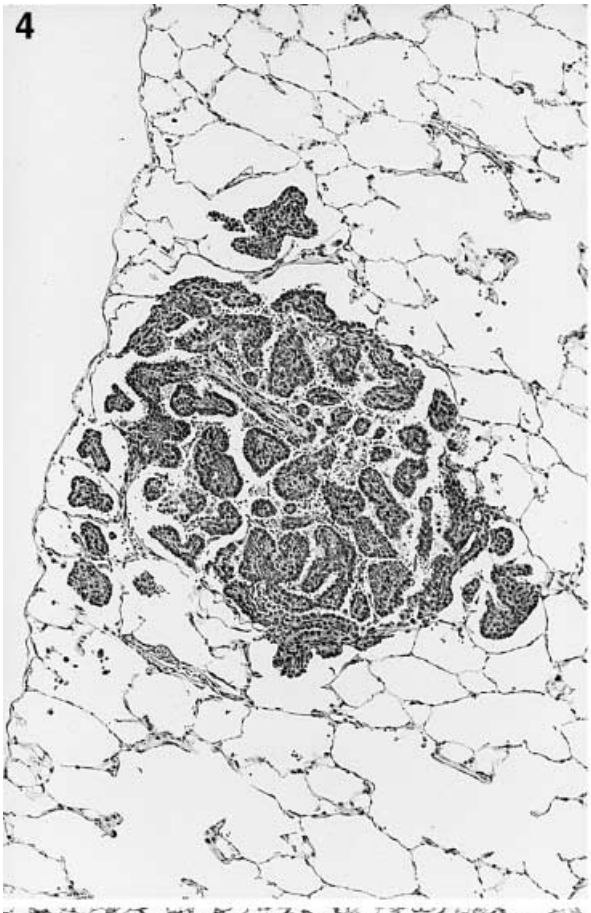
Haemosiderin was stored in alveolar macrophages and in the interstitium. In the top-dose group of the TNO study, it was not possible in haematoxylin-eosin (HE) stained slides to clearly distinguish between haemosiderin and yellow-brown particles presumably consisting of MDI-derived material. However, in the Fraunhofer study, haemosiderin could easily be distinguished from the light yellow, presumably MDI-related particles.

Aggregates of monocytes, lymphocytes and plasma cells were recorded under the term mononuclear cell infiltration.

Incidence and severity of microscopic lung findings

Results reported in this section are summarized in Table 7.

Three bronchiolo-alveolar adenomas were found, two in top-dose animals of the TNO study and one in a top-dose animal of the Fraunhofer study. Increased incidences of grade 4 and 5 alveolartype bronchiolo-alveolar hyperplasia (regarded as a preneoplastic change) were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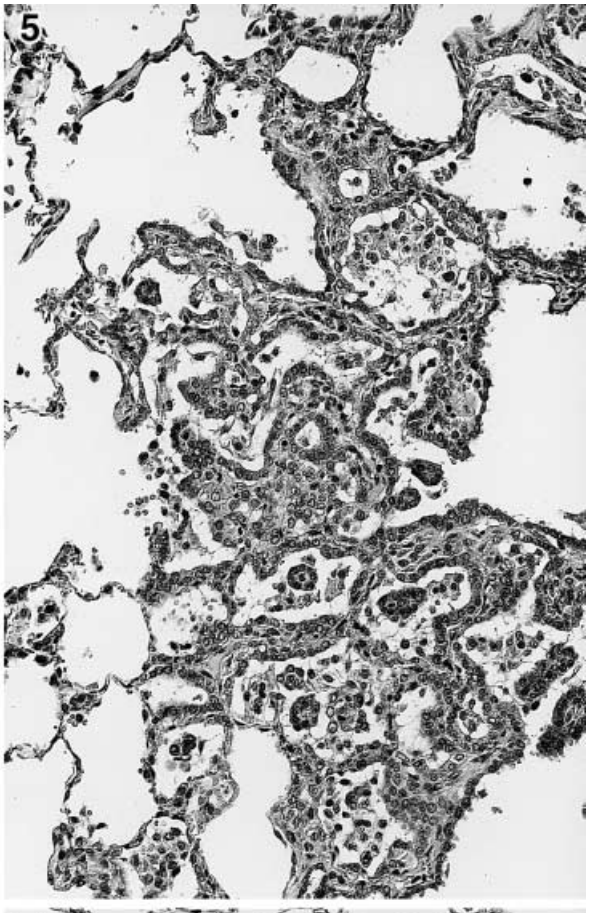
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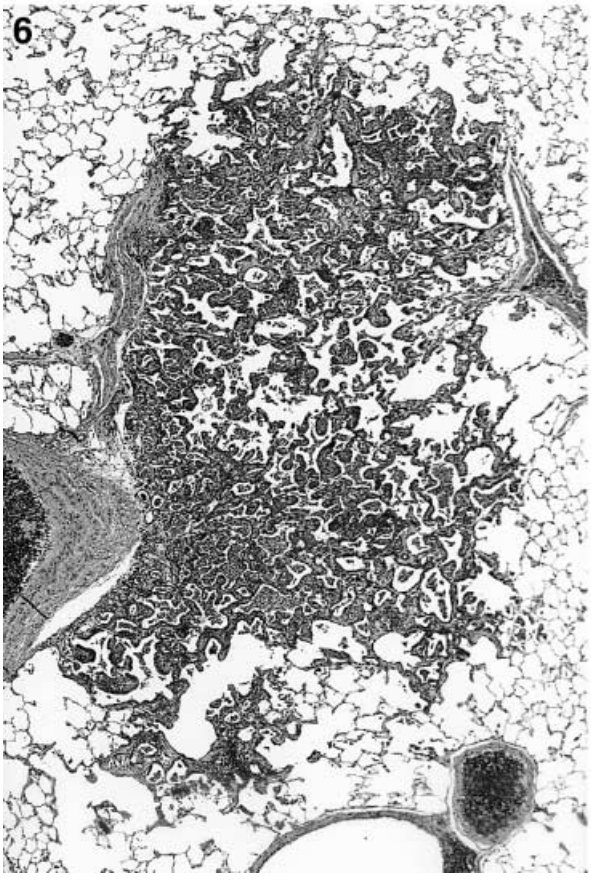
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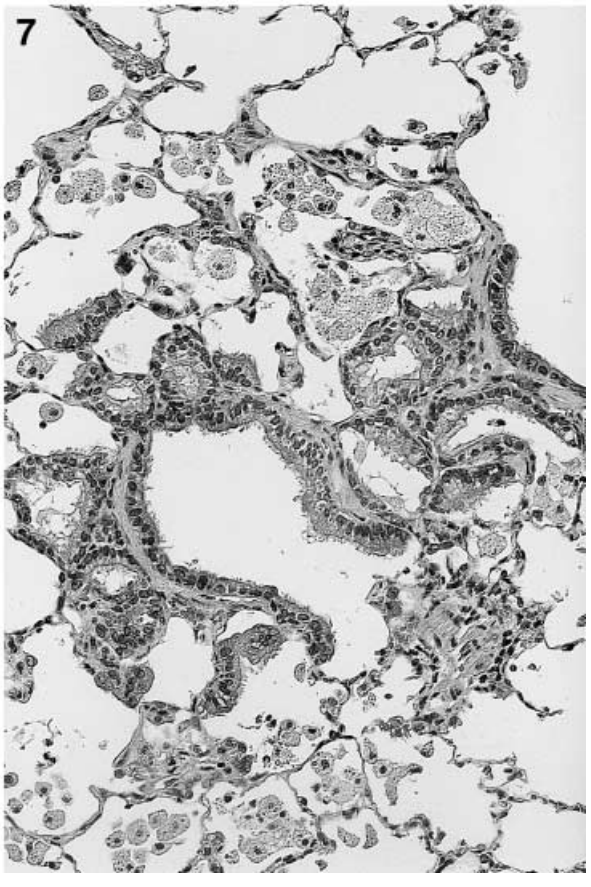
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Fig. 4 Bronchiolo-alveolar adenoma. Female rat from the high-dose group (17,575 mgh/m^3) of the Fraunhofer study, killed on day 505 (HE, ×104)

Fig. 5 Bronchiolo-alveolar hyperplasia, alveolar type, grade 3. Female rat from the high-dose group (17,575 mgh/m^3) of the Fraunhofer study, killed on day 352 (HE, ×208)

Fig. 6 Bronchiolo-alveolar hyperplasia, alveolar type, grade 5. Female rat from the high-dose group (17,575 mgh/m^3) of the Fraunhofer study, killed on day 671 (HE, × 42)

Fig. 7 Bronchiolo-alveolar hyperplasia, type, grade 3. Female rat from the high-dose group (17,575 mgh/m^3) of the Fraunhofer study, killed on day 545 (HE, × 208)

seen in the top-dose group of both studies and in the mid-dose group of the Fraunhofer study. Incidence and/or severity of lower grade (grades 1, 2 and 3) alveolar type bronchiolo-alveolar hyperplasia were increased in the top-dose group of the TNO study and in all dose-groups of the Fraunhofer study. Bronchiolar type bronchiolo-alveolar hyperplasia, predominantly of minimal or slight degree (grades 1 and 2), was increased in incidence and/or severity in the mid- and top-dose groups of the TNO study and in all dose groups of the Fraunhofer study. Mixed and flat type bronchiolo-alveolar hyperplasia were seen in low incidences in several groups with no obvious relationship with MDI treatment.

Interstitial fibrosis was increased in incidence (Fig. 12) and degree in both the mid- and top-dose group of the TNO study and in all dose groups of the Fraunhofer study, grade 3 lesions occurring even in the low-dose group of the Fraunhofer study.

Particle-laden macrophages were observed in all exposed groups of both studies, but incidence and extensiveness (including aggregate formation) were greater in mid- and top-dose groups of the TNO study than in these dose groups of the Fraunhofer study. In the TNO study, the macrophages were heavily laden with clear yellow particles, whereas in the Fraunhofer study the macrophages contained smaller numbers of yellowish-white particles that were not immediately apparent. Moreover, macrophage degeneration and disintegration were somewhat more conspicuous in rats from the Fraunhofer study than in rats from the TNO study, but this difference was also noticeable between ordinary alveolar macrophages from control rats of the TNO andFraunhofer study.

Accumulations of macrophages not containing particles were seen in control as well as in treated animals, the incidences in several of the exposed groups being clearly lower than those in the control groups.

Haemosiderin storage, mainly occurring in the interstitium, was increased in a dose-related manner in all treatment groups of the Fraunhofer study. In the TNO study, no increase in haemosiderin storage was observed.

Both studies showed a tendency towards increased focal mineralization in alveolar and bronchiolar lumen (calcified deposits and osseous metaplasia). However, whereas the incidence of calcified deposits was significantly increased in the top-dose group of both studies, a significantly increased incidence in osseous metaplasia occurred only in the top-dose group of the Fraunhofer study. On the other hand, in the top-dose rats from the TNO study, the calcified deposits were larger and occurred more frequently than in the rats from the Fraunhofer study.

In the top-dose group of the Fraunhofer study, the incidences of cholesterol granuloma and giant cells were distinctly increased.

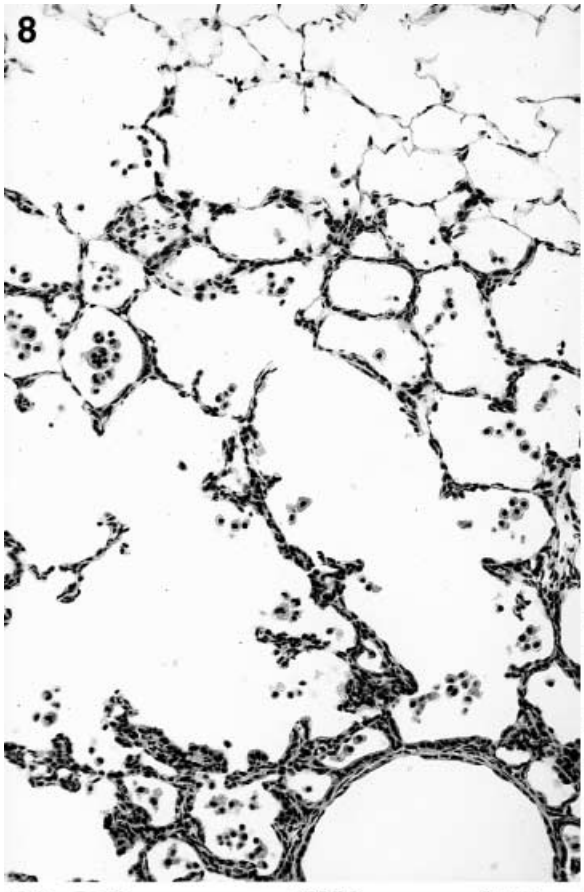
Mononuclear cell infiltration was clearly increased in incidence in the top-dose group of the TNO study, the increase being predominantly due to an increase in interstitial cell infiltration. In the Fraunhofer study, mononuclear cell infiltration was increased in incidence in all dose groups and was found to about the same incidence and degree in the perivascular/peribronchiolar area and in the interstitium.

The other findings listed in Table 7 did not show significant differences between groups or were considered incidental observations unrelated to MDI exposure.

Discussion

Looking at the overall design of these two long-term studies, a comparative evaluation seems to be rather difficult and there are indeed differences which may have had some influence on the results; e.g. exposure regimen and corresponding recovery periods, or chemical species (e.g. oligomers) presentin one test material but not the other. However, a detailed evaluation of the histopathological findings revealed considerable similarities in biological response. With respect to their chemistry and biochemistry both test materials can be expected to show a very similar reaction pattern and reaction rate in the biological environment. If inhalation parameters are compared and normalized, it is evident that both studies can be interrelated. Overall, with respect to inhalation parameters and the test materials used, there appeared to be no serious inconsistencies that might invalidate the subsequent comparison of the pathological findings.

Major effects of inhaled MDI on the lung included increased lung weights, bronchiolo-alveolar adenomas and hyperplasia, and interstitial fibrosis. These effects were seen consistently in both studies (see Table 8 for summary), indicating a similar qualitative response of the lung of female Wistar rats to polymeric and monomeric MDI. Quantitatively, lung responses were clearly dose-related in each study, and a reasonable overall dose-response relationship was apparent for the majority of the major lung lesions when the two studies were reviewed as a whole (Table 7). This is remarkable considering the differences in experimental conditions between the two studies, such as physicochemical status of the test material, exposure concentrations, daily exposure duration and source and lifespan of the rats. Some differences were apparent, however, and these will be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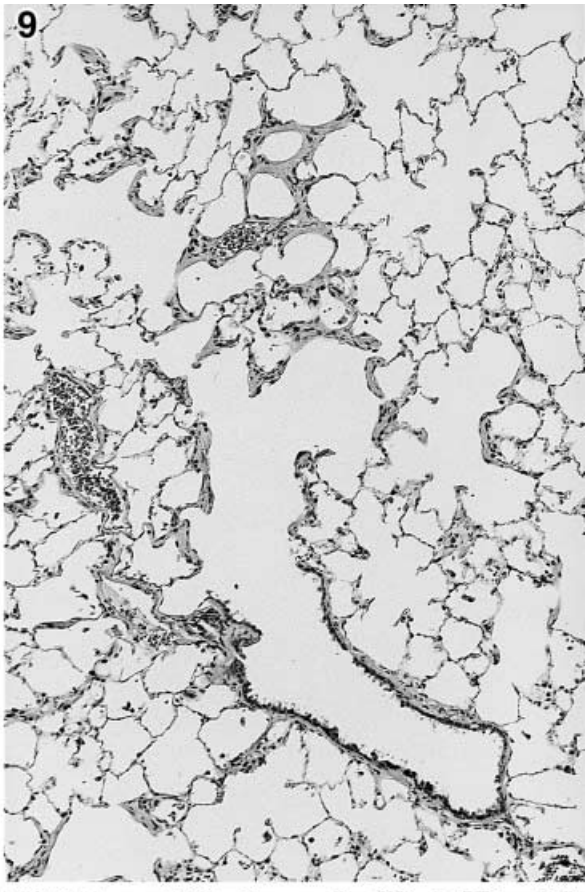
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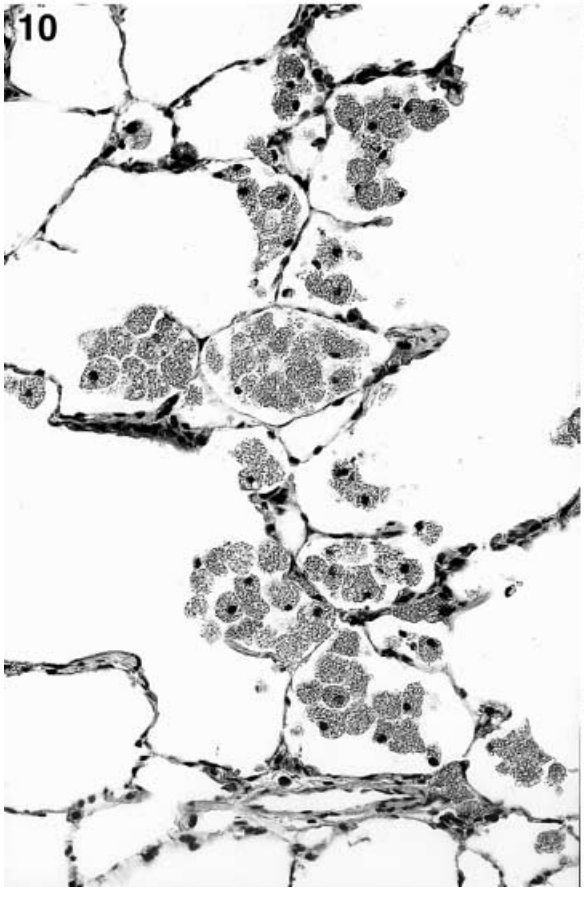
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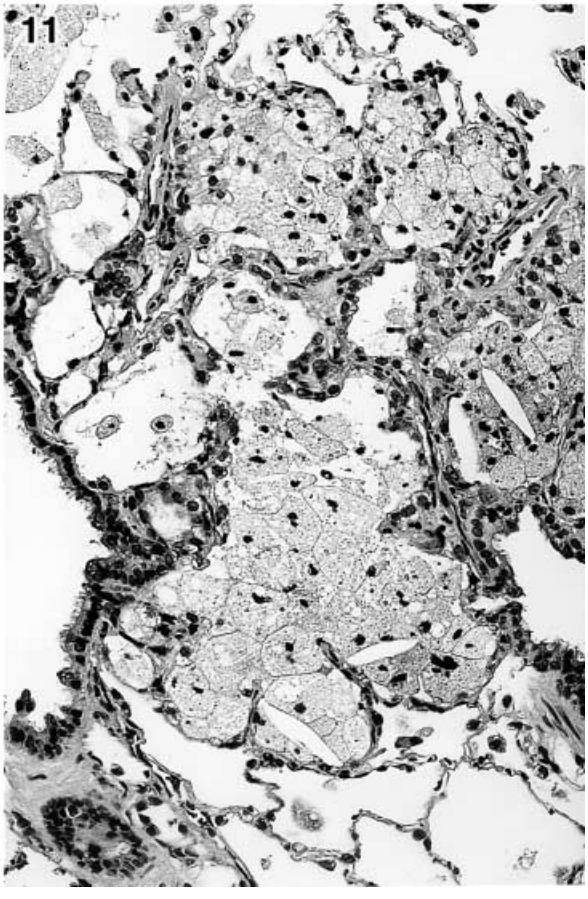
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Fig. 8 Bronchiolo-alveolar hyperplasia, flat type. Female rat from the mid-dose group (2881 mgh/m^3) of the TNO study, killed at the end of the study (HE, ×166)

Fig. 9 Interstitial fibrosis, grade 3. Female rat from the high-dose group (17,575 mgh/m^3) of the Fraunhofer study, killed on day 563 (He, × 104)

Fig. 10 Alveolar macrophages heavily laden with (truly yellow) particles. Note the normal shape and integrity of most of the macrophages. Female rat from the high-dose group 17,728 mgh/m^3 of the TNO study, killed at the end of the study (HE, × 260)

Fig. 11 Large foamy alveolar macrophages containing small numbers of (light yellow) particles. Note the vacuolation, disintegration of cell membranes and fusion of the macrophages. Female rat from the high-dose group (17,575 mgh/m^3) of the Fraunhofer study, killed on the day 650 (HE, × 266)

discussed systematically, on a lesion-by-lesion basis, in the following paragraphs. Possible explanations for these differences will also be presented. The incidences of bronchiolo-alveolar adenomas and preneoplastic lesions (bronchiolo-alveolar hyperplasia, alveolar type degrees 4 and 5) were slightly higher in the top-dose group of the TNO study (adenoma incidence: 3%; increase in incidence of preneoplasia: 11%) relative to the top-dose group ofthe Fraunhofer study (adenoma incidence: 1%; increase in incidence of preneoplasia: 9%), as was the incidence and/or degree of bronchiolo-alveolar hyperplasia, bronchiolar type (relative incidences of 100% and 53% for the TNO and Fraunhofer study, respectively). These differences intarget tissue response occurred despite similarities in dose (17,728 and 17,575 mgh/m^3 for the top-dose group of the TNO and Fraunhofer study, respectively). The higher incidence and greater extent of the proliferative epithelial changes in the rats from the top-dose group of the TNO study was most probably due to their better survival (700 days versus 518 days for the rats from the Fraunhofer study), giving more time for development of these chronic epithelial reactions. The low survival rate of the rats in the Fraunhofer study is probably due to a genetic drift of the Wistar rat strain used. Furthermore, the 3-fold greater exposure concentration in the TNO study (6.03 mg/m^3 versus 2.05 mg/m^3 for the high-dose animals from the Fraunhofer study) most probably resulted in a higher local dose and tissue deposition during actual exposure, again potentially leading to a stronger response of the bronchiolo-alveolar epithelium in top-dose animals of the TNO study. It may be emphasised that in both studies lung tumours were only seen in the top dose group.

The induction of bronchiolo-alveolar hyperplasia in the mid- and low-dose groups also differed between the two studies. Thus in the Fraunhofer study, the incidence and degree of both types of bronchiolo-alveolar hyperplasia were clearly and dose-relatedly greater for the low- and mid-dose groups (incidences: alveolar type 14 and 16%, bronchiolar type 10 and 25%, respectively) relative to the controls (incidence: 3% for both the alveolar and bronchiolar type). In contrast, the incidence and degree of bronchiolar type hyperplasia was only increased in the mid-dose group of the TNO study (incidence: 20% versus 0% in the control group) with no significant response in the low-dose group (incidence: 2%).

A qualitative comparison of the hyperplastic response of the bronchiolo-alveolar epithelium for the mid-concentration group showed differences between the studies. Rats from the Fraunhofer study exhibited a clearly increased incidence and degree of both types of bronchiolo-alveolar hyperplasia, including an increase in preneoplastic lesions (increased 13 and 23% for the alveolar and bronchiolar type, respectively), whereas in the mid-concentration rats from the TNO study only the bronchiolar type showed an increase in incidence and degree (increased incidence: 20%). It is possible, however, that the high background incidence of alveolar type bronchiolo-alveolar hyperplasia in the rats from the TNO study (12% in the concurrent control group) might have obscured any treatment-related increase in mid-concentration animals. A quantitative comparison revealed there was a similar hyperplastic response in bronchiolo-alveolar epithelium in both mid-concentration groups (increase inincidence 23 and 24% in the TNO and Fraunhofer study, respectively), despite the animals from the TNO study receiving a lower total dose than their Fraunhofer counterparts (2881 mgh/m^3 versus 6001 mgh/m^3 for mid-concentration groups of the TNO and Fraunhofer study, respectively). It seems probable that this difference in total dose may have been offset by the slightly higher exposure concentration (0.98 mg/m^3 versus 0.70 mg/m^3) and the much longer survival (709 days versus 520 days) for the animals from the mid-dose group of the TNO study.

In the low dose-groups, only animals from the Fraunhofer study showed a hyperplastic response of the bronchiolo-alveolar epithelium. It seems likely that the slightly higher exposure concentration (0.23 and 0.19 mg/m^3 in the Fraunhofer and TNO study, respectively) and the more than 3.5-fold greater MDI dose (1972 mgh/m^3 versus 559 mgh/m^3) were responsible for the difference.

Incidence and/or degree of interstitial fibrosis were clearly higher in the Fraunhofer studythan in the TNO study. Mononuclear cell infiltration (indicative of an inflammatory response) also was markedly greater in the top-dose group of the Fraunhofer study than in the top-dose group of the TNO study. In this instance, the stronger fibrotic response was probably a response to the considerably longer daily exposure period for the rats in the Fraunhofer study which were exposed for 18 h each day versus a daily 6-h exposure in the TNO study. As a consequence the animals in the Fraunhofer study have experienced a much longer deposition of freshly inhaled MDI in the alveolar duct region, most likely leading to greater damage of the local tissue, with a subsequent shorter (overnight) recovery period (6 h recovery/non-exposure in the Fraunhofer study versus 18 h in the TNO study). Clearly, the more pronounced interstitial fibrosis in the Fraunhofer study cannot be related to longer survival or higher exposure concen-

Table 7 Type and incidences (%) of microscopic findings in the lungs of female rats exposed to MDI

	TNO study				Fraunhofer study			
Exposure concentration (mg/m^3)	0	0.19	0.98	6.03	0	0.23	0.7	2.05
Dose (mgh/m^3)	0	559	2881	17,728	0	1972	6001	17,575
No. of animals in selected group	60	60	60	60	80	80	80	80
No. of lungs examined	59	60	60	59	80	80	80	80
Adenoma, bronchiolo-alveolar	0	0	0	3	0	0	0	1
Hyperplasia, brochiolo-alveolar	19	17	42	100	10	20	34	66
Alveolar type	12	8	13	51	3	14	16	36
Grade 1	5	0	3	8	1	10	5	15
Grade 2	2	7	3	14	1	3	6	4
Grade 3	3	2	5	15	0	1	3	9
Grade 4	2	0	2	10	0	0	1	8
Grade 5	0	0	0	3	0	0	1	1
Bronchiolar type	0	2	20	100	3	10	25	53
Grade 1	0	0	15	29	3	8	23	36
Grade 2	0	2	5	63	0	3	3	13
Grade 3	0	0	0	8	0	0	0	4
Mixed type	0	0	2	2	0	0	1	0
Flat type	7	5	13	7	5	0	0	3
Hyperplasia, bronchial epithelium	2	0	2	0	3	0	1	0
Fibrosis	3	3	32	100	16	79	96	99
Interstitial	3	3	32	100	13	79	96	99
Grade 1	3	3	32	12	9	45	33	3
Grade 2	0	0	0	75	4	31	51	36
Grade 3	0	0	0	12	0	3	11	43
Grade 4	0	0	0	2	0	0	1	18
Scar/malformation	0	0	0	2	3	0	1	0
Particle-laden macrophages	0	52	93	100	0	66	88	100
Alveolar	0	52	92	100	0	63	86	99
Grade 1	0	45	58	2	0	59	64	41
Grade 2	0	7	33	12	0	4	21	49
Grade 3	0	0	0	78	0	0	1	9
Grade 4	0	0	0	8	0	0	0	0
Interstitial	0	0	35	90	0	6	19	73
Grade 1	0	0	28	29	0	6	16	51
Grade 2	0	0	7	51	0	0	3	19
Grade 3	0	0	0	10	0	0	0	3
Macrophage accumulation	31	25	2	3	34	18	20	33
Grade 1	14	8	0	2	23	10	8	15
Grade 2	12	13	2	0	11	8	10	11
Grade 3	5	2	0	2	0	0	3	6
Grade 4	0	2	0	0	0	0	0	0
Osseous metaplasia	2	5	3	3	6	9	9	19
Calcified deposits	8	2	12	64	0	5	1	16
Grade 1	8	2	12	29	0	5	1	13
Grade 2	0	0	0	20	0	0	0	4
Grade 3	0	0	0	15	0	0	0	0
Granuloma, focal	2	2	0	0	0	1	0	4
Granuloma, cholesterol	8	5	8	0	1	4	6	14
Granuloma, foreign body	2	3	5	2	0	0	0	1
Giant cells, multinucleated	2	2	3	0	0	0	3	21
Grade 1	2	2	2	0	0	0	3	15
Grade 2	0	0	2	0	0	0	0	4
Grade 3	0	0	0	0	0	0	0	3
Haemosiderin storage	47	47	38	10	13	29	36	56
Alveolar (macrophages)	5	5	5	2	4	14	20	40
Grade 1	2	2	2	0	1	4	8	16
Grade 2	2	2	3	2	3	3	8	16
Grade 3	0	0	0	0	0	4	4	8
Grade 4	2	2	0	0	0	4	1	0
Interstitial	47	45	35	8	11	28	26	36
Grade 1	34	40	32	5	9	18	16	24
Grade 2	12	3	2	2	3	10	8	13
Grade 3	0	0	2	2	0	0	3	0
Grade 4	2	2	0	0	0	0	0	0
Mononuclear cell infiltration	46	55	52	88	30	45	63	88
Perivascular/peribronchiolar	42	38	33	61	18	21	30	56

Table 7 (Contd.)

	TNO study				Fraunhofer study			
Grade 1	17	27	25	17	11	18	19	24
Grade 2	24	8	8	42	6	4	10	33
Grade 3	2	3	0	2	0	0	1	0
Interstitial	14	32	32	71	16	31	43	60
Grade 1	8	27	23	29	11	20	34	46
Grade 2	5	5	8	37	5	11	9	13
Grade 3	0	0	0	5	0	0	0	1
Haemorrhage, focal	3	3	3	2	5	5	8	8
Aspiration pneumonia	2	3	2	0	5	1	1	0
Abscess	2	0	0	0	0	0	0	0
Pleural fibrosis	2	0	0	0	5	1	0	0
Inflammatory cells	0	7	2	3	1	0	0	3
Congestion	5	5	2	2	0	1	3	3
Alveolar edema	3	2	2	0	1	0	0	0
Pleuritis	2	0	0	0	3	0	0	0
Lymphoma/histiocytic sarcoma	3	2	0	0	1	3	3	1
Metastasis	3	0	2	0	1	1	1	1

tration since, as already noted, survival was longer and the exposure concentration was higher in the top-dose group of the TNO study.

While the explanations for the differences in proliferative epithelial and fibrotic responses between the two studies described in the preceding paragraphs are straightforward and plausible other factors should also be considered such as differences in toxicity between monomeric and polymeric MDI, or differences in sensitivity between the two sources of rats. These factors could account, for example, for the different appearances of the particle-laden macrophages from the two studies. Difference in sensitivity between the two sources of rats is a potentially important factor, given that the ordinary alveolar macrophages from untreated controls from the two studies also differed in appearance. Though not fully comparable, it may be relevant in this respect to refer to a recent paper on source related variations in incidence and severity of granulomatous pneumonia in Brown Norway rats (Germann et al. 1998). Moreover, McKenna et al. (1997) performed a short-term inhalation study with cadmium oxide fumes in mice and observed that the alveolar macrophages from C57Bl/6 mice responded with a faster and greater proliferation than did alveolar macrophages from DBA/2NCr mice. It is also well knownthat the microbiocidal activity of alveolar phagocytes varies widely among different strains of mice (Albright and Goldstein 1996). Other treatment-related lung changes (e.g. haemosiderin storage, osseous metaplasia, cholesterol granuloma, giant cells) that occurred only in rats from the Fraunhofer study, or were clearly more pronounced in only one study (e.g. mononuclear cell infiltration in rats from the Fraunhofer study, calcified deposits in rats from theTNO study) were considered minor findings in comparison to the major lesions viz. increased lung weight, bronchiolo-alveolar adenoma and hyperplasia, and interstitial fibrosis. Nevertheless, they are toxicologically relevant, because they invariably are manifestations of lung toxicity of MDI. Overall, the complexity of responses of lung tissue to airborne chemicals, in particular particulate materials, is well known and suggests that an intricate combined action of many factors ultimately determines the nature and severity of the lung response. This is most probably applicable to the major lung tissue responses observed in rats exposed to MDI.

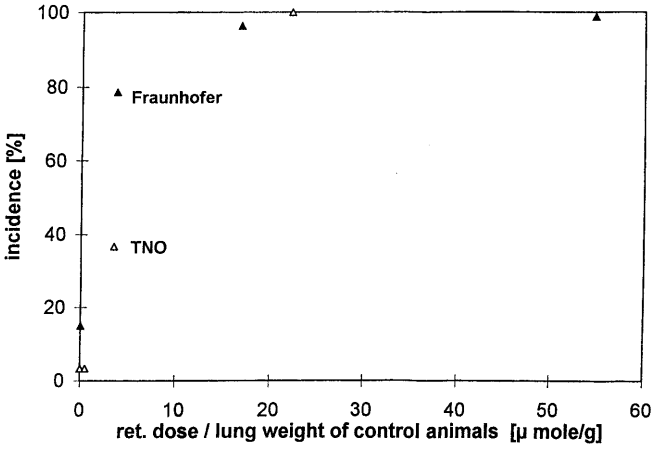

Fig. 12 Incidence of fibrosis as a function of the retained dose, measured in terms of NCO concentration per lung weight of the control animals (*open triangles* TNO study, *full triangles* Fraunhofer study)

In the Fraunhofer study, a range of pulmonary lesions was still seen at the lowest exposure level (0.23 mg/m^3). In the TNO study, the only treatment-related effect seen at the lowest exposure level (0.19 mg/m^3) was the occurrence of (grade 1 and 2) particle-laden alveolar macrophages. These macrophages had a normal appearance without any sign of degeneration. Therefore, this macrophage reaction is considered a physiological response to the deposition of particles in the lungs. Since, moreover, in the lowest dose group the presence of these viable particle-laden macrophages was not seen to be accompanied by any tissuedamage or inflammatory reaction, the lowest exposure level in the TNO study is considered

Table 8 Summary of treatment-related lung findings of female rats exposed to MDI

	TNO study			Fraunhofer study		
Exposure concentration (mg/m^3)	0.19	0.98	6.03	0.23	0.7	2.05
Dose (mgh/m^3)	559	2881	17,728	1972	6001	17,575
Findings in both studies						
Increased lung weight	–[a]	–	+[b]	–	+	+
Adenoma and/or pre-neoplastic lesions (bronchiolo-alveolar hyperplasia: alveolar type, grade 4 and 5)	–	–	+	–	+	+
Hyperplasia, bronchiolo-alveolar: alveolar type (grade 1, 2 and 3)	–	–	+	+	+	+
Hyperplasia, bronchiolo-alveolar: bronchiolar-type	–	+	+	+	+	+
Interstitial fibrosis	–	+	+	+	+	+
Calcified deposits	–	–	+	–	–	+
Mononuclear cell infiltration	–	–	+	+	+	+
Particle-laden macrophages	+	+	+	+	+	+
Findings only in Fraunhofer study						
Osseous metaplasia	–	–	–	–	–	+
Haemosiderin storage	–	–	–	+	+	+
Granuloma, cholesterol	–	–	–	–	–	+
Giant cells, multinucleated	–	–	–	–	–	+

[a] Absent or not significantly different from controls
[b] Present and/or significantly increased compared to controls

a dose level without any observed treatment-related adverse effect. Taking both studies as a whole, would it be toxicologically justifiable to consider this dose level in the TNO study (0.19 mg/m^3; 559 mgh/m^3) also a dose level without any observable adverse effect for inhalation of monomeric MDI aerosol? The following arguments would suggest a positive answer to this question: (a) the lung appeared to be the main target organ in both studies,(b) the major MDI-related adverse effects (increased lung weight, bronchiolo-alveolar adenoma and hyperplasia, and interstitial fibrosis) were very similar in both studies, (c) differences in experimental design (e.g. exposure concentration, exposure period, recovery period, total dose) appear responsible for many of the qualitative and quantitative differences between study findings and (d) from the dose-response relationship obtained in the Fraunhofer study, it appears that themild tissue effects recorded at 0.23 mg/m^3 for 18 h would not have been triggered at lower combinations of exposure level and exposure time. It seems probable, therefore, that 0.23 mg/m^3 over 6 h would have been an exposure at which no adverse effects would occur. This exposure is also very close to 0.19 mg/m^3 over 6 h, the exposure at which no adverse effect was observed in the TNO study.

Overall, many similarities in toxicological profiles emerged from this review of the two studies. Dissimilarities were most probably a consequence of the experimental protocols used rather than due to differences in intrinsic toxicity of the test materials. Low incidences of lung tumours occurred at the highest dose level in both studies but no lung tumours were foundat lower dose levels. For inflammatory and other non-neoplastic pulmonary changes establishment of a no-observed-adverse-effect-level of 559 mgh/m^3 for both polymeric and monomeric MDI (exposure conditions: 0.19 mg/m^3, 6 h/day, 5 days/week for 24 months) was considered scientifically justifiable. It was concluded that the results of both studies could be combined to serve as a basis for human risk assessment.

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