

Diesel engine emissions

Supplement 2008

MAK value	–
Peak limitation	–
Absorption through the skin	–
Sensitization	–
Carcinogenicity (1987)	Category 2
Prenatal toxicity	–
Germ cell mutagenicity	–

Further animal and epidemiological studies and data about the mode of action have been published since the last documentation from 1987 (see documentation “Diesel engine emissions” 1990). The potential lung cancer risk for humans must be re-evaluated. For this purpose, the original epidemiological studies and data from animal studies were supplemented and reviews on this subject considered. This Supplement also assesses the allergenic effect of diesel engine emissions. It does not contain data for reproductive toxicity or genotoxicity, except for those obtained in human lymphocytes.

The documentation is based on data that were obtained under exposure to the old diesel engine emissions. There have been considerable qualitative and quantitative alterations in the emissions due to the new diesel engine technology. Since these new diesel engines were not in use before the end of the 1990s, all available epidemiological studies that were assessed in 2007 are based on exposures to previous diesel engine emissions. The new diesel engine emissions can be assessed only when suitable studies are available.

Mechanism of action

In spite of the great deal of available data, the mechanism of action of the toxicity of diesel engine emissions is not fully understood. Two hypotheses have been used to explain the development of lung tumours in animals after exposure to diesel engine emissions.

One hypothesis says that the mechanism of action mainly consists of inflammatory proliferative effects of soot core particles on lung cells (Heinrich et al. 1986, 1995). The soot core is a poorly soluble particle of low toxicity. The pulmonary toxicity typical of poorly soluble particles is characterized by an inflammatory proliferative and subsequently fibrotic effect. This process is caused by macrophages that are activated after the particles have been absorbed and secrete various cytokines and chemokines to an increased extent. These signalling proteins lead to the migration of inflammatory cells, which in their turn produce oxygen and nitrogen radicals. The radicals cause damage and proliferation of the pulmonary epithelial tissue and pulmonary parenchymal tissue. The indirect mutagenic and proliferative stimuli promote the development of lung tumours (for details see documentation "General threshold limit value for dust (R fraction) (Biopersistent granular dusts)" 2014, a translation of the German from 2012). Studies have demonstrated the formation of reactive oxygen species in the inflamed lungs using various approaches.

Evidence of a good correlation between the formation of 8-hydroxydeoxyguanosine and an increased incidence of lung tumours was provided after instillation of non-extracted diesel soot particles.

The second hypothesis says that inorganic and organic substances (nitro-PAH and PAH adducts) attached to the soot core are mainly responsible for the development of lung tumours. The fraction of PAH only accounts for a small percentage of the diesel soot mass. It is assumed that the metabolism of the substances attached to the diesel soot core and released in the organism leads to mutagenic metabolites. Several studies thus detected DNA adducts in the lung cells of rats and monkeys after exposure to diesel engine emissions.

Effects in humans

Repeated exposure

Volunteer studies

A cohort of 15 healthy non-smokers was exposed to diesel engine emissions of an aerodynamic diameter of $< 10 \mu\text{m}$ [PM_{10}] at $300 \mu\text{g}/\text{m}^3$ for 1 hour. Endobronchial mucosal biopsies were taken from the volunteers three times 6 hours after each exposure and bronchial cells were obtained by rinsing with $3 \times 60 \text{ ml}$ sterile buffer. Proteins and biological effectors were also washed out with this lavage fluid. Mucosal biopsies were examined with monoclonal antibodies for the presence of specific cell markers, markers for neutrophilic leukocytes, lymphocytes, mast cells, macrophages and eosinophilic leukocytes. An analysis of various adhesion molecules and their ligands was also performed. Further analyses included differential counting of the rinsed cells and identification of the lymphocyte subpopulations. Conventional methods were used to determine adhesion molecules and interleukin-8, lactate

dehydrogenase, albumin and protein in culture supernatant. The volunteers showed pronounced inflammation of the lungs, which was characterized by neutrophilic leukocytes, mast cells and lymphocytes and was accompanied by an increased expression of endothelial adhesion molecules and their ligands. At the same time, the number of neutrophils and platelets increased in the peripheral blood. There was a significant increase in the number of neutrophilic leukocytes and B lymphocytes in the lavage fluid. Methylhistamine and fibronectin were also increased. Both neutrophilic leukocytes and platelets significantly increased in the peripheral blood, whereas the number of lymphocytes was reduced. These systemic effects seem to be due to the migration of cytokines secreted from interstitial inflammatory cells into the blood. The standard pulmonary function parameters remained unchanged in the volunteers. Immunohistochemical staining of the bronchial epithelium for proteins showed an increased expression of interleukin-8 of 200% and of growth-regulated oncogene- α protein (GRO- α) of 230%. The authors concluded from their methodologically very elaborate studies that 1-hour exposure to diesel engine emissions at a concentration of $300 \mu\text{g}/\text{m}^3$ produced a defined and marked pulmonary inflammatory reaction in healthy volunteers, which was mainly mediated by the chemokine interleukin-8 and the cytokine GRO- α . No lung burden effects were found by means of standard pulmonary function tests (Pourazar et al. 2004; Salvi et al. 1999, 2000).

A group of 10 healthy non-smokers was exposed to diesel engine emissions of an aerodynamic diameter of $< 10 \mu\text{m}$ [PM_{10}] at $200 \mu\text{g}/\text{m}^3$ for 2 hours. Spirometry was carried out and pulse, blood pressure, exhaled carbon monoxide concentration, metacholine reactivity, sputum and blood were examined 4 and 24 hours after exposure. There were no changes in lung function, pulse, blood pressure or bronchial reactivity to metacholine. Levels of exhaled carbon monoxide increased within 24 hours and, with an increase of 50%, were at their maximum during the first hour. Increases in the number of neutrophilic leukocytes in the sputum and of the myeloperoxidase level were observed while macrophages decreased. The authors concluded from these results that these were the early stage of an airway inflammatory response at a concentration of diesel engine emissions of $200 \mu\text{g}/\text{m}^3$ (Nightingale et al. 2000).

A group of 25 healthy non-smokers and 15 volunteers with mild asthma (normal lung function) was exposed to diesel engine emissions at a particle concentration of $108 \mu\text{g}/\text{m}^3$ with a 50% aerodynamic diameter of $10 \mu\text{m}$ [PM_{10}] for 2 hours. Biopsies and bronchio-alveolar lavage samples were obtained 6 hours after exposure. FEV₁ and FVC remained unchanged in both groups. Specific airway resistance was increased in both groups, the increase being significant in the healthy subjects. The proportion of neutrophilic leukocytes and lymphocytes – even in absolute terms – significantly increased after exposure. The accumulation of neutrophilic leukocytes and lymphocytes in the airways was accompanied by a significant increase in the pro-inflammatory cytokines interleukin-6 and interleukin-8. Interleukin-8 mRNA transcripts were also increased specifically in the bronchial tissue. This was associated with a significantly increased expression of P-selectin (endothelial adhesion

molecule of the leukocytes) and another adhesion molecule VCAM-1 (vascular cell adhesion molecule) in the bronchial mucosa. There were differences in the lavage samples obtained from the asthmatic group with regard to several parameters even after inhalation of pure air. These included baseline increases of eosinophilic leukocytes, mast cells, methyl histamine and eosinophilic cationic protein. Lower baseline lymphocyte counts and slightly lower eosinophilic leukocytes were recorded. The biopsies revealed an increased incidence of eosinophilic leukocytes, a reduced CD³⁺ lymphocyte count, an increased expression of TNF- α , but a decreased expression of interleukin-10. After exposure, interleukin-10 significantly increased in the biopsies of the asthmatics, whereas it decreased in those of the healthy volunteers. There were no changes in any other parameters of the asthmatic group. This study showed that initial airway inflammation response occurs as early as after 2-hour exposure to diesel engine emissions at 100 $\mu\text{g}/\text{m}^3$ (Stenfors et al. 2004).

Epidemiological studies

Non-neoplastic disorders

No changes were found in 38 men who worked as highway toll collectors in Turkey as compared with a control group from the same company with regard to vitamin E levels in the serum or the lung function parameters FVC, FEV₁ and MEF₂₅₋₇₅. The peak expiratory flow was significantly reduced by an average of 15% as compared with the control group. The serum levels of malondialdehyde, nitrite and nitrate were significantly higher than in the control group after smoking habits were taken into account. The clinical findings observed, such as coughing, sputum, dyspnoea, wheezing and chest tightness did not correlate with the increased serum levels of malondialdehyde, nitrite or nitrate. The study was carried out from September to December 2002. There is no information about the exposure level or exposure duration (Arbak et al. 2004).

In a case-control study, railroad workers (engineers and conductors) were found to have an increased risk to die from obstructive pulmonary disease after exposure to diesel engine emissions. The relative risk increased with an increasing period of exposure and was statistically significantly increased in those exposed longest (0–10 years, 48 cases: RR 0.75 CI: 0.51–1.1; 11–15 years, 59 cases: 1.33 CI: 0.93–1.91; > 16 years, 75 cases: 1.61 CI: 1.12–2.3). Adjustment was made for age, race, healthy worker effect and smoking habits (Hart et al. 2006).

Lung cancer

The results of cohort and case-control studies relevant for assessment regarding an association between the incidence of lung cancer and diesel engine emissions are shown in Table 1 and Table 2. The tables are mainly based on a review of the data

of the WHO Report 1996 and from the meta-analysis of Bhatia et al. 1998; recent studies were supplemented.

The following studies were not regarded as relevant for assessment when the individual studies were reviewed: Edling et al. 1987 (number of cases too small < 10); Lerchen et al. 1987 (number of cases too small < 10); Howe et al. 1983 (mixed exposure to coal mine dust; not adjusted for smoking; exposure period unclear); Gustafsson et al. 1986 (unclear whether dock workers were exposed); Kaplan 1959 and Milne et al. 1983 (exposure period too short); Buiatti et al. 1985 (exposure not specified exactly); Leupker and Smith 1978 and Waller 1981 (both studies are only based on active workers: selection bias). Studies from mining were not used for assessment because of mixed exposure to mine dust and quartz.

Sufficient data for the determination of exposure are only available for the period of exposure, if at all, but not for the exposure level. Therefore, the term exposure-response relationship rather than dose-response relationship is used in the following.

Cohort studies

The cohort studies relevant for assessment are shown in Table 1.

Table 1 Cohort studies on the relative risk of developing lung cancer

Study	Cases	Exposure/occupation	Relative risk (95% CI)
Ahlberg et al. 1981 Sweden	161	truck drivers	1.3 (1.1–1.6)
Balarajan and McDowall 1988 England	280	truck drivers	1.59 (1.00–2.53)
Bender et al. 1989 USA	54	road construction workers	0.69 (0.52–0.9)
Boffetta et al. 1988 USA		(1982–1988)	
	18	truck drivers	1.22 (0.77–1.95)
	5	heavy equipment operators	2.60 (1.12–6.06)
	14	railroad workers	1.59 (0.94–2.69)
Boffetta et al. 2001 Sweden	men	various occupations	
	3705	low	0.95 (0.9–1.0)
	1181	middle	1.1 (1.1–1.2)
	1058	high	1.3 (1.3–1.4)
	women		
	38	low	0.8 (0.58–1.1)
	13	middle–high	1.1 (0.62–1.84)

Table 1 (Continued)

Study	Cases	Exposure/occupation	Relative risk (95% CI)
Garshick et al. 1988 USA	1694	(1959–1980)train staff/railroad repair workers 1–330 µg/m ³ 40–44 years old in 1959 60–64 years old in 1959(not exposed to DEE) exposed for 1–4 years exposed for 5–9 years exposed for 10–14 years exposed for ≥ 15 years	 1.45 (1.11–1.89) 0.99 (0.74–1.33) 1.20 (1.01–1.44) 1.24 (1.06–1.44) 1.32 (1.13–1.56) 1.72 (1.27–2.33)
Garshick et al. 2004 USA		(1959–1996) conductors and engineers	
	884	40–44 years old in 1959	1.49 (1.3–1.7)
	732	45–49 years old in 1959	1.37 (1.18–1.58)
	456	50–54 years old in 1959	1.39 (1.18–1.64)
	286	55–59 years old in 1959	1.34 (1.09–1.64)
	121	60–64 years old in 1959	0.99 (0.75–1.3)
Gustafsson et al. 1986 Sweden		(1961–1980) dock workers	
	86	new cases	1.68 (1.36–2.07)
	70	persons who died from lung cancer	SMR: 1.32 (1.05– 1.66)
Emmelin et al. 1993 Sweden(nested case- control study)	50	dock workers exposure:	
		low	1.0
		middle	2.7 (0.6–11.3)
		high	6.8 (1.3–4.9)
		smokers:	
		low (10 cases)	3.7 (0.9–14.6)
		middle (17 cases)	10.7 (1.5–78.4)
		high (17 cases)	28.9 (3.5–240)
		non-smokers:	
		low	1.0
		middle (2 cases)	1.6 (0.2–12.5)
		high (2 cases)	2.9 (0.2–39)
Gubéran et al. 1992 Switzerland	77	professional drivers (diesel exposure unclear)	1.5 (1.23–1.81)
Gustavsson et al. 1990 ⁵ Sweden	17	(1958–1970)bus drivers/bus garage workers0.3–1.4 mg/m ³ (total dust) 0–10 ¹ 10–20 20–30 > 30	SMR (total) 1.22 (0.71–1.96) 1.0 ² 1.27 (0.21–7.72) 1.56 (0.34–7.16) 2.63 (0.74–9.42)

Table 1 (Continued)

Study	Cases	Exposure/occupation	Relative risk (95% CI)
Hansen 1993 Denmark	76	truck drivers	1.6 (1.26–2.0)
Howe et al. 1983 Canada		railroaders	
	933	total	1.06 (0.99–1.13)
	297	probably exposed	1.35
Jakobsson et al. 1997 ⁴⁾ Sweden	144	short-distance truck drivers	1.2 (1.0–1.4)
	304	long-distance truck drivers	1.1 (0.9–1.2)
Magnani et al. 1988 England	379	job-exposure matrix	0.97 (0.94–0.99)
Menck and Henderson 1976USA	109	truck drivers	1.65 (1.35–1.99)
Nokso-Koivisto and Pukkala 1994 Finland		railroad workers	
	6	exposed for 0–14 years	1.02 (0.37–2.22)
	43	exposed for 15–29 years	0.73 (0.53–0.97)
	187	exposed for > 30 years	0.89 (0.77–1.02)
Raffle 1957 England	30	bus drivers	1.4 (0.94–2.02)
Rafnsson and Gunnarsdottir 1991 Iceland	24	(1951–1988)	
		truck drivers	SMR:
		exposed for < 2 years	2.7 (0.74–6.92)
		exposed for 2–10 years	2.46 (0.99–5.08)
		exposed for 11–30 years	0.68 (0.01–3.76)
		exposed for > 30 years	2.32 (0.85–5.04)
Rushton et al. 1983 England	102	(1967–1975)	
		bus mechanics (total)	1.01 (0.82–1.22)
		unskilled workers	1.33 (0.98–1.76)
Säverin et al. 1999 ⁴⁾ Germany	38	(1970–1994)	
		potash minersconcentration of total carbon in fine dust in 1992: 0.12 mg/m ³ (workshop) 0.23 mg/m ³ (maintenance) and 0.39 mg/m ³ (mining) range: 0.038– 1.28 mg/m ³	2.2 (0.8–6.0) ³

Table 2 (Continued)

Study	Cases	Exposure/occupation	Relative risk (95% CI)
Wong et al. 1985 USA	309	(1967–1978)	
		heavy equipment operators	SMR:
		total	0.99 (0.88–1.1)
		exposed for < 5 years	0.45 ⁶ (n.s.)
		exposed for 5–9 years	0.74 (n.s.)
		exposed for 10–14 years	1.08 (n.s.)
		exposed for 15–19 years	1.02 (n.s.)
		exposed for > 20 years	1.07 (0.92–1.25)

¹ exposure intensity from 1–6 multiplied by years of exposure² control³ 90% confidence interval⁴ adjusted for smoking⁵ adjusted for exposure to asbestos⁶ significant $p < 0.01$

RR: relative risk

SMR: standard mortality rate

n.s.: not specified

In the studies by Boffetta et al. 1988 and Rushton et al. 1983, no significantly increased lung cancer risk was observed for the occupational groups of truck drivers, heavy equipment operators, railroad workers and bus mechanics. The number of cases included in the study of Boffetta et al. 1988 is small (limited validity), but adjustment was made for smokers. Boffetta et al. 1988 indicated that a suspected association between lung cancer and exposure to diesel engine emissions cannot be ruled out due to the increased relative risk. In the study by Jakobsson et al. 1997, short-distance and long-distance truck drivers were found to have a slightly increased relative lung cancer risk, which was statistically significant for short-distance truck drivers.

The nested case-control study by Emmelin et al. 1993 is not appropriate for assessment either, although an exposure assessment was made, since the numbers of cases were too small in the different exposure groups.

An increased relative lung cancer risk was observed for dock workers and truck drivers in other studies (Ahlberg et al. 1981; Hansen 1993; Menck and Henderson 1976). No adjustment was made for smoking in all three studies, nor was an exposure-response relationship established.

Exposure-response relationship

The study by Garshick et al. 1988 is relevant for the assessment of diesel engine emissions on the basis of its cohort size and exposure periods and will therefore be described in detail below. The relative lung cancer risk was examined in a cohort of

55,407 white, male railroad workers who were 40–64 years old in 1959, were exposed to diesel engine emissions and had started railroad service 10–20 years earlier. The cohort was traced until the end of 1980 and death certificates were obtained for 88% of 19,396 deaths. Lung cancer was identified as the cause of death for 1694 persons. Annual job identifications from 1959 to retirement or death were available from the U.S. Railroad Retirement Board. This information served as an index of exposure to diesel engine emissions. Directly standardized rates and proportional hazard models were used to calculate the relative risk of lung cancer based on an exposure to diesel engine emissions at the workplace beginning in 1959. A relative risk of 1.45 (95% CI: 1.11–1.89) of developing lung cancer was obtained for workers who were 40 to 44 years old in 1959 and thus belonged to the group with the longest possible duration of diesel engine emission exposure. There were no details about smoking habits. The smoking habits in 1981/82 were assumed to have existed throughout the study period. The cohort was selected to minimize the effect of past asbestos exposure. An analysis with workers that excluded possible asbestos exposure resulted in a similarly elevated risk. Workers of the cohort with 20 or more years of exposure to diesel engine emissions had the highest relative risk. However, no quantitative data on exposure were obtained within the study period. Individual exposure before 1959 was only substantiated by sparse qualitative data, and there was no information about the variation of exposure according to place and season or about the whole period. The relevance of simultaneous exposure (*e.g.* to lubricants, dust, other vapours, asbestos and tobacco smoke) was not analyzed in detail. The exposure data on diesel engine emissions are only suitable for a gross classification according to job titles. Other scores such as duration of occupation with a specific job title, intensity of exposure [$\mu\text{g}/\text{m}^3$] and lifetime exposure ($[\mu\text{g}/\text{m}^3]\cdot\text{years}$) are not sufficient for a quantitative exposure-response analysis. Evidence of a positive association between lung cancer and cumulative exposure to diesel engine emissions is based exclusively on the different relative risks for various categories of occupation. Train staff (with higher exposure) had an increased relative risk as compared with office workers (with low or no exposure). However, the association between the relative lung cancer risk and the duration of occupation was negative within one job category. Although the estimated exposure levels are not correct, a positive trend as a function of the duration of occupation would be expected for the exposed group. This is to be regarded as strong evidence of a systematic error.

The following factors may explain the negative association between the duration of occupation and lung cancer incidence: a bias that was introduced by a wrong differential classification of exposure within and among the occupations, incomplete verification of lung cancer as the cause of death, lack of information regarding other occupational exposures and air pollution, a healthy worker survivor effect, confounding by smoking and analysis of the relative instead of the absolute risk (HEI 1999).

The data reviewed by Garshick et al. 1988 were reanalyzed in the studies by Crump et al. 1991 and 1999. This reanalysis was based on exposure data from In-

dustrial Hygiene Studies (Hammond et al. 1988; Woskie et al. 1988 a, b). Different analyses for an exposure-response relationship were carried out based on information about the particle concentration, taking into account climatic conditions, age, calendar year and occupational categories. Most models yielded an inverse relationship between exposure to diesel engine emissions and the relative risk of dying from lung cancer. It was also pointed out that observation was incomplete in the years from 1976 to 1980, which was confirmed by Garshick. In a new analysis, Garshick concluded that the relative risk was increased in all four exposure groups, but there was no evidence of an exposure-response relationship (HEI 1999).

In another reanalysis of the study by Garshick et al. 1988, indirect adjustment for smoking was made retrospectively. For this analysis, exposed persons and their relatives were questioned and job-specific data about cigarette consumption used. It was assumed that smoking habits in the individual occupational groups had not changed throughout the years. When the adjustment for smoking was taken into account, the relative lung cancer risk of those with the longest duration of exposure decreased by 4–12% in every occupational group. After adjustment for smoking, it was no longer possible to establish an exposure-response relationship (Larkin et al. 2000).

Another reanalysis of the study by Garshick et al. 1988 used an improved statistical evaluation and a 7-stage model to be able to better assess exposure. The authors concluded that a positive exposure-response relationship can be derived from the data of the study by Garshick et al. 1988 (Dawson and Alexeeff 2001). Crump (2001) considered this result to be questionable since the assumptions for this model did not seem plausible to him. Another follow-up study of the Garshick study until 1996 by Garshick (2004) himself showed that although the group of conductors and engineers exposed for the longest duration had an increased relative lung cancer risk, no exposure-response relationship was found. According to the author, this is mainly due to the healthy worker effect and a lack of information about changes in exposure situations and the potential contribution of an effect of coal combustion before the transition to diesel as fuel (Garshick et al. 2004). Another reanalysis with improved exposure assessment that considered the year of hirement before or after 1945 (change from steam to diesel locomotives in the United States) confirmed that it was not possible to provide evidence of an exposure-response relationship (Laden et al. 2006). The influence of smoking habits was taken into account in another publication (Garshick et al. 2006). For this purpose, data from an independent case-control study for which the smoking habits were available were transferred to the cohort of railroad workers in line with age and birth. Data were available on the smoking habits of 2470 men, *i.e.* exclusively of persons who had died. Therefore, only the about 40,000 persons who had died were considered from the cohort study comprising about 55,000 employed persons. The relative lung cancer risk of 1.33 after exposure to diesel engine emissions without considering smoking decreased to a level of 1.2 (95% CI: 1.11–1.3) after the data on smoking habits had been included.

The relative risks were lower when smoking habits were taken into account, but they were still elevated. The fact that there was no evidence of a dose-response relationship is problematic. These analyses cannot be made since there are no data on the exposure of the engine drivers.

The following studies were considered in the assessment, but they are only of subordinate relevance because of the considerably smaller number of cases.

In the study by Rafnsson and Gunnarsdottir (1991), clearly increased standardized mortality ratios were observed in truck drivers, but these were not significant. Since no increase in the standardized mortality ratio was observed with an increase in the duration of exposure, the authors assessed this study as negative. The study by Wong et al. 1985 supports this result; no significant increase in the standardized mortality ratio with an increasing duration was observed here either.

However, the study by Gustavsson et al. 1990 describes an elevated relative lung cancer risk in bus drivers and bus garage workers with increasing exposure (exposure index: assumed exposure level multiplied by the duration of exposure) to diesel engine emissions, but this was not significant. The authors stated that the increased relative risks of 1.56 (middle exposure group) and 2.63 (highest exposure group) cannot be explained by smoking alone. On the basis of the small number of 2, 3 and 10 cases in the individual exposure groups, no significant result is expected, nor can definite conclusions about the relative lung cancer risk be drawn from this study.

A cohort study carried out in Germany included 5536 potash miners who had been exposed to diesel engine emissions underground from 1970 onwards. The data of the cohort were based on records of the routine occupational medical examinations and on interviews. Measurements of the total carbon concentration in fine dust of the inhaled air, which were carried out afterwards under comparable exposure conditions, were used for the assessment of exposure. A total of 424 deaths were recorded, including 38 from lung cancer. Calculation of the standardized mortality ratios revealed that the mortality in the total cohort was only about half as high as in the male population. This did not only apply to all causes of death together but, to a restricted extent, also to cancer and especially to lung cancer. To restrict this pronounced healthy worker effect, a comparison was made within the cohort between highly exposed mining workers and maintenance workers with low exposure. The relative lung cancer risk was 2.2 (95% CI: 0.8–6.0) for workers with high exposure. Cox regression yielded a relative risk of 1.2 (95% CI: 0.4–3.5) for 20-year work in the highest exposure category. As the wide confidence intervals have shown, the estimate of the risk involves substantial uncertainties on account of the small number of 38 lung cancer cases. Therefore, further follow-up of the deaths is planned (Säverin et al. 1999).

Case-control studies

All case-control studies relevant for assessment are shown in Table 2.

Table 2 Case-control studies on the relative risk of developing lung cancer

Study	Cases/controls	Exposure/occupationexposure period	Relative risk (95% CI)
Benhamou et al. 1988 ^{2,4} France 1976–1980	128/167	motor vehicle drivers	1.42 (1.07–1.89)
	157/224	transport equipment operators	1.35 (1.05–1.75)
	65/96	motor vehicle mechanics	1.06 (0.73–1.54)
Boffetta et al. 1990 ^{2,3} USA 1977–1987	210/324	probable occupational exposure	0.95 (0.78–1.16)
	240/473	possible occupational exposure	0.92 (0.76–1.1)
	114/176	truck drivers	0.88 (0.67–1.15)
Brüske-Hohlfeld et al. 1999 ^{2,3} Germany	534/337	professional drivers	1.25 (1.05–1.47)
	99/60	other traffic-related jobs	1.53 (1.04–2.24)
	81/32	machine operators	2.31 (1.44–3.7)
	52/36	tractor drivers	1.29 (0.78–2.14)
Brüske-Hohlfeld et al. 2000 ^{2,3} Germany		professional drivers and machine operators after estimated cumulative exposure level:	
	2816/3123	not exposed	1
	208/158	1st tertile	1.29 (1.01–1.65)
	229/138	2nd tertile	1.32 (1.03–1.70)
	245/122	3rd tertile	1.71 (1.33–2.22)
Coggon et al. 1984 England	172/281	evaluation of death certificates	total:
	32/57	exposure not defined in detail	1.3 (1.0–1.6) high exposure 1.1 (0.7–1.8)
Damner and Larsson 1985 Sweden		professional drivers	not adjusted
	63/95	1–10 years	1.36 (0.97–1.91)
	43/60	10–20 years	1.47 (0.97–2.2)
	33/42	> 20 years truck drivers	1.61 (1.01–2.57)
	35/57	1–10 years	1.26 (0.81–1.95)
	26/32	10–20 years	0.98 (0.98–2.81)
	20/24	> 20 years	0.94 (0.94–3.1)
Damner and Larsson 1987 ² Sweden		professional drivers	
	64/44 33/20	> 1 year > 20 years	1.0 (0.7–1.6) 1.1 (0.6–2.2)

Table 2 (Continued)

Study	Cases/controls	Exposure/occupationexposure period	Relative risk (95% CI)
Garshick et al. 1987 ^{2,3} USA 1959– 1982	1256/ 2385	railroad workers (87–322 µg/m ³) workers < 65 years old: 0–4 years 5–19 years > 20 years workers > 65 years old: 5–19 years > 20 years	1.0 1.02 (0.72–1.45) 1.41 (1.06–1.88) 0.95 (0.79–1.13) 0.94 (0.56–1.59)
Gustavsson et al. 2000 ² Sweden	180–200/842	bus drivers, truck drivers, mechanics and heavy equipment and forklift operators 0–9 years 10–29 years > 30 years	0.76 (0.51–1.13) 1.21 (0.88–1.65) 1.38 (0.97–1.97)
Hall and Wynder 1984 ^{2,5} USA	45/24	bus drivers, truck drivers, railroad workers and mechanics of heavy equipment	1.4 (0.8–2.4)
Hayes et al. 1989 ² USA	1444/1893	truck drivers (exposure unclear) mechanics	1.5 (1.1–2.0) 2.1 (0.9–5.2)
Jöckel et al. 1998 ^{2,3} Germany	72/44	mechanics	1.27 (0.82–1.96)
	18/16	< 3 years	0.94 (0.44–2.05)
	21/14	3–10 years	0.83 (0.39–1.73)
	33/14	> 10 years	2.32 ⁶ (1.12–4.8)
	396/292	truck drivers	1.48 ⁶ (1.18– 1.86)
	94/87	< 3 years	1.23 (0.86–1.76)
	99/78	3–10 years	1.37 (0.94–2.01)
	203/127	> 10 years	1.72 ⁶ (1.29– 2.31)
Parent et al. 2006 Canada	74/25	> 5 years	1.6 (0.9–2.8) ^{2,3,5}
Richiardi et al. 2006 ^{2,5} Italy	159/196		0.95 (0.72–1.26)
	47/67	< 6.5 years	0.83 (0.53–1.29)
	59/66	6.5–19 years	1.02 (0.67–1.54)
	53/63	> 20 years	1.01 (0.65–1.57)
Siemiatycki et al. 1988 ^{2,5} Canada	81/n.s. (squamous cell carcino- mas)	exposure period and level unclear	1.2 (0.9–1.6) ¹

Table 2 (Continued)

Study	Cases/controls	Exposure/occupationexposure period	Relative risk (95% CI)
Steenland et al. 1990 ^{2,3} USA	996/1085	truck drivers: long-haul truck driving city truck driving 1–24 years 25–34 years > 35 years mechanics (total) 1–24 years 25–34 years > 35 years	 1.27 (0.83–1.93) 1.31 (0.81–2.11) 1.27 (0.72–2.27) 1.26 (0.74–2.16) 1.89 (1.04–3.42) 1.69 (0.92–3.09) 1.69 (0.61–4.67) 1.39 (0.63–3.07) 1.09 (0.44–2.66)
Steenland et al. 1998	see Steenland et al. 1990	workers in trucking industry cumula- tive exposure [$\mu\text{g}/\text{m}^3$ per year of elemental carbon] 0–174 174–268 268–360 > 360	 1.2 (0.79–1.81) 1.16 (0.77–1.75) 1.39 (0.91–2.11) 1.72 (1.11–2.64)
Swanson et al. 1993 ² ; Burns and Swanson 1991 USA	121/31	truck drivers	2.5 (1.1–4.4)
	40/15	railroad workers	2.4 (1.1–5.1)

¹ 90% confidence interval² adjusted for smoking³ adjusted for asbestos exposure⁴ adjusted for alcohol consumption⁵ adjusted for social status⁶ $p < 0.05$, two-sided test

n.s.: not specified

The study by Coggon et al. 1984 can be used for assessment only with reservations since only exposed persons were used who died below 40 years of age. Exposure may thus have lasted no more than 20 years. No adjustment was made for smoking habits. There was a slightly significantly increased relative lung cancer risk of 1.3, but the relative risk decreased in the group with the longest possible duration of exposure and was no longer significant.

Although the studies by Benhamou et al. 1988 and Siemiatycki et al. 1988 substantiate a significant increase in the relative lung cancer risk, neither study provides accurate data about the duration of exposure or whether there was any exposure to diesel engine emissions at all.

No increased lung cancer risk was found for occupational groups such as truck and professional drivers after adjustment for smoking (Damberg and Larsson 1985) or for bus and heavy equipment operators after adjustment for smoking, asbestos

exposure, and education and training (Boffetta et al. 1990). The relative risk became even lower with an increasing duration of exposure (Damber and Larsson 1985).

In a population-based study by Richiardi et al. 2006, no definite association between exposure to diesel engine emissions and an increased relative lung cancer risk was found, nor was an exposure-response relationship established. No significantly increased lung cancer risk was established even after classification according to 9 different occupational groups with different exposure levels. In this study, adjustment was made for age, cigarette smoking and educational level (Richiardi et al. 2006).

Hall and Wynder (1984) observed a not significantly increased relative lung cancer risk of 1.4 for bus drivers, truck drivers, railroad workers and mechanics of heavy equipment. However, the authors assessed the study as inadequate for providing evidence of an increased relative lung cancer risk after exposure to diesel engine emissions since the information about period and level of exposure was insufficient.

The relative lung cancer risk was significantly increased in truck drivers who had been exposed for more than 10 years. Adjustment was made for smoking, and the authors explained that the increased risk could not be attributed to smoking (Hayes et al. 1989).

Swanson et al. 1993 found a significantly increased relative lung cancer risk for the occupational group of truck drivers and railroad workers. The validity of the study is limited since there are no data on exposure.

A pooled analysis of two case-control studies on the lung cancer risk in West and East Germany comprised 3498 male patients with lung cancer and 3541 male population controls. An increased relative lung cancer risk with a high odds ratio of 1.91, which dropped to 1.43 (95% CI: 1.23–1.67) after adjustment for smoking and asbestos exposure, was found for the total group of occupations with exposure to diesel engine emissions. Professional drivers (including truck, bus and taxi drivers) with an OR of 1.25 (95% CI: 1.05–1.47) were the largest individual group. Persons exposed to diesel engine emissions in different traffic-related jobs (including diesel engine locomotive drivers, switchmen and forklift operators) had an increased OR of 1.53 (95% CI: 1.04–2.24). The most marked relative risks were observed for machine operators (including drivers of bulldozers, graders, excavators and other earth-moving machinery, transport equipment operators and machine tenders) (OR: 2.31; 95% CI: 1.44–3.70). With an increasing duration of occupation, tractor drivers had an increased relative risk with an OR of 6.81 after exposure of more than 30 years (95% CI: 1.17–39.51). All listed risks had been adjusted for smoking and asbestos (Brüske-Hohlfeld et al. 1999).

Exposure-response relationship

A significantly increased relative lung cancer risk was found for railroad workers who were below 65 years old and had been exposed for more than 20 years, but not for the exposure period of 5–19 years or for workers over 65 years in either exposure category (Garshick et al. 1987). The authors pointed out that only 3% of the more than 65-year-old workers had been exposed to diesel engine emissions for 20 years

or longer; this is due to the fact that diesel locomotives were introduced nationwide in the United States only towards the end of the 1950s.

The study by Gustavsson et al. 2000 also provides evidence of an exposure-response relationship. The relative risk of developing lung cancer increased after evaluation of the results according to duration of exposure, but was not statistically significant. If the evaluation was based on μg nitrogen dioxide/ m^3 or mg years/ m^3 (cumulative exposure: product of intensity, probability and duration of exposure), the relative risks were not increased.

On the basis of the data from the above-mentioned study (Brüske-Hohlfeld et al. 1999), another study (Brüske-Hohlfeld et al. 2000) was intended to provide a retrospective quantitative assessment of the exposure level to diesel engine emissions for various occupational groups by means of a job exposure matrix (JEM) covering all occupations and another risk calculation using the JEM. In relation to cumulative exposure, the relative risk of lung cancer increased by 13% (95% CI: 4%–23%) per estimated diesel soot year for all occupations exposed to diesel engine emissions together or by 53% (95% CI: 13%–106%) for professional drivers and 11% (95% CI: 1%–21%) for other occupations exposed to diesel engine emissions. One diesel soot year corresponds to exposure to an estimated total carbon concentration of 1 mg/m^3 year. These risk coefficients should be interpreted with great caution. Since there were no measured values of total carbon or elemental carbon for most work areas, the assumptions involved in the job-exposure matrix were based on estimates and could not be validated by means of specific measurements at the workplace (Brüske-Hohlfeld et al. 2000).

The case-control study by Jöckel et al. 1998 gives additional information as to how hours of occupational use of vehicles can be determined and about exposure to diesel engine emissions in storehouses. The relative lung cancer risk was statistically significantly increased for truck drivers overall; for mechanics it was increased, but not statistically significantly. Evaluation based on hours of exposure yielded an increased lung cancer risk for truck drivers, which at 1.31 was not significant for those exposed < 10,000 hours and at 1.88 (1.27–2.8) was significant for those exposed > 10,000 hours. An increase in the relative lung cancer risk was obtained for truck drivers with an increasing duration of exposure in years (see Table 2). It was statistically significant in the group exposed longest, thus indicating an exposure-response relationship. The authors specify that, even after adjustment for smoking and asbestos exposure, there was a significantly increased lung cancer risk after exposure to diesel engine emissions that cannot be explained by other confounders (Jöckel et al. 1998).

The case-control study by Steenland et al. 1990 is of special relevance. Cases and controls were selected from 10,699 men who had filed claims for pension benefits from the Teamsters Union and had died in 1982 and 1983. Death certificates were obtained for 98% of these persons. A total of 1288 lung carcinoma cases were identified. Controls were every 6th death excluding the diagnoses of lung carcinoma, bladder carcinoma and motor vehicle accident. The next of kin of the case or control person was questioned in detail on work history, asbestos exposure, smoking and

diet. The response rate was 81%. Most non-response was due to the fact that the next of kin could not be located. The analysis included 994 lung carcinoma cases and 1085 controls. A statistically significant increase in risk was obtained for long-haul drivers of diesel trucks (OR: 1.89; 95% CI: 1.04–3.42) who had been exposed longer than 35 years. The risk of developing lung cancer was increased for the other occupational categories, although it was not statistically significant. It is unusual that the relative risk was clearly lower for mechanics who had been exposed longer than 35 years than for mechanics who had worked fewer than 35 years in this job, although it is assumed that mechanics were exposed to higher concentrations of diesel engine emissions than truck drivers. No definite exposure-response relationship can be derived from this study for any job category.

On the basis of this case-control study, Steenland et al. 1998 carried out an analysis on exposure-response relationships. Elemental carbon with an aerodynamic diameter $< 1 \mu\text{m}$ [EC_1] was selected as a marker of diesel engine emission exposure. This marker is more sensitive and more specific than respirable-sized particles (RSP). Nevertheless, some restrictions must be considered; for example, the contribution of diesel engine emissions to the EC_1 over time was not constant.

The industrial hygiene study (Zaebst et al. 1991), which was based on the case-control study by Steenland et al. (1990), defined an exposure range for various job titles, but did not consider some factors: variations from place to place, seasonal fluctuations, simultaneous exposure to other substances, historical particle concentrations in the ambient air and intra- and interindividual differences. Assessment of historical exposure must consider the measured values of diesel vehicles that currently in use and the time of change to diesel vehicles. Alternatives to the miles driven per vehicle and regional historical measured values on environmental pollution are required. Taking past exposure into account, all analyses showed a significantly positive trend between the relative lung cancer risk and cumulative exposure (Steenland et al. 1998).

A Swedish register study reviewed the association between occupational exposure to diesel engine emissions and the cancer incidence of various organs. In 1960 and 1970, information was gathered about the employment status of Sweden's population. Mortality was observed from 1971–1989. A statistically significantly increased relative lung cancer risk of 1.1 (1.08–1.21) in the middle exposure group, and of 1.3 (1.26–1.42) in the high one was obtained for the men exposed to diesel engine emissions in 1960. No increased relative lung cancer risk was found for the exposed women. Registration of exposure, which was based on the workers' own information in 1960 and 1970, is the main problem of this study. There are no data about the exposure period, smoking habits or diet. Therefore, this study is not suitable for assessing the lung cancer risk after exposure to diesel engine emissions (Boffetta et al. 2001).

Another case-control study is available from Canada (Parent et al. 2006). A cohort of 857 male patients with lung cancer was compared with 533 population controls and 1349 patients with other tumour localizations. All persons were questioned about their work histories and possible confounders. In order to supple-

ment the data available from similar studies of this working group, work histories were recorded in more detail, on the one hand, and smoking habits were described by several factors, on the other hand. No increased relative lung cancer risk (OR: 1.0) was found among the patients with other tumour localizations. An OR of 1.2 (95% CI: 0.8–1.8) was observed for exposure to diesel engine emissions overall as compared with the population controls. Limitation to all cases and controls with substantial exposure led to an increase in the OR to 1.6 (95% CI: 0.9–2.8). No exposure-response relationship was found when exposure was classified according to frequency, concentration and duration. According to the authors, the results support the hypothesis of an association between exposure to diesel engine emissions and lung cancer.

Meta-analyses

In a meta-analysis, 30 studies were assessed on the relative lung cancer risk after exposure to diesel engine emissions. Studies from mining were not included in the meta-analysis because of possible confounders such as radon, arsenic and quartz. The studies had to meet the following criteria: (1) A relative risk with standard deviation had to be available or derivable from the available information; (2) the latency had to be less than 10 years; (3) there should be no systematic error resulting from case ascertainment; (4) the studies should be independent from each other. The relative risks of the highest exposure group and of those exposed longest were used from each of the studies. Important aspects regarding the heterogeneity of the studies were taken into account in certain methods of analysis (subset analysis) and linear metaregression. Indicator variables were created to characterize the studies for occupational categories, reference population, latency (10 years or undefined), duration of exposure, method of identifying cases, year of publication, location and healthy worker effect. The meta-analysis yielded an increased relative lung cancer risk of 1.29 (95% CI: 1.14–1.47) for the cohort studies and of 1.44 (1.33–1.56) for the case-control studies. A smoking-adjusted relative lung cancer risk of 1.47 (95% CI: 1.29–1.67) was obtained after all studies had been considered (Lipsett and Campleman 1999).

In a meta-analysis by Bhatia et al. 1998, 29 studies were assessed on the relative lung cancer risk after exposure to diesel engine emissions. A significantly increased relative lung cancer risk of 1.33 (1.27–1.4) was established after evaluation of the highest exposure group in the specific studies. A statistically significantly increased relative lung cancer risk of 1.35 (1.22–1.49) was also obtained for 16 studies adjusted for smoking. There was no essential difference from the relative lung cancer risk (1.33 CI: 1.25–1.41) obtained in 13 studies that were not adjusted for smoking. However, in the assessment of this meta-analysis it must be borne in mind that the re-evaluated data of the study by Garshick (2004, 2006) have not yet been taken into account.

Furthermore, an analysis of 15 cohort studies showed that using an internal control group instead of an external one had no influence on the increased relative lung cancer risk. The authors conclude from the result of the meta-analysis that there is

a clear association between the increased relative lung cancer risk and exposure to diesel engine emissions.

Experience shows that smoking habits are very similar among workers with low and high exposure, *i.e.* smoking and the level of occupational exposure are substantially independent of each other. Relative risks in a range between 1.5 and 2 cannot be explained by smoking alone, since smoking habits differed only slightly within the categories of sex, age and time (Hertz-Picciotto 1995). The publication by Bathia et al. 1998 gives three reasons why the increased relative risk is not due to smoking: (1) The pooled relative risks for studies adjusted for smoking are the same as for those not adjusted for smoking; (2) in the studies with information about smoking, the relative risk changes only slightly if the evaluation is carried out without taking smoking into account; (3) in studies with internal reference populations (as in the cohort studies), confounding by smoking is unlikely (Table 3) (Bhatia et al. 1998).

The studies listed in Table 1 and Table 2 were also assessed by Cohen and Higgins (1995). Their assessment of the studies until 1995 led the authors to conclude an increase in the relative lung cancer risk of 20% to 40%, which cannot be attributed to confounding by smoking or any other systematic error. No meta-analysis was calculated.

Various international organizations have assessed the lung cancer risk after expo-

Table 3 Review of the relative lung cancer risk (Bhatia et al. 1998)

	Number of studies	RR ¹	95% CI ²	Heterogeneity	Adjusted RR
all studies	29	1.33	1.27–1.40	58.0	1.24–1.44
case-control studies	14	1.33	1.21–1.47	20.5	1.18–1.51
cohort studies	15	1.33	1.26–1.42	37.5	1.21–1.47
internal reference group	8	1.43	1.32–1.55	11.0	1.29–1.58
external reference group	7	1.22	1.12–1.34	20.0	1.04–1.44
adjusted for smoking	16	1.35	1.22–1.49	23.4	1.20–1.52
not adjusted for smoking	13	1.33	1.25–1.41	34.5	1.20–1.47
analysis according to occupational groups	24	1.37	1.30–1.46	48.4	1.27–1.49
railroad workers	6	1.44	1.30–1.59	5.6	1.30–1.60
machine operators	3	1.11	0.95–1.29	4.3	0.89–1.38
truck drivers	10	1.49	1.36–1.64	9.8	1.36–1.65
bus drivers	5	1.24	1.07–1.43	14.8	0.93–1.64

¹ relative risk

² confidence interval

sure to diesel engine emissions. Older assessments concluded that, although epidemiological studies indicate a carcinogenic risk, human evidence is limited (IARC 1989; NIOSH 1988). Recent assessments have attributed clearly greater importance to epidemiology and consider carcinogenicity in humans caused by diesel engine emissions to be very likely, although the available studies are not suitable for a quantitative risk assessment (HEI 1999; WHO 1996). The USEPA also concluded that no exposure-response relationship can be derived between exposure to diesel engine emissions and lung cancer (USEPA 1999).

Bladder cancer

The results of cohort and case-control studies regarding an association between the incidence of bladder cancer and diesel engine emissions are shown in Table 4 and Table 5.

The cohort studies do not provide any evidence of a significantly increased blad-

Table 4 Cohort studies on the relative risk of developing bladder cancer or of dying from it (Boffetta and Silverman 2001)¹

Study	Cases	Exposure/occupation	RR (95% CI)
Boffetta et al. 2001	men		
	2453	low	0.91 (0.87–0.95)
	725	middle	1.0 (0.95–1.11)
	491	high	0.91 (0.83–1.00)
	women		
	27	low	0.82 (0.56–1.2)
	6	middle–high	0.84 (0.38–1.87)
Boffetta et al. 1988	13	JEM ²	1.04 (0.55–1.78) ³
		> 16 years	0.94 (0.32–2.51)
Gustavsson et al. 1990	4	mechanics in bus garages	0.66 (0.18–1.68)
Howe et al. 1983 Rushton et al. 1983	175	railroad workers	1.03 (0.88–1.2) ⁴
	12	mechanics in bus garages	1.39 (0.72–2.43)
Schenker et al. 1984	30	railroad workers	0.76 (0.15–2.21)
Soll-Johanning et al. 1998		bus drivers	
	177	men	1.4 (1.2–1.6)
	2	women	1.3 (0.2–4.7)
Wong et al. 1985	27	heavy construction equipment operators	1.18 (0.78–1.72)
		> 20 years	1.15 (0.63–1.92)

¹ All cohort studies listed have been considered in the meta-analysis by Boffetta and Silverman 2001.

² JEM: job-exposure matrix: occupations with exposure to diesel engine emissions

³ adjusted for smoking

⁴ Study has not been included in the assessment because of mixed exposure to coal mine dust.

Table 5 Case-control studies on the relative risk of developing bladder cancer

Study	Cases	Exposure/occupation	RR (95% CI)
Bonassi et al. 1989 ¹	3	truck drivers	1.88 (0.44–8.00)
Cordier et al. 1993 ^{1,2}	33	railroad workers	0.8 (0.49–1.3)
NIOSH 1977 ¹	no exact specification possible	truck drivers	1.67 (0.94–2.98)
		bus drivers	2.89 (0.86–9.73)
		railroad workers	1.63 (0.66–4.04)
Hoar and Hoover 1985 ^{1,2}	32	truck drivers	
	9	1–4 years	1.4 (0.6–3.3)
	12	5–9 years	2.9 (1.2–6.7)
	11	> 10 years	1.8 (0.8–4.1)
	23	JEM ³	1.5 (0.9–2.6)
	5	1–19 years	0.9 (0.3–2.8)
	5	20–29 years	2.1 (0.5–8.6)
	6	30–39 years	3.2 (0.8–13.7)
Howe et al. 1980 ¹	7	> 40 years	1.7 (0.5–5.0)
	9	railroad workers	9.0 (1.2–395)
	11	JEM ³	2.8 (0.8–11.8)
Iscovich et al. 1987 ^{1,2}	20	railroad workers, drivers	4.16 (1.82–9.53) ⁴
Iyer et al. 1990 ²	41	various occupations exposed to diesel engine emissions	1.24 (0.77–2.0)
Jensen et al. 1987 ^{1,2}		truck/bus/taxi drivers	1.29 (1.05–1.59)
	11	1–9 years	0.7 (0.4–1.5)
	13	10–19 years	1.6 (0.8–3.4)
	9	20–29 years	3.5 (1.1–11.6)
	9	> 30 years	2.4 (0.9–6.6)
Kunze et al. 1992 ^{1,2}	51	truck drivers	1.8 (1.1–2.8)
	14	1–9 years	2.1 (n.s.)
	14	10–19 years	1.5 (n.s.)
	10	20–29 years	1.7 (n.s.)
	13	> 30 years	3.0 (n.s.)
	12	engine drivers	3.0 (1.0–8.8)
Porru et al. 1996 ¹		truck drivers, van drivers	
	7	short distances	0.5 (0.2–1.6)
	23	long distances	1.1 (0.5–2.2)
Risch et al. 1988 ¹	113	railroad workers	1.07 (0.71–1.61)
	309	men (exposed occupationally)	1.53 (1.17–2.00)
	19	women (exposed occupationally)	0.62 (0.23–1.57)

Table 7 (Continued)

Study	Cases	Exposure/occupation	RR (95% CI)
Schoenberg et al. 1984 ¹	70	truck drivers	1.06 (0.76–1.48)
	20	bus drivers	1.17 (0.63–2.17)
Siemiatycki et al. 1994 ¹		truck drivers	
	22	1–10 years	1.2 (0.7–1.9)
	56	> 11 years	1.3 (0.8–1.5)
Silverman et al. 1983 ^{1,2}	42	truck drivers	2.1 (1.4–4.4)
	23	< 10 years	1.4 (n.s.)
	16	> 10 years	5.5 (n.s.)
	6	bus drivers	1.5 (0.4–5.3)
Silverman et al. 1986 ^{1,2}	488	truck drivers (ever exposed; no other details)	1.3 (1.1–1.4)
	74	< 5 years	1.2 (n.s.)
	32	5–9 years	1.4 (n.s.)
	33	10–24 years	2.1 (n.s.)
	22	> 25 years	2.2 (1.1–4.2)
	49	bus drivers	1.3 (0.9–1.9)
Steenland et al. 1987	6	truck drivers	
		> 20 years	12 (2.3–62.9)
	22	railroad workers	
		> 20 years	2.2 (1.2–4.0)
Steineck et al. 1990 ²	25	low exposure	1.3 (0.6–3.1)
		middle exposure	2.2 (0.7–6.6)
		high exposure	2.9 (0.3–30)
Vineis and Magnani 1985 ^{1,2}	16	truck drivers	1.2 (0.6–2.5)
	7	railroad workers	0.5 (0.2–1.4)
Wynder et al. 1985 ¹		exposed occupationally	0.87 (0.47–1.58)
	10	truck/bus drivers	0.9 (0.4–1.9)
	2	railroad workers	2.0 (0.3–11.6)
	2	heavy construction equipment operators	0.7 (0.2–3.5)

¹ in the meta-analysis by Boffetta and Silverman 2001² adjusted for smoking³ JEM: job-exposure matrix: occupations with exposure to diesel engine emissions⁴ calculated according to Boffetta and Silverman 2001

n.s.: not specified

der cancer risk after exposure to diesel engine emissions. A not statistically significantly increased bladder cancer risk was found in three studies, but the number of cases was very small in one study (Rushton et al. 1983) and exposure was very unclear in the study by Wong et al. (1985). The authors of the study by Soll-Johanning

et al. 1998 stated that the increased relative risk may also have been due to other factors, *e.g.* smoking.

A not statistically significantly increased bladder cancer risk was established in some case-control studies for the occupational group of truck drivers.

A trend for an increase in the relative bladder cancer risk was derived from some studies as a function of the duration of exposure (Hoar and Hoover 1985; Jensen et al. 1987; Kunze et al. 1992; Silverman et al. 1986; Steineck et al. 1990), the highest relative risk not always being observed in the group of persons exposed for the longest period. Since the number of cases is low, an assessment of the results is difficult. Most studies do not provide information about the duration and level of exposure or possible confounders such as smoking and exposure to aromatic amines, which are known to be a risk for the development of bladder cancer. Assignment to the individual occupational groups and the related level of exposure is unclear in most studies. The results indicate that truck drivers apparently have an increased bladder cancer risk, although no evidence exists to date whether this can be attributed to diesel engine emissions (WHO 1996; USEPA 1999).

Meta-analyses were carried out on the increased bladder cancer risk in various occupational groups after exposure to diesel engine emissions (Boffetta and Silverman 2001). A total of 35 relevant studies were identified for these meta-analyses.

Only studies in which there were 5 years between the exposure to diesel engine emissions and the occurrence of bladder cancer were included. The mentioned studies (Andersen et al. 1999; Claude et al. 1988; Malke et al. 1987; Schuhmacher et al. 1989; Silverman et al. 1989; Steenland et al. 1987) were not used for assessment for the following reasons: the results had already been mentioned in major studies, and there was no specification of a certain occupational group. A statistically significantly increased relative bladder cancer risk of 1.17 (CI: 1.06–1.29) was specified for truck drivers and of 1.33 (1.22–1.45) for bus drivers. Furthermore, a meta-analysis of the results was carried out for the occupational exposure groups that were exposed for the longest period in each case. A statistically significantly increased relative bladder cancer risk of 1.44 (1.18–1.76) was obtained for these groups. The studies used as a basis for the meta-analyses are shown in Table 4 and Table 5.

Biomonitoring

Lymphocyte DNA adducts and haemoglobin adducts (hydroxyethylvaline) as well as urinary 1-hydroxypyrene excretion were measured among 10 workers (non-smokers) who worked as mechanics in bus garages. Persons of the control group were employed in administration, which was located 5 to 10 kilometres away from the garages.

The employment period was 9–31 years for both groups. The workshops were well aerated. There are no data available for exposure. Age, passive smoking, living area, use of medications, tar ointment, diet and previous employment were taken into account.

Table 6 DNA adducts, haemoglobin adducts in lymphocytes and urinary 1-hydroxypyrene excretion (median values; range) (Nielsen and Autrup 1994; Nielsen et al. 1994)

	DNA adducts (butanol) [fmol/ μ g DNA]	DNA adducts (P1 nuclease) [fmol/ μ g DNA]	Haemoglobin adducts [pmol/g haemoglobin]	Urinary 1-hydroxy- pyrene [μ mol/mol creatinine]
exposed persons	0.84 (0.3–1.88)	0.65 (0.15–3.33)	33.3 (25.4–58.8)	0.11 (0.05–0.16)
control group	0.26 (0.13–1.4)	0.08 (0.03–0.29)	22.1 (8.0–37.0)	0.05 (0–0.11)

The results were significantly higher in the persons exposed than in the control persons for all three end points (Table 6). The results of DNA adduct formation did not correlate with those of haemoglobin adduct formation. The authors concluded from the results that there was increased exposure to genotoxic compounds, but their origin is unclear. They consider PAH from diesel engine emissions and from lubricating oils to be mainly responsible for the increase of the adducts (Nielsen and Autrup 1994; Nielsen et al. 1996).

Blood samples of 29 bus garage workers (non-smokers) were analyzed for haemoglobin adducts of nitro-PAH. Control groups consisted of 20 urban hospital workers and 14 rural workers. The haemoglobin adduct concentration revealed no difference between the mechanics who were classified as highly exposed to diesel engine emissions and the persons from urban areas (middle exposure). However, the haemoglobin adduct concentration was significantly increased among the urban workers as compared with the rural workers (low exposure) (Zwirner-Baier and Neumann 1999).

The excretion of 1-aminopyrene, a metabolite of 1-nitropyrene and marker of exposure to diesel engine emissions, was significantly increased in the 24-hour urine of 3 mechanics (non-smokers) who repaired locomotive engines as compared with 2 control persons. The measured values were obtained by means of an ELISA. The total particle concentration was 0.18–1.01 mg/m³, and a 1-nitropyrene level of 3.6–15.0 μ g/g dust or 0.5–5.6 ng/m³ was specified (Scheepers et al. 1994).

Lymphocyte DNA adducts (³²P-postlabelling) and urinary 1-hydroxypyrene were measured, and micronucleus tests were carried out in a total of 48 mechanics in 3 garages for buses and vans as compared with 2 control groups. PAH profiles were measured in the 3 garages and at 2 different sites with much and little traffic in Budapest. The concentrations of pyrene and benzo[a]pyrene were 250–2600 ng/m³ and 51–184 ng/m³, respectively, in the garages and 0.3–1.1 ng/m³ and 0.62–0.85 ng/m³ at the above-mentioned sites. This leads to a clearly higher exposure of the mechanics to these 2 PAH as compared with the control group. No statistically significant difference between the mechanics and the control group and between the mechanics of the 3 different garages were observed regarding DNA adduct and micronucleus formations after adjustment for smoking. However, 1-hydroxypyrene excretion was statistically significantly increased in the mechanics as compared with the control group even after adjustment for smoking (Schoket et al. 1999). B[a]P-7,8-diol-9,10-epoxide adduct formation in

globulin and albumin was additionally evaluated in 15 mechanics. The lobulin adduct rate was increased 2.4 times as compared with the control. The publication includes no data about adjustment for smoking (Melikian et al. 1999).

A group of 45 mechanics who worked for more than 5 years in bus garages and were all non-smokers was exposed to particle concentrations of 0.22–0.91 mg/m³. The concentration of benzene in the particle fraction was 0.11–0.27 mg/g. 5-Aminolaevulinic acid synthesis activity and concentration were increased in the lymphocytes of the mechanics as compared with the control group, and haemoglobin formation and ferrochelatase activity were decreased. The protoporphyrin levels showed no significant differences between exposed and non-exposed persons. A significant increase of porphyrine DNA adducts was found. The authors specify that these results indicate that the mechanics have a higher relative cancer risk (Muzyka et al. 1998).

DNA adducts, urinary 1-hydroxypyrene excretion, PAH plasma protein adducts and haemoglobin adducts were measured among 26 bus drivers (urban), 23 bus drivers (suburban), 19 taxi drivers and 22 control persons. No differences were measured between the groups regarding 1-hydroxypyrene excretion and haemoglobin adducts. The DNA adduct level was statistically significantly increased in the taxi driver and bus driver (suburban) groups as compared with the control persons. The increase of PAH plasma protein adducts was statistically significantly increased in the taxi drivers as compared with the control group (Hemminki et al. 1994 a).

DNA adduct levels (³²P-postlabelling) were measured from bus maintenance and truck terminal workers. No data were available about the level of exposure. In 1981, benzo[*a*]pyrene concentrations of 30–40 ng/m³ were measured, which decreased to 15 ng/m³ in 1989. Workers who washed and maintained the buses had the highest number of DNA adducts (3.73 adducts/10⁸ nucleotides). It was also significantly increased as compared with the control group (Hemminki et al. 1994 b). The urine of 18 salt miners who were exposed to diesel engine emissions underground was examined during and after their shift for levels of 1-hydroxypyrene, hydroxylated metabolites of phenanthrene, aromatic amines such as 1-nitropyrene and 3-nitro-benzanthrone. Half of the workers were smokers. The non-smokers excreted phenanthrene metabolites in a range of 4 µg/l in the urine, whereas the urinary levels in smokers were up to 3 times higher. In summary, it may be stated that there was an increase of PAH metabolism in the workers, probably by an induction of cytochrome P450. The smokers were identified by their higher amounts of excreted phenanthrene metabolites and 1-naphthylamine. The excreted amounts of aromatic amines were 5–10 times higher than expected (Seidel et al. 2002).

The urine of 40 underground workers aged between 23 and 54 years who had been exposed to diesel engine emissions in an oil shale mine for 2 years and more was examined over one working week. Adjustment was made for smoking, eating habits, exposure to lubricating oil and the use of open fire. The control group consisted of 38 surface workers. The underground concentration of 1-nitropyrene in the air was 8 times higher than above ground; measurement was person-related. The excretion of S-phenyl mercapturic acid and trans,trans-muconic acid significantly increased in

the underground workers during the working week. A higher O⁶-alkylguanine DNA adduct rate was also measured in the leukocytes of the underground workers as compared with the surface workers. No differences were found in the measurement of other DNA adducts (Scheepers et al. 2002). An accumulation of 5-aminolaevulinic acid, a significant increase of 5-aminolaevulinic acid activity and a significant increase of protoporphyrine was also observed in the lymphocytes of the underground workers. Ferrochelatase activity was significantly reduced in the lymphocytes of the underground workers. A comparison between smokers and non-smokers of the two groups (surface and underground workers) revealed a significant difference for all measured parameters. No difference between the measured parameters was observed within the group of underground or surface workers in relation to the period of exposure (more than 10 years or less), but there was a significant difference between the groups (Muzyka et al. 2004).

The biomonitoring data can be assessed only to a very limited extent since, because of PAH exposure, the increase in the DNA adduct rate was significant only at much higher concentrations when the different confounders were taken into account.

Genotoxicity

Urinary samples of 87 railroad workers and employees categorized according to their workplace were examined for mutagenicity. A modified *Salmonella* mutagenicity test was used as a method of detection. The number of cigarettes smoked and diet were taken into account on the day of sampling. The workers were questioned for the use of medications, exposure apart from the workplace, diet, cigarette consumption and lifestyle. Urinary mutagenicity was not increased in the exposed persons as compared with the non-exposed persons (Schenker et al. 1992).

No significant increase in the urinary thioether concentration or mutagenicity was measured in a total of 89 workers in bus garages, on roll-on roll-off ships or on car ferries. No increase in the urinary thioether concentration or mutagenicity was found 4 and 8 hours and on the morning following exposure in 6 workers who were directly exposed to diesel engine emissions for 3 hours and 40 minutes (Ulfvarson et al. 1987).

The 24-hour urine and faeces of 8 car mechanics exposed to diesel engine emissions during their work were examined for mutagenicity. No increased mutagenicity was observed as compared with a control group of office workers; faecal mutagenicity was even higher in the office workers. The authors attributed this to their different diet (Willems et al. 1989).

The urinary excretion of 8-oxo-2-deoxyguanosine and a UDS test in lymphocytes was carried out in a total of 57 bus drivers (all non-smokers; 43 men/14 women; 30 from central Copenhagen and 27 from rural/suburban areas). A significantly increased excretion of 8-oxo-2-deoxyguanosine was found in bus drivers from central

Copenhagen as compared with those from rural/suburban greater Copenhagen. The UDS test was negative (Loft et al. 1999).

Allergenic effects

Epidemiological studies on asthma and diesel engine emissions show that particularly children living near busy roads have an increased risk of developing asthma and asthma-like symptoms (Brunekreef et al. 1997; Ciccone et al. 1998; Duhme et al. 1996). It must be considered that diesel engine emissions are a complex mixture of substances, some of these individual substances being known irritants (*e.g.* SO₂, NO₂ and PM₁₀). The question whether the increase of allergic diseases, in particular asthma, in industrial nations is caused by diesel engine emissions remains unanswered (Parnia and Frew 2001). The impact of occupational exposure to diesel engine emissions was associated in 3 case reports (Wade and Newman 1993). In short-term human studies, asthmatics showed increased bronchial hyperreactivity and an increase in sputum levels of IL-6 after inhalation of diesel engine emissions (Nordenhäll et al. 2001); no allergic mechanism is assumed here. A great deal of studies are available regarding the modes of action of diesel engine emissions on the respiratory immune system. Diesel engine emissions can stimulate the formation of TH2 lymphocytes (IL-4, IL-5, IL-6 and IL-10) and IgE production, have an effect stimulating eosinophils and increase the expression of chemokines and the formation of oxidants by diesel engine emissions. Furthermore, an adjuvant effect of diesel engine emissions has been demonstrated in sensitization studies in various animal models and humans (reviewed in Pandya et al. 2002).

In spite of these associations between diesel engine emissions and asthma, diesel engine emissions are not designated with “Sa” since they are not allergens themselves.

Animal Experiments and in vitro Studies

Subacute, subchronic and chronic toxicity

Inhalation

Only those studies using several concentrations will be described below. Studies with only one concentration are shown in Table 7.

Groups of 62 male and 62 female F344 rats were exposed to the diesel engine emissions of a 1.8-litre light duty (LD) engine at soot particle concentrations of 0.1, 0.4, 1.1 and 2.3 mg/m³ or to the diesel engine emissions of a 11-litre heavy duty (HD) engine at soot concentrations of 0.5, 1.0, 1.8 and 3.7 mg/m³.

Table 7 Effects of diesel engine emissions after repeated inhalation

Species, strain, No. per group	Exposure	Findings	References
rat, F344, No. of animals n.s. ♂	24 months, 2 mg soot/m ³ , 7 h/d, 5 d/w	slight effects on viability, mean cell count, oxygen consumption, membrane integrity, lysosomal enzyme activity and protein content of macrophages; dose-dependent decrease in the phagocytic activity of the alveolar macrophages	Castranova et al. 1985
rat, F344, 24 ♀	24 months, total exhaust: haust: 4.9 mg soot/m ³ , gaseous phase 8 h/d, 7 d/w 6-month observation	total exhaust 6 months: body weight gain ↓; type II cell proliferation with adenomatous metaplasia 12 months: rel. lung weight doubled; increased formation of foci; deposited particle mass ↑; degenerative alveolar macrophages ↑ 24 months: rel. lung weight tripled; fibrous thickening of the alveolar walls; infiltration of mast cells; epithelial hyperplasia gaseous phase 24 months: rel. lung weight ↑; no histopathological effects	Iwai et al. 1986
rat, Wistar, 92–96 ♀	lifetime, 4.2 mg total exhaust/m ³ , gaseous phase 19 h/d, 5 d/w	total exhaust: body weight gain ↓; mortality not affected; 94/95 bronchiolo-alveolar hyperplasia; 62/95 metaplasia of the bronchoalveolar epithelium 3 months: lung clearance already significantly ↓ 12 months: rel. lung weight ↑; inflammatory, hyperplastic and metaplastic changes; septal thickening; airway resistance ↑; dynamic lung compliance ↑ 21 months: lung lavage: LDH ↑; alkaline phosphatase ↑; acid phosphatase ↑; G6PDH ↑; total protein ↑; protease pH 5.1 ↑; collagen ↑; leukocytes ↑; granulocytes ↑; number of macrophages ↓ gaseous phase: no effects	Heinrich et al. 1986

Table 7 (Continued)

Species, strain, No. per group	Exposure	Findings	References
rat, F344, 221–230 ♂ and ♀ (n.s. how many ♂ and ♀ per group)	30 months, 0.35, 3.5, 7.1 mgsoot/m ³ ; 7 h/d, 5 d/w	no effects on body weight or survival from 0.35 mg/m³ in relation to the concentration: accumulation of soot in the lung; chronic inflammation; epithelial hyperplasia; squamous cell metaplasia adjacent to fibrotic areas; focal fibrotic and proliferative changes of the lung; major fraction of the pulmonary parenchyma normal	Mauderly et al. 1987
rat, F344, 144♂ and ♀	24 months, 2 mg/m ³ , 7 h/d, 5 d/w	alveolar type II cell hyperplasia; inhibition of long-term clearance; time-related increase of accumulations of black macrophages in the alveolar ducts; pulmonary lipidosis; increase of banded cells; no effects on body weight or mortality immunological parameters: aggregations of particle-loaded macrophages particularly in the alveoli, slightly in the interstitium; inflammatory reaction and septal fibrosis (see also monkey below)	Lewis et al. 1986, 1989 Nikula et al. 1997 (assessment of material of study by Lewis et al. 1986, 1989)
rat, F344, 34–80 ♂	24 months, 3.5 mg/m ³ , 7 h/d, 5 d/w	comparison of normal and emphysematous rats: emphysematous animals showed no increased susceptibility since less soot accumulated	Mauderly et al. 1990
rat, F344, 48–168 ♀	6, 12 months, 9.4 mg soot/m ³ , 9.4 mg/m ³ filtered exhaust, 8 h/d, 7 d/ w, 3–6-month observation	soot: 6 months: hyperplasia of the terminal bronchial epithelia, with dilation into the alveolar ducts, associated with bronchiolization; cuboidal hyperplasia with particle accumulations in the alveolar region; fibrosis of the alveolar walls with mast cell infiltration 12 months: proliferative areas in the alveolar walls, probably early stages of neoplastic changes; early neoplastic changes (adenoma) after 15 months filtered exhaust: no effects	Iwai et al. 1997

Table 7 (Continued)

Species, strain, No. per group	Exposure	Findings	References
rat, F344, 48–50 ♀	1–12 months, 3.5 mg soot/m ³ , 17 h/d, 3 d/w, 18-month observation	1 month: few particle-loaded macrophages; maximum DNA adduct formation, then decreasing 3 months: many particle-loaded macrophages, hypertrophy of the alveolar epithelium 6 months: further increase in particle retention; bronchiolization of the alveolar ducts; hyperplasia of the cuboidal alveolar epithelium; slight fibrotic thickening of the alveolar walls; pre-neoplastic changes 12 months: macrophages severely particle-loaded; hyperplastic, slightly atypical alveolar epithelia, in some cases adenomatous; associated with interstitial fibrosis; still about 60% of DNA adducts 1 month after exposure; observation of fibrosis of the alveolar walls after 30 months; neoplastic changes 30 months: particle load 58–78% of the specific values at the end of exposure	Iwai et al. 2000
mouse, NMRL , 96 ♀	lifetime, total exhaust: 4.2 mg soot/m ³ , gaseous phase 19 h/d, 5 d/w	total exhaust: body weight gain ↓; mortality not affected; rel. lung weight ↑; 64% bronchiolo-alveolar hyperplasia; 71% multifocal alveolar lipoproteinosis; 43% multifocal interstitial fibrosis gaseous phase: no effects	Heinrich et al. 1986
mouse, no other details	34 weeks, 0.3, 1.0, 3.0 mg/m ³ , 12 h/d, 7 d/w	dose-dependent increase in cell proliferation of non-ciliated cells and hyperthrophy of epithelial cells in the airways; in BALF: total cell count ↑; macrophages ↑; neutrophils ↑	Ichinose et al. 1998
Syrian golden hamster, 48 ♀	lifetime, total exhaust: 3.9 mg soot/m ³ , gaseous phase 7 h/d, 5 d/w	total exhaust: proliferative changes in the lung; 60% adenomatous gaseous phase: no effects	Heinrich et al. 1982

Table 8 (Continued)

Species, strain, No. per group	Exposure	Findings	References
Syrian golden hamster , 96 ♀	lifetime , total exhaust: 4.2 mg soot/m ³ , gaseous phase 19 h/d, 5 d/w	total exhaust : mortality not affected; rel. lung weight ↑; 24 months : lung lavage: LDH ↑; alkaline phosphatase ↑; acid phosphatase ↑, G6P DH ↑ and total protein ↑; protease pH 5.1 ↑; collagen ↑, leukocytes ↑, granulocytes ↑, lymphocytes ↑, bronchiolo-alveolar hyperplasia, emphysematous lesions and al- veolar septal thickening; increase of airway resistance and slight reduction of lung compliance gaseous phase : no effects	Heinrich et al. 1986
monkey , cynomolgus , 15 ♂	24 months , 2.0 mg/m ³ , 7 h/d, 5 d/w	perivascular, peribronchial and alveolar particle accumulation; slight respiratory obstruction multifocal particle distribution, more in the interstitium than in the alveoli; no hyperplastic, inflammatory or fibrotic reactions	Lewis et al. 1989 Nikula et al. 1997 (as- sessment of material of study by Lewis et al. 1989)
cat , n.s. 25 ♂	24 months , 6.0–12 mg/m ³ , 8 h/d, 7 d/w	123 weeks : signs of restrictive lung changes: inspiration capacity ↓; vital capacity ↓; total lung capacity ↓; diffusion capacity ↓; respiratory mechanics not affected; reversible bronchiolar epithelial metaplasia; fibrosis	Moormann et al. 1985

n.s.: not specified
LDH: lactate-dehydrogenase
G6P DH: glucose-6-phosphate-dehydrogenase

For exhaust generation, the engines were operated at constant engine speed and load. Furthermore, groups of 64 male rats were exposed to soot at 0.4 or 4 mg/m³ and particle-free diesel engine emissions of the HD engine at 0.4 or 4 mg/m³. The exposure period was consistently 16 hours/day, 6 days/week, for 6 to 30 months. Inhalation of the HD diesel engine emissions led to reduced body weight gain in relation to the concentration in both sexes; the body weight of the high concentration groups was 10% to 20% below that of the controls. Feed and water consumption were also lower. Mortality was not affected in any group.

The following effects were observed in relation to the dose from 0.4 mg/m³ LD engine and 0.5 mg/m³ HD engine. The only findings among the numerous haematological and clinicochemical parameters that were measured were decreases in the cholinesterase activity and in free cholesterol and phospholipid. After 6 months, anthracosis initially occurred as a black pigment in the alveolar spaces and gradually appeared as black discolouration of the lung surface. Diesel particles were found in the interstitial tissue, penetrated the alveolar walls and were deposited in regional and mediastinal lymph nodes. The most intense discolouration was observed in the animals of the 1.8-mg/m³ HD group. Particle-loaded macrophages covered proliferating type II epithelial cells of the alveolar walls; the latter developed into "glandular" metaplastic foci. The alveolar walls were thickened by infiltrating macrophages, plasma cells and locally increased collagen fibres. The changes were only slightly pronounced at sites where no alveolar macrophages had accumulated. However, type I epithelial cells phagocytized the particles here and absorbed them into the cytoplasm. Type II epithelial cells also showed hypertrophy with increased microvilli and distended lamellar inclusion bodies. Carbon-loaded macrophages were occasionally found in the interstitium of the alveolar walls accompanied by oedematous swelling and a slight increase of collagen fibres. Together with anthracosis, hyperplasia of type II epithelial cells and the bronchial epithelium occurred focally in the first 12 months, but spread to the alveolar spaces after 18 months and merged with other focal areas to form a diffuse pattern. In regions of diffuse hyperplasia, there were sometimes papillary and sometimes extensive epithelial proliferations, which were difficult to differentiate from adenomas. Epithelial metaplasia with focal interstitial fibrosis was observed in hyperplastic regions of the subpleural zone. The epithelial cells normally lined with cilia were shortened and showed absence of their cilia in the trachea and main bronchi. Clara cells of the distal region of the respiratory tract revealed irregular protrusions or hypertrophic foci in the middle of areas of cilia-free epithelial cells. These lesions occurred in both the animal groups exposed to total diesel engine emissions and the groups exposed only to the gaseous components of the diesel engine emissions. The authors concluded that the described pathological changes were caused mainly by the gaseous components.

The explanation given in the discussion of the publication is important for establishing a NOAEL. It is based on the finding that, in the LD groups, hyperplasia of type II epithelial cells was observed in 70% of the animals of the 2.3-mg/m³ group, in 9.8% of the 1.1-mg/m³ group and in fewer than 5% of the animals of the 0.4-mg/

m^3 group, 0.1-mg/m^3 group and control group after 30-month inhalation. The corresponding values for the HD groups were: 20.2% in the 3.7-mg/m^3 group, 11.4% in the 1.8-mg/m^3 group and less than 6% in the 1.0-mg/m^3 and 0.5-mg/m^3 groups and in the control group.

The incidences of hyperplasia of type II epithelial cells observed after 30 months were far higher than the specific values measured after 24 months. This underlines the relevance of this hyperplasia. The authors did not specify the distribution of hyperplasia of type II epithelial cells among the individual groups; it probably started at a lower exposure concentration and must definitely also be regarded as an adverse effect. In particular, the question remains as to whether the 0.1-mg/m^3 group revealed any degree I hyperplasia (Ishinishi et al. 1986, 1988). Groups of 60 male Wistar rats were exposed to diesel engine emissions at a soot level of 0.2, 1.2 or 3.0 mg/m^3 or the gaseous phase of the diesel engine emissions with NO_2 at 1.1 ml/m^3 for 16 hours/day, 6 days/week, for 24 months. The histopathological examination revealed slight anthracosis in the bronchioles from 0.2 mg/m^3 after 12, 18 and 24 months. Anthracosis resulted from infiltrations of a large number of macrophages that had phagocytized diesel particles. Goblet cells with increased mucus formation were observed after 12 and 18 months; however, only few cells of this type were found. In the alveolar region, type II epithelial cells and hyperplasia of type II epithelial cells increased with slight severity after 6, 12 and 18 months and with the next higher severity of mild after 24 months. Hyperplasia covered the walls of the alveoli in the regions where alveolar macrophages had accumulated. Anthracosis with slight severity was found after 6, 12 and 18 months and with the next higher severity of mild after 24 months. Slight bronchiolization occurred in relation to the concentration and time after 24 months. This change was not observed either in the control animals or in the animals that were exposed only to the gaseous phase. After 18 months, inflammatory cells such as particle-loaded alveolar macrophages, mast cells and lymphocytes infiltrated the interstitium of the alveolar walls. The mentioned cells showed cell-to-cell contact, which the authors considered to be unusual. Furthermore, holes were found in the alveolar walls as a sign of lung destruction.

No alveolar changes occurred in the rats that were only exposed to the gaseous phase. Shortening of the cilia (starting with the 6th month), hyperplasia of Clara cells (after 18 and 24 months) and flattening of the epithelial cells (after 18 and 24 months) were observed in the trachea, bronchioles and terminal bronchioles. Furthermore, the rats of this group showed no effects in the bronchus-associated lymphoid tissue, which is a sign that the particles themselves or particle-loaded macrophages induced the infiltration of inflammatory cells (Kato et al. 2000). No NOAEL can be derived from this study either.

Groups of 24–30 male Wistar rats were exposed to diesel engine emissions at a soot level of 0.2, 1.1 or 2.8 mg/m^3 for 16 hours/day, 6 days/week, for up to 24 months. The diesel engine emissions were produced by 2 engines that ran under standard conditions. The emissions had to be diluted. The mass median diameter of 50% of the particles was between 0.3 and $0.5\text{ }\mu\text{m}$. Another animal group was

exposed to the particle-free gaseous phase, which corresponded to a mean particle concentration of 1.1 mg/m^3 . In the 2.8-mg/m^3 group, the concentrations of these gases were about 2 to 3 times higher. Nevertheless, they did not reach the concentration ranges that caused irritation, except for NO_2 (3.2 ml/m^3). After 6, 12, 18 and 24 months, some animals were sacrificed and bronchoalveolar lung fluid and blood were examined for various biomarkers of inflammation. Animals of the 0.2-mg/m^3 group showed no deviation from the control animals at any time of observation: total cell count, number of macrophages, leukocytes, lymphocytes, total protein, prostaglandin E_2 , fucose, sialic acid and phospholipid.

In the 1.1-mg/m^3 group, the total cell count increased from the 12th month, the number of macrophages and leukocytes increasing as early as from the 6th month; lymphocytes were increased in the 6th, 12th and 24th months, but not in the 18th month. An increase of fucose was recorded from the 12th month; it did not continue in the observation period. Phospholipid was increased in the 12th and 18th months, but not in the 6th or 24th months. The 2.8-mg/m^3 group showed increases in the total cell count and in the number of macrophages and leukocytes at all times of observation, the intensity of the elevation increasing with time. The number of lymphocytes was slightly elevated only after 12 months; total protein showed an increase from the 12th month, whereas fucose increased from the 6th month as a function of time, and phospholipid and sialic acid were elevated from the 12th month.

In the animal group that inhaled particle-free diesel engine emissions, only a slight increase in the number of leukocytes was observed after 24 months. According to the authors, this finding shows that the particles rather than the accompanying gases induce the inflammatory process. Interaction of the particles with the alveoli is decisive, with the toxic effects occurring earlier in the alveolar region than in the tracheobronchial tree anyway. The increase of phospholipids in the lavage fluid is also a sign of this interaction. Phospholipids are secreted from alveolar epithelial cells. Typically, exposure to the gaseous fraction of diesel engine emissions does not lead to an increase of phospholipids. No deviations from the control values were detected either for the histamine marker or for arachidonic acid in any of the animal groups exposed or at any time of observation. The authors considered the NOAEL in the rat lung to be between 0.2 mg/m^3 and 1.0 mg/m^3 particle concentration of diesel engine emissions (Ishihara and Kagawa 2003).

F344 rats and CD-1 mice of both sexes were exposed to diesel engine emissions at a soot concentration of 0, 0.35, 3.5 or 7.0 mg/m^3 for 7 hours/day, 5 days/week, for 24 months. Unfortunately, no control group was used concurrently, which would have shown the effect of the particle-free gaseous phase of the diesel engine emissions. Lavage parameters were determined and the lungs examined histopathologically at intervals of 6 months. The authors stated that no significant biochemical or cytoplasmic changes whatsoever were found at the low soot concentration in either species. The two higher exposure concentrations of 3.5 mg/m^3 and 7.0 mg/m^3 induced a chronic inflammation of the lungs in rats and mice. The following signs of inflammation were found in the lavage fluid: increase of inflammatory cells,

with the number of neutrophilic leukocytes increasing far more than that of macrophages; increase of total protein; increase in the activity of cytoplasmic enzymes, such as lactate dehydrogenase and glutathione reductase; increase in the activity of lysosomal enzymes such as β -glucuronidase. An increase of hydroxyproline was observed in mice of the 3.5-mg/m³ and 7.0-mg/m³ groups at all times of examination; an increase was also observed in rats of the 7.0-mg/m³ group after 12, 18 and 24 months and in the 3.5-mg/m³ group after 16 and 24 months. The increase of hydroxyproline in the lavage fluid is a sign of degradation and conversion of the extracellular collagen matrix of the lungs. The low exposure concentration caused deviations of only individual parameters of the lavage fluid from the normal values. These parameters are considered to be sensitive markers.

In **mice**, major amounts of diesel soot in the lungs, increased values of β -glucuronidase, although only after 12 months, and a significant increase of the glutathione level were observed from the lowest exposure concentration of 0.35 mg/m³ as a function of the dose. At 0.35 mg/m³, an occasional accumulation of large soot-loaded macrophages, which in some cases filled the alveoli, occurred in rats and mice. The effect was observed more often at the next higher concentration of 3.5 mg/m³. In **rats**, increased values of β -glucuronidase and acid phosphatase were observed, although only after 18 months. Among the lavage parameters examined, β -glucuronidase was affected most in rats, too, and correlated with the degree of lung fibrosis. The authors arranged the observed changes chronologically and gave the following mechanistic explanation of their occurrence: macrophages were activated in the lungs to the same extent as soot accumulated in the lungs. They released chemotactic factors that attracted neutrophilic leukocytes. Then both leukocytes and macrophages produced mediators of inflammation and oxygen radicals. After exposure to 0.35 mg/m³, borderline signs of inflammation could still be detected. The authors derived a NOAEL of 0.35 mg/m³ for rats and mice from the study (Henderson et al. 1988).

In another inhalation study, female Wistar rats (24–200 per group), NMRI and C57BL mice (40–120 per group) were exposed to diesel engine emissions. The rats inhaled diesel engine emissions at a soot concentration of 0.8, 2.5 or 7.0 mg/m³ for 18 hours/day, 5 days/week, for 24 months and were observed in clean air for another 6 months at the most. NMRI and C57BL mice inhaled diesel engine emissions at soot concentrations of 4.5 and 7.0 mg/m³ or a specific dilution of the gaseous phase; control groups with the same number of animals were exposed to clean air. The NMRI mice of the high concentration group were exposed for 13.5 months and kept in clean air for another 9.5 months; the NMRI mice of the low concentration group were exposed for 23 months. The C57BL mice were exposed for 24 months and observed in clean air for another 6 months.

In **rats**, the body weight gain of the middle and high concentration groups was reduced from days 440 and 200, respectively. The lung weights of the high concentration group were increased from the 3rd month of the study and of the middle concentration group only after 22 to 24 months of exposure. After 24 months, the particle load of the lungs of the 3 exposure groups was 6.3, 23.7 and 63.9 mg/lung.

The half-life of lung clearance was prolonged in all 3 groups from the 3rd month of exposure related to time and concentration; in the high concentration group, it was prolonged by a factor of 7 after 18 months as compared with the controls. Observation for 3 months did not lead to any effect of recovery. Analysis of the lung lavage fluid after 24-month exposure showed distinct effects on the parameters of differential cell count, lactate dehydrogenase, glucuronidase, hydroxypyroline and total protein in the middle and high concentration groups. No more details were communicated. Histopathology revealed concentration-related incidences of bronchio-lo-alveolar hyperplasia and interstitial fibrosis as non-neoplastic changes; at the high concentration, they were even observed from the 6th month of exposure.

The **NMRI mice** that were exposed to diesel engine emissions at a soot concentration of 7.0 mg/m³ for 13.5 months had reduced body weight gain in the period from the 6th to 17th months; afterwards, there was no significant difference from the control. After 19 months, 50% of the mice that had only been exposed to the gaseous phase died; in the control animals, this was the case after 20 months. The particle load of the lungs was increased after 3, 6 and 12 months, *i.e.* 1.7, 4.1 and 7.0 mg/lung; the lung weights were increased after 3 and 12 months of exposure. **NMRI mice** that had inhaled diesel engine emissions at a soot concentration of 4.5 mg/m³ or a specific dilution of the particle-free gaseous phase showed reduced body weight gain as compared with the controls after 12 months. Mortality was slightly increased in the group exposed to diesel engine emissions; 50% was reached after 19 months as compared with 20 months in the gaseous phase and control. After 6 months, the lung weights were twice as high as those of the control and were increased by a factor of 3.5 after 18 months. The particle load of the lungs of this group was 0.9, 2.4, 4.0 and 5.9 mg/lung load after 3, 6, 12 and 18 months, respectively. **C57BL mice** that had inhaled diesel engine emissions with a soot level of 4.5 mg/m³ or the particle-free gaseous phase diluted to the same extent showed slightly reduced body weight as compared with the controls from day 400. In the diesel engine emission group, 50% mortality was reached after 25 months and in the two other groups after 27 months. In these mice, the particle load of the lungs was 0.8, 2.3, 3.5, 4.3 and 5.5 mg/lung after 3, 6, 12, 18 and 21 months, respectively (Heinrich et al. 1995).

ICR mice were exposed to diesel engine emissions at a soot concentration of 0.3, 1.0 or 3.0 mg/m³ for 34 weeks. Histopathology revealed a concentration-dependent increase of lymphocytes, proliferation of non-ciliated cells and epithelial cell hypertrophy in the airways from a soot concentration of 1.0 mg/m³. After exposure to diesel engine emissions, total cell counts, macrophages and neutrophils were increased in the lung lavage fluid in relation to the concentration (Ichinose et al. 1998).

In another study, the effects after exposure to diesel engine emissions were investigated in 72 female and 72 male F344 rats and Syrian golden hamsters using a 1.5-litre VW engine. Exposure was carried out for 16 hours/day, 5 days/week, for a maximum of 24 months. Some of the rats were observed in clean air for another 6 months. Controls were kept in clean air. The exposure concentrations to soot were

0.7, 2.2 or 6.6 mg/m³. No effects whatsoever were found in further groups that were exposed to the gaseous phase. In the course of the study, the **rats** of the middle and high concentration groups showed a time- and concentration-dependent reduction of body weight gain, which was more pronounced in the males than in the females. At necropsy, an increase in the lung weights was detected in both rats and hamsters. In rats, this effect was more pronounced and was time- and concentration-related. Urinalyses did not reveal any effects in either species. Clinicochemical and haematological examinations were carried out in 8 male and 8 female rats after 6, 12, 18 and 24 months. After 24 months, the high concentration group showed reduced blood levels for glucose and cholesterol in both sexes, for total protein and triglycerides in the females and for cholinesterase in the males. The blood levels of urea nitrogen and alkaline phosphatase were increased in both sexes, and those of alanine and aspartate aminotransferase only in the females. After 18-month exposure, the haematological examinations of the high concentration group revealed increases in the erythrocyte and leukocyte counts, haemoglobin concentration, haematocrit value, prothrombin time and number of segmented neutrophils and decreases in the number of lymphocytes in both sexes in most cases. These effects were more pronounced in the females and were also observed after 24 months, whereas hardly any effects were detected in the males after 24 months. Cardiovascular examinations carried out in male rats after 24-month inhalation revealed a significantly increased body weight/heart weight ratio, an increased weight ratio of the right ventricle to total heart weight and reduced contractility of the left ventricle.

In **hamsters**, clinicochemical and haematological examinations were carried out after 6 and 16 months. Increased blood levels of γ -glutamyl transpeptidase were found in both sexes of the high concentration group after 16 months. Only the females revealed increased levels for albumin and reduced levels for potassium, lactate and α -hydroxybutyl dehydrogenase and aspartate aminotransferase. Haematologically, increased haemoglobin levels and an increased haematocrit were found only in the females (Brightwell et al. 1986, 1989).

Groups of 24 male **guinea pigs** were exposed to diesel engine emissions at particle concentrations of 0.25, 0.75, 1.5 or 6.0 mg/m³ for 20 hours on 5.5 days/week up to 24 months. Control animals inhaled clean air. A 5.7-l engine, which was operated at constant engine speed and load, was used for exhaust generation. The following effects were observed from 0.75 mg/m³. Examinations by light microscopy showed that pigmented macrophages were scattered in the lungs and occasionally aggregated at the end of the terminal bronchioles. Pigmentation caused by particles was also observed in lymphatic tissue close to the points of transition between bronchioles and alveoli. After 6-month exposure, there was pronounced proliferation of type II epithelial cells. In macrophages and type I epithelial cells, the phagocytized particles were not free in the cytoplasm, but were packed in lysosomes. No signs of cytotoxicity, such as cytolysis, were detected. There were hardly any changes in the alveolar wall structure after exposure. After 2-week exposure to 0.75 mg/m³, the number (hyperplasia) and size (hypertrophy) of type II epithelial cells increased in

several alveoli. Particle-containing phagosomes were detected in alveolar macrophages as early as 2 weeks after exposure to 0.75 mg/m^3 . Reactive monocytes, the precursor cells of macrophages, also contained diesel soot particles, although their number was smaller than in macrophages. The particles were not cytotoxic to either type of cells. Type I epithelial cells contained particles as early as 2 weeks after the beginning of exposure to 0.75 mg/m^3 , with the number of particle-containing type I cells increasing with the exposure concentration and duration. The fate of type I cells with phagocytic vesicles is not known. However, there are data indicating that the particles were discharged again, for example into the interstitium, where they found their way into peribronchial and perivascular lymphatic tissue after having been absorbed by macrophages. In guinea pigs, the inflammatory reaction was characterized by eosinophilic leukocytes rather than by neutrophilic leukocytes as in rats. The number of cells in the alveolar wall interstitium increased after exposure to both 1.5 mg/m^3 and 0.75 mg/m^3 . Specifically, these were fibroblasts, monocytes, eosinophilic leukocytes, plasma cells and macrophages. The number of cells that accumulated in the perivascular and peribronchiolar space of the interstitium was larger than in the alveolar wall interstitium. This alteration was not observed in the 0.25-mg/m^3 group.

The most pronounced lung changes were evident in the form of an increase in the tissue volume. In the 0.75-mg/m^3 group, increases of 56% were measured after 2 weeks of inhalation, 41% after 3 months and 39% after 6 months. The increase remained at this level during the 1-year exposure period. In the 1.5-mg/m^3 group, the increase was up to 112% within 6 months and levelled out to about 80% after 18-month exposure. No increase was recorded for the 0.25-mg/m^3 group either after 9 or after 24 months. Considerable increases were also observed for the areal density of the alveolar epithelial cells and their cell count. This applied particularly to type II epithelial cells, whose number doubled and tripled in the 0.75-mg/m^3 group. The tissue thickness of the alveoli-capillary barrier, which determines the gas exchange, also increased in the exposed animals: in the 0.75-mg/m^3 group, increases of 41% were measured after 2 weeks, 46% after 3 months and 77% after 6 months. The value for the 1.5-mg/m^3 group was 130% after 6 months. Exposure to 0.25 mg/m^3 induced relatively slight tissue changes, with the exception of a significant 30% increase in the number and volume of alveolar macrophages, type I and type II epithelial cells and endothelial cells after 9 months. Although these increases continued up to the 24th month, they were at a significant level only for type I epithelial cells and the number of alveolar macrophages. The authors attributed the observed lung changes to the soot fraction of the diesel engine emissions rather than to the gas fraction. They substantiated this by stating that lung effects described in other publications (e.g. proliferation of type II epithelial cells) were induced by NO_2 concentrations that were 33 to 83 times higher than those used in their own study (Barnhart et al. 1981, 1982).

Groups of 8–10 **guinea pigs** were exposed to diesel engine emissions at 0.2, 1.09 or 2.82 mg/m^3 on 6 days per week, 16 hours per day, for 24 months. A separate group was only exposed to the gaseous phase of the emissions. The diesel engine

emissions were produced by 2 engines (cubic capacity: 7.4 l), which ran without a break in line with a standardized load. The median of the particle diameters was between 0.3 μm and 0.5 μm . The lavage fluid was investigated for the cell count and further markers of inflammation after 6, 12, 18 and 24 months. The results demonstrated that long-term exposure to diesel engine emissions caused chronic inflammation in the lungs of guinea pigs, induced overproduction of mucus and phospholipids and led to an increase of the bronchoconstrictor leukotriene C_4 . The latter was also increased in the blood. Significant changes were only observed in the medium and high concentration groups after one year at the earliest. The gaseous phase without particles did not induce any significant findings except for an increase of leukotriene C_4 in the blood. This indicates that toxic irritation was almost only caused by the particles. The number of eosinophilic leukocytes – but not that of alveolar macrophages or neutrophilic leukocytes – increased in the medium and high concentration groups from the 12th month of exposure. The same applied to lactate dehydrogenase, the marker of cell damage. Total protein reacted less sensitively, its concentration increasing in the highest concentration group only from the 12th month of exposure and in the middle concentration group not before the end of exposure. Fucose, sialic acid and phospholipid were increased in the highest concentration group from the 12th month of exposure, and fucose and phospholipid were also increased in the middle concentration group from the 18th month. Sialic acid production was elevated in the middle concentration group only after 2 years. Among the various leukotrienes and prostaglandins that were investigated, significant changes were only observed for leukotriene C_4 : in the high and middle exposure groups, it was increased both in the blood and lavage fluid after 2 years, and in the blood of the animals of the high exposure group as early as after 18 months. The authors considered the NOAEL in the guinea pig lung to be 1.0 mg/m^3 diesel particles of diesel engine emissions (Ishihara and Kagawa 2002).

Summary of the animal studies:

In rats, hyper- and metaplastic changes and fibroses were the most frequent changes found (Heinrich et al. 1986; Ishinishi et al. 1986, 1988; Lewis et al. 1986, 1989; Mauderly et al. 1987). Other commonly observed findings were body weight loss at high concentrations (Ishinishi et al. 1986, 1988; Iwai et al. 1986; Brightwell et al. 1986, 1989), increases in lung weights (Brightwell et al. 1986, 1989; Heinrich et al. 1986; Henderson et al. 1988; Iwai et al. 1986) and proliferative changes (Iwai et al. 1986; Ishinishi et al. 1986, 1988; Mauderly et al. 1987). Changes in mechanical lung function parameters (Heinrich et al. 1986) and lung lavage fluid parameters (Henderson et al. 1988), inhibition of long-term clearance (Lewis et al. 1989), a decrease in the phagocytic activity of the alveolar macrophages (Castranova et al. 1985) and oedematous effects (Ishinishi et al. 1986, 1988) were described in further studies. Rats with emphysematous damage did not react more sensitively than ani-

mals without emphysematous damage since they accumulated less soot (Mauderly et al. 1990).

Genotoxicity

Human lymphocytes were incubated with diesel particle extraction at 1 µg/ml for 18 hours. Various DNA adducts were detected, but not specified in detail. On the basis of its migration rate in the autoradiogram, one adduct was identified as the benzo[*a*]pyrene DNA adduct as compared with the adducts obtained with benzo[*a*]pyrene after incubation of human lymphocyte DNA (Gallagher et al. 1993).

Lymphocyte cultures of 4 volunteers (non-smokers) were exposed to diesel engine emissions for 16, 48 and 160 minutes. The SCE rate was significantly increased in lymphocytes from 2 volunteers after 160 minutes (Tucker et al. 1986).

Carcinogenicity

Inhalation studies in rats

The results of a number of inhalation studies in rats, mice, hamsters and monkeys were published in 1986. However, evidence of a carcinogenic potential of diesel engine emissions has up to now only been provided in rats. Table 8 shows the exposure conditions and results of the studies in rats. In this species, significantly increased lung tumour incidences occurred at a particle concentration of about 2 mg/m³ and above after exposure periods of at least 24 months. Adenomas, squamous cell tumours and adenocarcinomas were the types of tumours mentioned most frequently (Brightwell et al. 1986, 1989; Heinrich et al. 1986, 1995; Ishinishi et al. 1986, 1988); squamous cell carcinomas also occurred (Mauderly et al. 1987; Nikula et al. 1994, 1995).

In several studies, evidence of a concentration-response relationship was provided between tumour incidences and soot diesel lung burden (Brightwell et al. 1986, 1989; Heinrich et al. 1995; Mauderly et al. 1986, 1987; Nikula et al. 1994, 1995). Only these studies will be described in detail below.

Groups of 72 female and 72 male F344 rats were exposed to diesel engine emissions (soot) at 0, 0.7, 2.2 or 6.6 mg/m³ using a 1.5-litre VW engine. Groups of 16 animals were exposed for 6, 12, 18 and 24 months and then sacrificed; the remaining animals either died during the study or were sacrificed after the 6-month observation period. Incidences of primary lung tumours of 1.4, 0.7, 9.9 and 38.5% were obtained for both sexes together. The tumour incidences of the female rats were higher than those of the males in relation to the concentration. They were 1, 0, 15 and 54% and 2, 1, 4 and 23%, respectively. The tumour data included animals that were sacrificed after 6, 12, 18 and 24 months. Therefore, they do not correctly show

the incidences that would have been obtained if all animals had survived up to their natural death. If the animals of the last interval had only been evaluated, tumour incidences of 96% (24/25), 76% (19/25) of which were classified as malignant, would have been obtained for the females of the highest concentration group, and a total of 44% (12/27), 37% (10/27) of which were malignant, would have been obtained for the males of the same group. In this study, too, different types of tumours occurred in several animals. The types of tumours that were detected included 40 adenomas, 35 squamous cell carcinomas, 19 adenocarcinomas, 9 adenomas or adenocarcinomas (definite classification not possible) and 1 mesothelioma (Brightwell et al. 1986, 1989).

Female Wistar rats were exposed to diesel engine emissions diluted at about 1:17 (4.2 mg soot/m³) or to the particle-free gaseous phase in the same dilution. A 1.6-litre engine was used for the generation of exhaust. No lung tumours occurred either in the animals of the control group or in those exposed to the gaseous phase.

Table 8 Carcinogenicity studies with diesel engine emissions in rats

Author:	Brightwell et al. 1986, 1989																							
Substance:	diesel engine emissions																							
Species:	male and female F344 rats																							
Administration:	inhalation																							
Concentration:	140–142 ♂ and 140–142 ♀: clean air; 72 ♂ and 72 ♀: 0.7, 2.2 or 6.6 mg soot/m ³																							
Duration:	16 h/d, 5 d/w, 24 months, 6-month observation, controls 30 months																							
Toxicity:	see text; no data on surviving animals																							
Tumours:	<table><tr><th colspan="5">exposure concentration (mg/m³)</th></tr><tr><th></th><th>0</th><th>0.7</th><th>2.2</th><th>6.6</th></tr><tr><td rowspan="2">primary lung tumours</td><td>♂ 3/140 (2%)</td><td>1/72 (1%)</td><td>3/72 (4%)</td><td>16/71 (23%)¹</td></tr><tr><td>♀ 1/142 (1%)</td><td>0/72</td><td>11/72 (15%)¹</td><td>39/72 (54%)¹</td></tr></table>					exposure concentration (mg/m ³)						0	0.7	2.2	6.6	primary lung tumours	♂ 3/140 (2%)	1/72 (1%)	3/72 (4%)	16/71 (23%) ¹	♀ 1/142 (1%)	0/72	11/72 (15%) ¹	39/72 (54%) ¹
exposure concentration (mg/m ³)																								
	0	0.7	2.2	6.6																				
primary lung tumours	♂ 3/140 (2%)	1/72 (1%)	3/72 (4%)	16/71 (23%) ¹																				
	♀ 1/142 (1%)	0/72	11/72 (15%) ¹	39/72 (54%) ¹																				
Author:	Heinrich et al. 1986																							
Substance:	diesel engine emissions																							
Species:	female Wistar rats																							
Administration:	inhalation																							
Concentration:	96 ♀: clean air; 92 ♀: gaseous phase; 95 ♀: 4.2 mg/m ³ (particle concentration of the total exhaust)																							
Duration:	19 h/d, 5 d/w, 35 months																							
Toxicity:	see Table 7																							
Tumours:																								

Table 8 (Continued)

	exposure concentration (mg/m ³)				
	0	gaseous phase		4.2	
surviving animals(24 months)	54%	58%	60%		
bronchioalveolar adenomas	0/96 (0%)	0/92 (0%)	8/95 (8%) ¹		
squamous cell tumours	0/96 (0%)	0/92 (0%)	9/95 (9%) ¹		
Author:	Heinrich et al. 1995				
Substance:	diesel engine emissions				
Species:	female Wistar rats				
Administration:	inhalation				
Concentration:	220 animals: clean air; 198 animals: 0.8 mg/m ³ ; 200 animals: 2.5 mg/m ³ ; 100 animals: 7.0 mg/m ³ (particle concentration of the total exhaust)				
Duration:	18 h/d, 5 d/w, 24 months, 6-month observation				
Toxicity:	see text				
Tumours:					
	exposure concentration (mg/m ³)				
	0	0.8	2.5	7.0	
surviving animals(24 months)	42%	45%	52%	47%	
primary lung tumours	1/220 (0.5%)	0/198 (0%)	11/200 (5.5%) ¹	22/100 (22%) ¹	
Author:	Ishinishi et al. 1986				
Substance:	diesel engine emissions				
Species:	64 male and 59 female Wistar rats				
Administration:	inhalation				
Concentration:	1.8-l engine: clean air; 0.1, 0.4, 1.0 or 2.0 mg/m ³ 11-l engine: clean air; 1.0, 0.4, 2.0 or 4.0 mg/m ³				
Duration:	16 h/d, 6 d/w, 30 months				
Toxicity:	see text				
Tumours:					
	exposure concentration (mg/m ³)				
DEE of 1.8 litre engine	0	0.1	0.4	1.0	2.0
surviving animals	♂ 80%	91%	77%	88%	75%
(24 months)	♀ 76%	86%	76%	76%	78%
lung adenomas	♂ 0/64	0/64	1/64 (2%)	0/64	0/64
	♀ 1/59 (2%)	1/59 (2%)	0/61	0/59	1/60 (2%)
lung carcinomas	♂ 2/64 (3%)	1/64 (2%)	0/64	3/64 (5%)	2/64 (3%)
	♀ 1/59 (2%)	1/59 (2%)	0/61	2/59 (3%)	0/60

Table 8 (Continued)

		exposure concentration (mg/m ³)				
		0	0.4	1	2	4
DEE of 11 litre engine						
surviving animals	♂	80%	81%	81%	84%	75%
(24 months)	♀	76%	76%	85%	80%	76%
lung adenomas	♂	0/64	0/64	0/64	0/64	0/64
	♀	0/59	0/59	0/61	0/59	0/60
lung carcinomas	♂	0/64	1/64 (2%)	0/64	3/64 (5%)	5/64 (8%) ¹
	♀	1/59 (2%)	0/59	0/61	1/59 (2%)	3/60 (5%)
Author:	Mauderly et al. 1986					
Substance:	diesel engine emissions					
Species:	male and female F344 rats					
Administration:	inhalation					
Concentration:	141: clean air; 138: 0.35 mg/m ³ ; 131: 3.5 mg/m ³ ; 143: 7.0 mg/m ³					
Duration:	7 h/d, 5 d/w, 30 months					
Toxicity:	survival unaffected; see text					
Tumours:		exposure concentration (mg/m ³)				
		0	0.35	3.5	7	
epithelial cysts		0%	0%	0%		5.6% ¹
lung adenomas		0%	0%	3.8% ¹		0.7%
adenocarcinomas		1.4%	0.7%	0.8%		9.8% ¹
all tumours		1.4%	0.7%	4.6%		16.1% ¹
Author:	Mauderly et al. 1987					
Substance:	diesel engine emissions					
Species:	male and female F344 rats					
Administration:	inhalation					
Concentration:	230: clean air; 223: 0.35 mg/m ³ ; 221: 3.5 mg/m ³ ; 227: 7.0 mg/m ³					
Duration:	7 h/d, 5 d/w, 30 months					
Toxicity:	see text; no significant change in survival					
Tumours:						

Table 8 (Continued)

		exposure concentration (mg/m ³)			
		0	0.35	3.5	7.0
epithelial cysts		0%	0%	2/221 (0.9%)	11/227 (4.9%) ¹
lung adenomas		0%	0%	5/221 (2.3%) ¹	1/227 (0.4%)
adenocarcinomas & squamous cell carcinomas		2/230 (0.9%)	3/223 (1.3%)	1/221 (0.5%)	17/227 (7.5%) ¹
all tumours		0.9%	1.3%	3.6% ¹	12.8% ¹
Author:	Kawabata et al. 1993				
Substance:	diesel engine emissions				
Species:	42–49 female F344 rats				
Administration:	inhalation				
Concentration:	4.7 mg/m ³				
Duration:	15 h/d, 3 d/w, 6, 12 and 24 months, 6-month observation				
Toxicity:	no data on survival				
Tumours:					
		exposure concentration (mg/m ³)			
		0	4.7 (6 months)	4.7 (12 months)	4.7 (24 months)
lung adenomas & adenocarcinomas		5/48 (10%)	1/45 (2%)	8/42 (19%)	6/49 (12%)
Author:	Nikula et al. 1994, 1995				
Substance:	diesel engine emissions				
Species:	105–109; male and female F344 rats				
Administration:	inhalation				
Concentration:	0, 2.5 and 6.5 mg/m ³				
Duration:	16 h/d, 5 d/w, 24 months				
Toxicity:	see Table 7				
Tumours:					
		exposure concentration (mg/m ³)			
		0	2.5	6.5	
surviving animals(23 months)	♂	13.8%	14.4%	5.8%	
	♀	35.6%	30.9%	26.7%	
lung adenomas	♂	1/109	2/105	47/106 ¹	
	♀	0/105	5/105	19/106 ¹	
adenocarcinomas	♂	1/109	1/105	3/106	
	♀	0/105	3/105	19/106 ¹	

Table 8 (Continued)

	exposure concentration (mg/m ³)			
	0	2.5	6.5	
squamous cell carcinomas	♂ 1/109	2/105	2/106	
	♀ 0/105	1/105	1/106	
Author:	Iwai et al. 1986			
Substance:	diesel engine emissions			
Species:	16–22; female F344 rats			
Administration:	inhalation			
Concentration:	0, gaseous phase or 4.9 mg/m ³			
Duration:	8 h/d, 7 d/w, 24 months			
Toxicity:	see Table 7; no data on survival			
Tumours:	exposure concentration (mg/m ³)			
	0	gaseous phase	4.9	
lung tumours (adenomas and	1/22 (4.5%)	0/16	8/19 (42%) ¹	
adenocarcinomas) splenic	2/22 (9%)	4/16 (25%)	4/19 (21%)	
lymphomas				
Author:	Iwai et al. 1997			
Substance:	diesel engine emissions			
Species:	19–168; female F344 rats			
Administration:	inhalation			
Concentration:	1st study: 9.4 mg/m ³ ; 2nd study: 3.2 mg/m ³ ; 3rd study: 5.1 mg/m ³			
Duration:	1st study: 8 h/d, 7 d/w, 24 months, observation up to 30th month			
	2nd study: 8 h/d, 6 d/w, 24 months, observation up to 30th month			
	3rd study: 18 h/d, 3 d/w, 24 months, observation up to 30th month			
Toxicity:	see Table 7; no data on survival			
Tumours:	exposure concentration (mg/m ³)			
	0	9.4	3.2	5.1 gaseous phase
lung tumours	5/121 (4%)	8/19 (42%) ¹	5/43 (12%)	40/96 (42%) ¹ 4/108 (4%)

Table 8 (Continued)

Author:	Iwai et al. 2000																												
Substance:	diesel engine emissions																												
Species:	48–50; female F344 rats																												
Administration:	inhalation																												
Concentration:	0 or 3.5 mg/m ³																												
Duration:	17 h/d, 3 d/w, 3, 6, 9 and 12 months, observation up to 30th month																												
Toxicity:	see Table 7																												
Tumours:	<table><tr><th colspan="6">exposure period (months)</th></tr><tr><th></th><th>0</th><th>3</th><th>6</th><th>9</th><th>12</th></tr><tr><td>surviving animals (> 18 months)</td><td>48/50</td><td>48/48</td><td>43/48</td><td>47/48</td><td>44/48</td></tr><tr><td>lung tumours</td><td>1/48 (2%)</td><td>0/48 (0%)</td><td>6/43 (14%)¹</td><td>19/47 (40%)¹</td><td>10/44 (23%)¹</td></tr></table>					exposure period (months)							0	3	6	9	12	surviving animals (> 18 months)	48/50	48/48	43/48	47/48	44/48	lung tumours	1/48 (2%)	0/48 (0%)	6/43 (14%) ¹	19/47 (40%) ¹	10/44 (23%) ¹
exposure period (months)																													
	0	3	6	9	12																								
surviving animals (> 18 months)	48/50	48/48	43/48	47/48	44/48																								
lung tumours	1/48 (2%)	0/48 (0%)	6/43 (14%) ¹	19/47 (40%) ¹	10/44 (23%) ¹																								

¹ $p < 0.05$

Among the rats exposed to total exhaust, 17/95 animals developed lung tumours, 8 of them being classified as bronchiolo-alveolar adenomas and 9 as squamous cell tumours; 8 of the latter were assessed as keratinizing cysts and 1 as a squamous cell carcinoma (Heinrich et al. 1986).

In another study with exhaust generation comparable to that in the study by Heinrich et al. 1986, no lung tumours occurred in female Wistar rats in the low concentration group of 0.8 mg/m³. The animals of the middle concentration group (2.5 mg/m³) had a bronchiolo-alveolar papilloma, 2 adenomas, 1 adenocarcinoma and 7 benign squamous cell tumours. In the highest concentration group, 22 animals developed lung tumours, with some animals showing different types of tumours. There was evidence of 4 adenomas, 5 adenocarcinomas, 2 squamous cell carcinomas and 14 cystic keratinizing squamous cell tumours (Heinrich et al. 1995).

Male and female Wistar rats were exposed to the diesel engine emissions of a 1.8-litre light duty (LD) engine at particle concentrations of 0.1, 0.4, 1.0 or 2.0 mg/m³ or to the diesel engine emissions of a 11-litre heavy duty (HD) engine at soot concentrations of 0.4, 1.0, 2.0 or 4.0 mg/m³. The tumour incidences were not significantly increased in the LD groups as compared with the control group. No lung adenomas were observed in the animals of the HD groups, but the incidences of lung carcinomas were increased in the females at 4 mg/m³; this finding was significant in the males. According to Japanese definitions, adenocarcinomas and squamous cell carcinomas were established as tumour types. The publication only provides data on the number of animals bearing adenomas and carcinomas (Ishinishi et al. 1986, 1988).

Two further studies substantiated the carcinogenicity of diesel engine emissions in male and female F344 rats exposed to diesel engine emissions. A 5.7-litre engine was used for the generation of exhaust. The total tumour incidence was significantly increased in the middle and high concentration groups as compared with the values of the controls and low concentration group. Adenocarcinomas and squamous cell carcinomas were found as tumour types in 2 animals (0.9%) of the control group and 3 animals (1.3%) at 0.35 mg/m³. The middle concentration group (3.5 mg/m³) revealed adenomas in 5 rats (2.3%), 1 adenocarcinoma and 1 squamous cell carcinoma in 1 rat (0.5%) and epithelial cysts in 2 animals (0.9%). In the high concentration group (7 mg/m³), 1 adenoma (0.4%), 17 adenocarcinomas and 1 squamous cell carcinoma (7.5%) and 11 epithelial cysts (4.9%) occurred. There were no metastases in the lymph nodes or any other organs (Mauderly et al. 1987). A concentration-related increase of lung adenomas and adenocarcinomas was observed in male and female F344 rats after exposure to diesel engine emissions at soot concentrations of 0, 2.5 and 6.5 mg/m³. At the high concentration, the elevated incidences of lung adenomas were significant in both sexes and those of adenocarcinomas were significant in the females only. There was no increase of squamous cell carcinomas. At the end of the exposure period, the lung burden of the females was about 37 and 81 mg soot and that of the males about 45 and 90 mg in the specific concentration groups (Nikula et al. 1994, 1995).

In a workshop organized by the DFG (Deutsche Forschungsgemeinschaft) in February 1995, 11 international lung pathologists discussed the nature and classification of cystic keratinizing squamous lesions of samples from 13 different studies with 11 different components (Boorman et al. 1996). The pathologists jointly investigated and assessed 61 preparations of 56 rats showing about 100 keratinizing lesions. A consensus was reached that the spectrum of cystic keratinizing lesions represents a family of changes that shows many morphological similarities. These changes generally occurred at a low incidence (max. 20%), only after an exposure period of more than 20 months, at the highest exposure concentrations and mostly in females. The joint assessment showed that these were related morphological changes, varying from squamous cell metaplasia to invasive squamous cell carcinoma. The tendency to form large, keratin-containing cysts was their common feature. Diagnostic criteria were described and a uniform terminology suggested for the following variants: pulmonary squamous cell metaplasia, pulmonary keratin cysts, pulmonary cystic keratinizing epitheliomas and pulmonary squamous cell carcinomas (Boorman et al. 1996). In spite of these common definitions, there are still uncertainties and large individual differences, particularly as regards the classification of those changes that were described as pulmonary keratin cysts (benign) or pulmonary cystic keratinizing epitheliomas (malignant). Therefore, changes described prior to the publication of this uniform classification must be assessed with caution.

Intratracheal instillation

Several studies found significantly increased lung tumour incidences in female rats after intratracheal instillation of diesel soot (Dasenbrock et al. 1996; Iwai et al. 1997; Pott et al. 1994). On account of their unphysiological form of application, these studies will not be described in detail, although they also substantiate the carcinogenicity of diesel engine emissions in rats.

Inhalation studies in other species

A parent generation of male and female **Sencar mice** was exposed to diesel engine emissions at a soot concentration of 6 mg/m^3 until mating (5–10 weeks), birth of the offspring and weaning, for 8 hours a day, on 7 days per week. In the pups, the soot concentration was increased to 12 mg/m^3 from 12 weeks of age and exposure was continued until they were 15 months old. Exposure increased the lung adenoma incidence from 7.2% to 16.3% in the females and from 3.8% to 5.9% in the males. The summary increase in both sexes taken together was significant. The incidence of lung carcinomas was not affected by exposure. Under similar conditions (6 and 12 mg soot/m^3), **cats** exposed for 24 months developed no lung tumours (Pepelko and Peirano 1983).

In a study, 69–102 newborn male and female **ICR and C57BL mice** were exposed in addition to rats (Takemoto et al. 1986). The exposure conditions corresponded to those in rats (see Table 8), but exposure in mice started within 24 hours after birth and lasted up to 28 months. Animals kept in clean air were used as control. The animals that died or were sacrificed in the intervals from 3–6, 7–12, 13–18 and 19–28 months were examined by histopathology. In ICR mice, the first tumours were found in both groups after 12 months. At the end of the study, 8/102 (7.8%) of the exposed males and 6/72 (8.3%) of the exposed females had lung tumours as compared with 3/72 (4.2%) and 4/69 (5.8%) of the controls. Only 6 adenomas and 1 adenocarcinoma were observed in the controls, whereas the exposed animals revealed 10 adenomas and 4 adenocarcinomas. There were no significant differences in tumour incidences between the exposed animals and the control animals or between the sexes. Among the C57BL mice, only 1 male of 34 control animals had developed an adenoma by the end of the study, while there was an increase in tumour incidences in the exposed animals (9/126 males and 8/100 females), although it was not significant. Evidence was provided of 12 adenomas and 5 adenocarcinomas.

Groups of 96 female **NMRI mice** and female **Syrian golden hamsters** were exposed to 4.2 mg/m^3 diesel engine emissions of a 1.6-litre engine and to the particle-free gaseous phase of the emissions throughout their entire lives for 19 hours per day, 5 days per week. After 24-month exposure, the mortality rate of the mice was 63–95% and that of the hamsters was 67–100% (for further data on toxicity see Table 7). At the end of the study, the tumour incidence was 13% in the untreated

mice and 31% (gaseous phase) or 32% (diesel engine emissions) in the exposed animals. A differentiated assessment showed that there was no change in the incidence of adenomas versus the control animals, whereas the incidence of adenocarcinomas was significantly increased both by diesel engine emissions and by the gaseous phase alone. The spontaneous incidence of adenocarcinomas was 2.4% in the control animals. In the exposed animals, the tumour incidence was 19% for the gaseous phase and 17% for diesel engine emissions. No lung tumours occurred in the hamsters (Heinrich et al. 1986). In an earlier study with **Syrian golden hamsters** (Heinrich et al. 1982), no lung tumours were observed in this species either.

In another study, female **NMRI** and **C57BL mice** (40–120 per group) were exposed to diesel engine emissions at soot concentrations of 7.5 mg/m³ or 4.5 mg/m³ or the specific dilution of the gaseous phase. Control groups of the same size were kept in clean air (for further details see Section “Subacute, subchronic and chronic toxicity”). The C57BL mice were exposed for 24 months and observed in clean air for another 6 months. The lung tumour incidence of the NMRI mice exposed to soot at 7.5 mg/m³ (32.1%) did not differ from that of the controls (30.0%). Adenomas (diesel engine emissions: 21.8%; control animals: 25%) and adenocarcinomas were identified (diesel engine emissions: 15.4%; control animals: 15.4%). At 23% and 46.7% versus 30.0% in the control animals, the lung tumour incidences of the NMRI mice exposed to soot at 4.5 mg/m³ and the gaseous phase, respectively, were not statistically significantly increased. The proportions of adenomas/carcinomas were 18.3%/5% (total exhaust), 31.7%/15% (gaseous phase) and 25.0%/8.0% (clean air). Nor did C57BL mice show increased lung tumour incidences that were due to exposure. The tumour incidences were 8.5% (total exhaust), 3.5% (gaseous phase) and 5.1% (clean air) (Heinrich et al. 1995).

Another study found no evidence of carcinogenicity in mice. In addition to the above-mentioned rats (Mauderly et al. 1986, 1987), male and female **CD-1 mice** were also exposed for 7 hours per day, 7 days per week, for up to 24 months. There was no increase in lung tumour incidences caused by exposure (Mauderly et al. 1996).

The effects of inhaled diesel engine emissions on male and female **Syrian golden hamsters** were examined under the same exposure conditions as in rats (see above; 0.7, 2.2 or 6.6 mg/m³, 16 hours per day, 5 days per week, for 24 months). In this study, no increased lung tumour incidences occurred in hamsters either (Brightwell et al. 1986, 1989).

A group of 15 **cynomolgus monkeys** was exposed to diesel engine emissions at a soot concentration of 2 mg/m³ for 7 hours per day, 5 days per week, for 24 months. Control animals were kept in clean air. None of the monkeys developed pre-neoplastic or neoplastic lung changes (Lewis et al. 1986).

Summary

Evidence of a carcinogenic potential of diesel engine emissions has up to now only been provided in rats. In a study using diesel engine emissions at a very high soot concentration, Sencar mice reacted with a slightly increased tumour incidence, although only the females were affected (Pepelko and Peirano 1983). No increased tumour incidences were found in newborn ICR and C57B1/N mice after inhalation of diesel engine emissions at a soot concentration of 2–4 mg/m³ (Takemoto et al. 1986). In NMRI mice, a significant increase of lung tumour incidences was found after inhalation of diesel engine emissions at a soot concentration of 4 mg/m³ (Heinrich et al. 1986). However, it was not possible to confirm this result in a follow-up study under similar exposure conditions either for NMRI or for C57B1/6N mice (Heinrich et al. 1995). Nor were lung tumour incidences increased in CD-1 mice after exposure to diesel engine emissions (Mauderly et al. 1996). Moreover, no lung tumours were observed in Syrian golden hamsters (Brightwell et al. 1986, 1989; Heinrich et al. 1982, 1986), cats (Pepelko and Peirano 1983) or monkeys (Lewis et al. 1986).

Manifesto

The animal studies that have been published since the documentation “Diesel engine emissions” (1987 see documentation “Diesel engine emissions” 1990) substantiate the carcinogenicity in rats after exposure to diesel engine emissions. No increased lung cancer risk was established in mice, hamsters, cats or monkeys.

Almost all epidemiological studies have shown an increased relative lung cancer risk for occupations with exposure to diesel engine emissions. The problem is a lack of evidence of a dose-response relationship. Most studies do not provide any details about the level of exposure. For example, in the comprehensive study by Garshick et al. (2006), it was hardly possible to establish exposure data for the persons exposed. The exposure period was often used as a surrogate. There is the added problem that the term “diesel engine emission” is not unambiguous. Emission depends on the technology of the diesel engine. The old diesel engines are successively being replaced by engines featuring the new technology. Currently, both technologies are used to the same extent. The ratio will change in the course of the coming years. The epidemiological studies date back to the time before this change in technology. As yet there are no data available about the effects of the new engines because of the short latency. No definite exposure-response relationship was detected even in some well documented case-control studies. The increase in the relative lung cancer risk was statistically significantly increased only in the occupational group that had been exposed for the longest period (Gustavsson et al. 2000; Jockei et al. 1998; Steenland et al. 1990, 1998). The cohort studies allow no statement to be made about an exposure-response relationship.

Therefore, the data are not sufficient to classify diesel engine emissions in Carcinogen Category 1; they remain in Carcinogen Category 2.

Epidemiological studies have revealed a relationship between exposure to diesel engine emissions and asthma. Furthermore, an adjuvant effect of diesel engine emissions has been demonstrated in sensitization studies in various animal models and humans. In spite of these associations between diesel engine emissions and asthma, diesel engine emissions are not designated with "Sa" since they are not allergens themselves.

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