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149. Diesel Engine Exhaust

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Preface

An agreement has been signed by the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) and the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council of the Netherlands. The members of both committees are listed in Appendix 2. The purpose of the agreement is to write joint scientific criteria documents, which could be used by the national regulatory authorities in the Nordic countries and the Netherlands for establishing occupational exposure limits.

This document on *Diesel engine exhaust* was written by Drs Piia Taxell and Tiina Santonen at the Finnish Institute of Occupational Health and has been reviewed by NEG as well as by DECOS. Whereas the document was adopted by consensus procedures, thereby granting the quality and conclusions, the authors are responsible for the factual content of the document. The joint document is published separately by the two committees.

The NEG version presented herein has been adapted to the requirements of NEG and the format of Arbete och Hälsa. The editorial work and technical editing have been carried out by the NEG secretariat. All documents produced by NEG can be downloaded from *www.nordicexpertgroup.org*.

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Contents

Abbreviations and acronyms

ACES	Advanced Collaborative Emissions Study
BAL	bronchoalveolar lavage
CHO	Chinese hamster ovary
CI	confidence interval
COHb	carboxyhaemoglobin
COPD	chronic obstructive pulmonary disease
DECOS	Dutch Expert Committee on Occupational Safety
DEP	diesel exhaust particles
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
EC	elemental carbon
EPA	Environmental Protection Agency
EU	European Union
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
HDL	high density lipoprotein
HO-1	haem oxygenase 1
HPRT	hypoxanthine-guanine phosphoribosyltransferase
IARC	International Agency for Research on Cancer
Ig	immunoglobulin
IL	interleukin
IPCS	International Programme on Chemical Safety
LOAEL	lowest observed adverse effect level
MAK	Maximale Arbeitsplatzkonzentration (maximum workplace conc.)
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observed adverse effect level
8-OHdG	8-hydroxydeoxyguanosine
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
PM_X	particulate matter with a maximal aerodynamic diameter of x μm
PMN	polymorphonuclear leukocyte (granulocyte)
RNS	reactive nitrogen species
ROS	reactive oxygen species
RR	relative risk
SCE	sister chromatid exchange
SCOEL	Scientific Committee on Occupational Exposure Limits
SMR	standard mortality ratio
SRM	standard reference material
SVOC	semi-volatile organic compound
Th2	T-helper cell type 2
TNF-α	tumour necrosis factor alpha
US	United States
WHO	World Health Organization

1. Introduction

Diesel engines are widely used for transport and power supply. Occupational exposure to diesel exhaust occurs e.g. in mining, construction work, professional driving, agriculture, forestry, waste management, environmental remediation and other activities where diesel-powered vehicles and tools are applied. In a study carried out in 15 European union (EU) countries in 1990–1993, diesel exhaust was found to be the fourth most common carcinogenic agent in workplaces, with three million regularly exposed workers (187).

In 2012, the International Agency for Research on Cancer (IARC) classified diesel engine exhaust as carcinogenic to humans (Group 1) based on the evidence of a causal association between diesel engine exhaust exposure and increased risk of lung cancer in humans, and an association with cancer of the urinary bladder (167).

In addition to carcinogenicity, exposure to diesel exhaust is associated with inflammatory lung effects and cardiovascular effects. A role of diesel exhaust in the exacerbation of asthma and allergic diseases has also been suggested.

In the past two decades, tightened emission regulations in the EU and other parts of the world have caused a significant evolution of diesel technologies, resulting in changes in the emissions and composition of the exhaust. These changes are also expected to affect the health effects of diesel exhaust.

This document concerns exhaust produced by diesel engines which are fuelled with standard commercial types of petroleum-based diesel fuels. Exhausts from alternative fuels, such as biodiesel, are not included in the evaluation. Because of the extensive literature on the health effects of diesel exhaust, this document focuses mainly on studies related to inhalation exposure.

The present document is a co-production between the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) and the Dutch Expert Committee on Occupational Safety (DECOS). The joint document is published separately, and according to different formats, by NEG and DECOS.

As a basis for this document, we have used published reviews produced by the United States Environmental Protection Agency (US EPA) in 2002 (423), the World Health Organization/International Programme on Chemical Safety (WHO /IPCS) in 1996 (448), the Deutsche Forschungsgemeinschaft (DFG) in 2008 (82) and IARC in 1989 and 2013 (166, 167).

Of the constituents of diesel exhaust, carbon monoxide has been discussed in detail in a recent evaluation by NEG (395). The health effects of nitrogen dioxide have recently been reviewed by the DFG (83) and the EU Scientific Committee on Occupational Exposure Limits (SCOEL) (373).

2. Substance identification

2.1 Composition and characteristics

Diesel engine exhaust is a complex mixture of substances in gaseous and particulate phases produced during the combustion of diesel fuels. Diesel engines may be fuelled by petroleum-based diesel fuels, vegetable oil- or animal fat-based biodiesels, coal-, natural gas- or biomass-based synthetic fuels, natural gas or alcohols (96). The focus of the present document is on exhaust produced by diesel engines fuelled with petroleum-based diesel fuels (further referred to as diesel fuel). Petroleum-based diesel fuels belong to the middle distillates of crude oil (448).

The emission rate and exact composition of diesel exhaust depend, among others, on the type, age, operational condition and maintenance of the engine, on the composition and physical properties of the fuel, and on the exhaust after-treatment techniques applied (245, 248, 423). The present chapter gives a general review of the composition and characteristics of diesel exhaust. The influence of state-of-the-art exhaust after-treatment technologies on the exhaust composition is discussed further in Section 2.2.

The main components of the gas phase of diesel exhaust are nitrogen, carbon dioxide (CO_2), oxygen, water vapour, nitrogen oxides (NO_X) and carbon monoxide (CO) (423). These gases cover in fact over 99% of the mass of the whole diesel exhaust. In addition, small amounts of sulphur dioxide (SO_2) and various organic compounds, such as low-molecular-weight carbonyls, carboxylic acids, alkanes, alkenes and aromatics may be emitted in the gas phase (244).

Diesel exhaust particles (DEP) contain elemental carbon (EC), organic compounds, sulphates, nitrates and trace amounts of metals and other elements (423). Figure 1 presents a typical size distribution of DEP in untreated diesel exhaust (195). The size distribution has a bimodal character which corresponds to the formation mechanisms of the particles. In the field of vehicle exhaust studies, it is customary to refer to the two modes as the accumulation and nuclei (or nucleation) modes. The accumulation mode (aerodynamic particle diameter 0.03–0.5 μm) contains agglomerates of carbonaceous particles formed in the engine cylinders (196). The particles are composed of EC, metal oxides and adsorbed organic compounds. Particles in the nuclei mode (0.003–0.03 μm) are formed through nucleation and condensation of sulphur dioxide (sulphuric acid) and hydrocarbons, either through homogeneous nucleation or nucleation on solid core particles (146, 359). The core particles detected in the nuclei mode are suggested to be composed of (oxidised) metals and/or pyrolysed hydrocarbons (359). In addition to the nuclei and accumulation modes, DEP in untreated diesel exhaust may contain larger (≥ 1 μm) particles formed through deposition and subsequent release of carbonaceous particles from the walls of the engine or the exhaust system.

The accumulation mode contains most of the DEP mass. Nuclei mode particles account for more than 90% of the particle number concentration, but less than 20% of the particulate mass of untreated diesel exhaust (195).

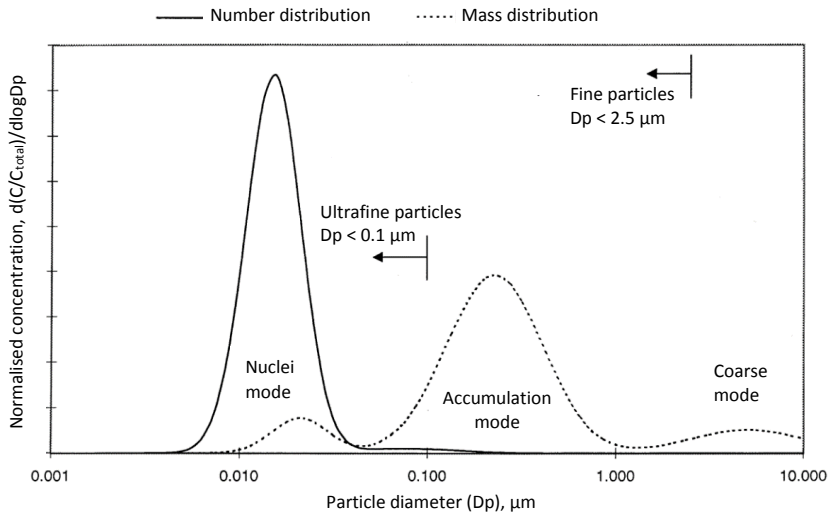

Figure 1. Typical mass and number size distributions of particles in untreated diesel exhaust. The mass or number concentration (C) of particles in any size range is proportional to the area under the corresponding curve in that range. Modified from Kittelson (195).

The organic material associated with DEP is a complex mixture of linear, branched and cyclic hydrocarbons originating mainly from unburned fuel and engine lubrication oil, with small quantities of partial combustion and pyrolysis products (195, 423). Polycyclic aromatic hydrocarbons (PAHs) and their oxygen and nitrogen derivatives may comprise up to 1% of the particulate mass of untreated diesel exhaust (423).

2.2 Influence of emission regulations

Exhaust emission standards for diesel engines have significantly tightened in the EU in the past two decades (96). Figure 2 presents the EU emission standards for heavy-duty diesel vehicle engines from 1992 to 2013 (engine power ≥ 85 kW). For example, the emission of DEP from these engines was regulated to 0.36 g/kWh in 1992 and to 0.01 g/kWh in 2013, meaning a 36-fold reduction of the allowed emissions over 20 years.

Similarly, for non-road engines (e.g. industrial, construction and agricultural equipment), the emission limits of DEP declined from 0.54–0.85 g/kWh in 1999 to 0.025 g/kWh in 2011–2014 for all engines with a power of at least 37 kW (96). However, for non-road engines with a net power below 37 kW, a higher particle emission, 0.6 g/kWh, is allowed, and for the smallest engines (< 19 kW) the emissions are not regulated at all.

A limit for the number of solid particles in diesel vehicle engine exhaust was also included in the recent emission regulation (Euro 5/6): the emission of solid particles (above the size of 23 nm) was regulated to 6.0–8.0 × 10^{11} particles/kWh

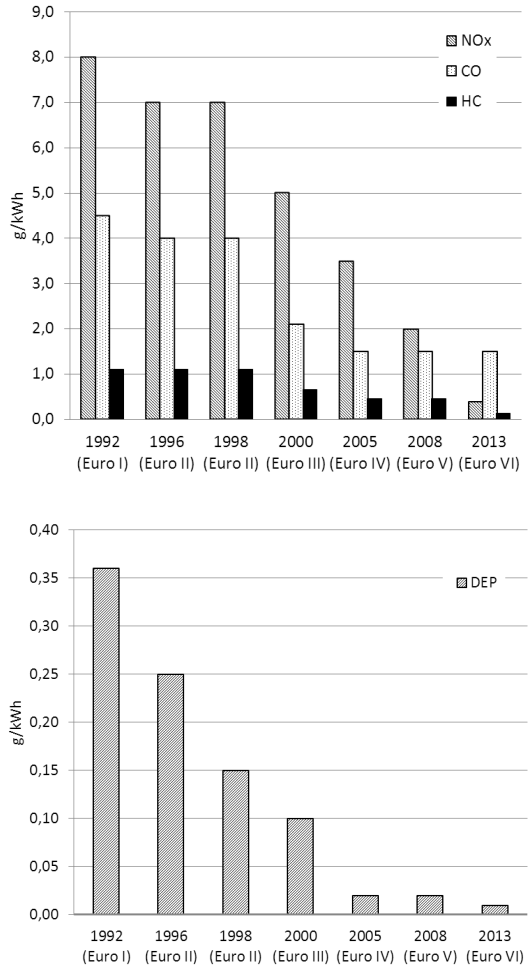

Figure 2. Development of emission standards for heavy-duty diesel engines in the EU. Euro I–VI refers to the European emission standards for heavy-duty diesel engines. Redrawn from data presented by ECOpoint (96). CO: carbon monoxide, DEP: diesel exhaust particles, HC: total hydrocarbons, NO_X: nitrogen oxides.

for heavy-duty engines and to 6.0×10^{11} particles/km for light-duty engines (96, 422). All standards apply to new vehicles/engines only.

The tightened emission regulations in the EU and other parts of the world have fostered a significant evolution of diesel engine and exhaust after-treatment technologies. The key developments include electronic high-pressure fuel injection systems, cooled exhaust gas recirculation and crankcase filtration in 1990–2000, and diesel oxidation catalysts and (wall-flow) diesel particulate filters in the late 2000s (243). The introduction of wall-flow diesel particulate filters and catalysts was enabled by the reduction of the sulphur content of diesel fuels. In the EU, "sulphur-free" diesel fuel (< 10 mg S/kg) became mandatory for highway vehicles

in 2009 and for non-road vehicles in 2011, with certain exemptions (96). A sulphur content up to 1 000 mg/kg is allowed for marine fuels.

Exhaust composition of state-of-the-art diesel engines with multi-component emissions reduction systems differs from that of older diesel engines (156, 243). Especially, DEP emissions are reduced by more than 90% by mass. Considering the DEP number concentration, diesel oxidation catalyst + diesel particulate filter systems have been shown to efficiently remove non-volatile particles present in the nuclei mode (146, 185). Instead, the number concentration of semi-volatile nuclei mode particles may in some cases even increase due to storage and release of sulphur compounds of the catalyst, and removal of larger particles on which the semi-volatiles could condensate (185).

Application of exhaust after-treatment systems (diesel oxidation catalyst + diesel particulate filter) changes also the composition of the particles. The proportion of EC in the particles is reduced and that of sulphates increased, reflecting the reduction of carbonaceous particles from the exhaust (Figure 3). Depending on the type and operational condition of the engine, EC comprises 30–90% of the particulate mass of pre-2000 diesel engine exhaust, with a typical proportion of 75 ± 10% for heavy-duty diesel engines (423). By contrast, the average EC percentage of the particle mass emitted by four heavy-duty diesel engines fulfilling the current emission standards was only 13% (191).

For the gas phase of the exhaust, the emissions of organic compounds, such as PAHs, aromatics and aldehydes, are significantly reduced with state-of-the-art diesel engines (225). Also, the proportion of nitrogen dioxide (NO_2) and nitrogen monoxide (NO) in the exhaust differs; although the total emission of NO_X has decreased, NO_2 may account for up to 50% of the NO_X in the exhaust of a state-of-the-art diesel engine, in comparison with older engines which produce exhaust in which NO_2 typically accounts for 10% of the NO_X (246).

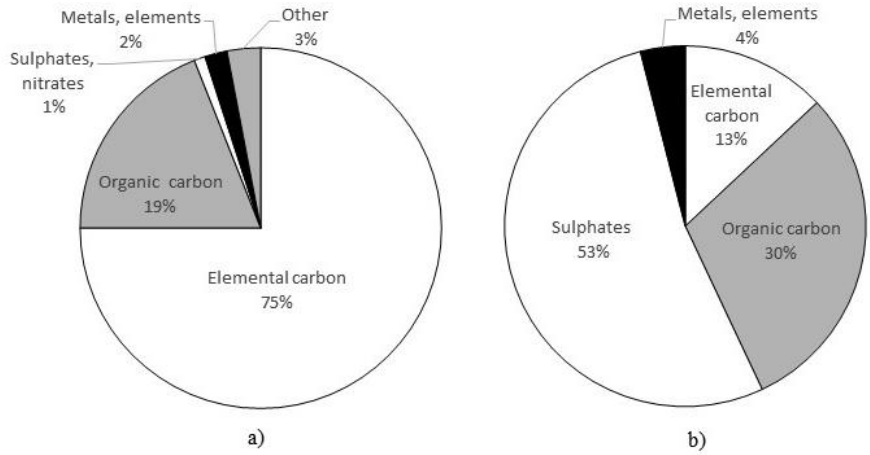

Figure 3. Typical composition of diesel exhaust particles (DEP) emitted by a) 1990–2000 diesel engine and b) post-2006 diesel engine. Redrawn from US EPA (423) and Khalek *et al*. (191).

Table 1. Average emissions from US 2004 compliant (corresponding to EU 1998–2000) and US 2007 compliant (corresponding to EU 2013) heavy-duty diesel engines (191).

Compound	US 2004 (EU 1998–2000) compliant engines (average ± SD, mg/h)	US 2007 (EU 2013) compliant engines (average ± SD, mg/h)	Reduction of emissions (%)
Elemental carbon	3 445 ± 1 110	23 ± 4.7	99
Organic carbon	1 180 ± 71	53 ± 47	96
Inorganic ions	320 ± 156	92 ± 38	71
Metals and elements	400 ± 141	6.7 ± 3.0	98
PAHs	325 ± 106	70 ± 24	79
Nitro-PAHs	0.3 ± 0.0	0.1 ± 0.0	81
Single-ring aromatics	405 ± 149	72 ± 33	82
Alkanes	1 030 ± 240	155 ± 78	85
Hopanes/steranes (polycyclic hydrocarbons)	8.2 ± 6.9	0.1 ± 0.1	99
Alcohols and organic acids	555 ± 134	107 ± 25	81
Carbonyls	12 500 ± 3 536	255 ± 95	98
Dibenzodioxins and furans	nd	$6.2 \times 10^{-5} \pm 5.2 \times 10^{-5}$	nd

EU: European Union, nd: no data, PAH: polycyclic aromatic hydrocarbon, SD: standard deviation, US: United States.

Table 1 gives an example of the emissions from heavy-duty diesel engines from the early 2000s in comparison with state-of-the-art diesel engines.

2.3 Standard reference materials

The US National Institute of Standards and Technology (NIST) provides two standard reference materials (SRMs) for DEP (290-292). One of the materials (SRM 1650; 1650a; 1650b) originates from several heavy-duty diesel engines and was produced in the mid-1980s. The other material (SRM 2975) was collected from an industrial diesel powered forklift. Although these materials are primarily intended for evaluation of analytical methods for the determination of selected PAHs and their nitrogen derivatives in diesel particulate matter and similar matrices, the materials have also been applied in toxicological studies focusing on the health effects of DEP.

2.4 Ambient air pollution versus diesel engine exhaust

Ambient air pollution is a complex and variable mixture of primary pollutants emitted in the atmosphere, e.g. primary particles, SO_2, NO_X and CO, and secondary pollutants formed within the atmosphere, e.g. secondary particles and ozone (451). Sources of atmospheric air pollution include traffic, power stations and other combustion plants, industrial plants, domestic heating and cooking, deliberate and unintended biomass burning, agriculture and natural sources (e.g. vegetation, soil and sea).

Based on a meta-analysis of 108 studies and air quality reports, the main sources of particulate emissions in Europe comprise atmospheric formation of secondary inorganic aerosols of ammonia (NH_3), SO_2 and NO_X; traffic-related primary particles (i.e. particles emitted from vehicle engines and formed through the wear of brake linings, clutch and tyres, together with road dust); soil/mineral dust; biomass burning; industrial point sources; and sea/road salt (30). The median contribution of traffic-related primary particles in the particulate air pollution (particulate matter with a maximal aerodynamic diameter of ≤ 2.5 µm, $PM_{2.5}$) is in the order of 20–30% at urban sites, and that of secondary inorganic aerosols in the order of 40%. The main sources of the gaseous precursors of the secondary inorganic aerosol include catalysed gasoline engines and farming activities for NH_3, vehicle exhausts and energy production for NO_X, and combustion of sulphur containing fuels (e.g. coal) for SO_2 (30). Traffic and other combustion sources comprise the main sources of CO in ambient air (450).

Although diesel exhaust contributes to ambient air pollution in particular at traffic-intensive urban sites, data on the health effects related to ambient air pollution cannot be directly applied for the health risk assessment of diesel exhaust due to the significant contribution of other emissions, both traffic-related and other, to the ambient air pollution. Studies related to ambient air pollution are, therefore, only shortly cited in the relevant sections of the present document.

3. Occurrence, production and use

As already indicated, only diesel exhaust produced by diesel engines which are fuelled with mineral oil (petroleum) based diesel fuels is within the scope of this review. Diesel engines are widely used for transport and power supply, and are dominating power-sources for heavy-duty vehicles. The main advantages of diesel engines include high efficiency, robustness and durability. In particular, the high energy efficiency makes the diesel engine an attractive alternative for many applications. In comparison with gasoline engine exhaust, diesel engine exhaust contains considerably less CO which makes it possible to run diesel engines in enclosed worksites where gasoline engines cannot be used.

The general population is mainly exposed to diesel exhaust by road traffic, but the working population may be additionally exposed to exhaust emitted by:

- on-road vehicles (e.g. passenger cars, buses, trucks, vans)
- off-road vehicles (e.g. forklift trucks, tractors, harvesting machines, excavators, military vehicles)
- sea-going and inland water vessels
- locomotives
- stationary equipment (compressors, pumps, building equipment, electricity generators, cranes and other machinery used in the industry and agriculture).

Exposed worker groups include mine and construction workers, warehouse workers, mechanics, emergency workers, professional drivers, and shipping and

railroad workers. Exposure to diesel exhaust may also occur in agriculture, forestry, waste management, environmental remediation, and other industries where diesel-powered vehicles and tools are applied.

The demand for diesel fuels has increased in Europe during the past decades. The annual consumption of diesel fuels in North West Europe increased from approximately 90 million tonnes in 2000 to 110 million in 2010 (463). In Norway, Sweden, Denmark and Finland, the total reported annual use of diesel fuels increased from 9.4 million tonnes in 2003 to 15 million in 2010 (386).

4. Measurements and analysis of workplace exposure

Because of the complex composition of diesel exhaust, varying exposure indicators have been applied for the measurements of diesel exposure at workplaces (39, 336).

Particulate phase

For the particulate fraction of diesel exhaust, gravimetric methods, such as determination of respirable particle mass of a size-selectively collected filter-sample (EN 481:1993), have been applied. Also other particle size fractions, e.g. "fine" ($PM_{2.5}$) or "submicron" ($PM_{1.0} \leq 1.0$ μm) particles, have been measured. The challenge with the gravimetric methods is, however, that they do not allow the separation of DEP from other particles in the workplace air (39). In addition, their sensitivity to small particle masses is insufficient.

EC is considered to be a more specific and sensitive marker of DEP (39). EC constitutes a large portion of the particulate mass, especially in the exhaust produced by older diesel engines where particle mass is of significance, and it can be quantified at low levels. In most workplaces, diesel engines are the only significant sources of EC. EC is determined by thermal-optical analysis of filter-collected DEP. The US National Institute for Occupational Safety and Health (NIOSH method 5040) reports a limit of detection (LOD) of ~ 2 μg EC/m^3 for a 960-litre air sample collected on a 37-mm filter with a 1.5 cm^2 punch from the filter. A lower LOD can be achieved by a larger sampling volume and/or a 25-mm filter, e.g. a 1 920-litre sample on a 25-mm filter gives a LOD of 0.4 μg EC/m^3 (285). Mechanically generated particles containing EC, such as coal dust, can be efficiently separated from DEP by size-selective sampling. For the new technology diesel engine exhaust with significantly reduced particle mass and EC concentration, EC may not be an equally useful marker.

In addition to EC, specific organic constituents of DEP, such as PAHs may be determined from the filter-collected DEP sample, e.g. by gas chromatography-mass spectrometry (308).

Recently, methodologies for determination of size-resolved DEP mass and number concentration with real-time aerosol monitors have been developed (223, 236). Experience on the applicability of these methodologies for workplace measurements is, however, limited.

Gas phase

For the gas phase of diesel exhaust, NO_X and CO are commonly applied exposure indicators (336). For NO_X, the highest sensitivity is reached with chemiluminescence analysers with a LOD of 0.002 ppm for both NO_2 and NO (78). The techniques used for determination of CO are often based on the principle of electrochemical detection or non-dispersive infrared detection (395).

5. Occupational exposure data

Tables 2–5 list personal measurement data for occupational exposure to diesel exhaust (measured as EC, CO, NO or NO_2). As described below, the highest exposure levels have been found in underground mines and tunnel construction sites, i.e. enclosed underground work sites where heavy diesel equipment is used. Intermediate levels were reported e.g. for warehouse, dock and terminal workers and vehicle mechanics, and the lowest levels for outdoor workers and drivers of diesel vehicles.

In a large survey conducted at seven non-metal mining facilities in the US in 1998–2001, the average exposure of underground workers to EC (respirable particles) ranged from 31–58 to 313–488 μg EC/m^3 across the facilities and of surface workers from 2 to 6 μg EC/m^3. The average levels of NO_X were 0.2–1.5 ppm NO and 0.1–0.6 ppm NO_2 for underground work, and 0.02–0.1 ppm NO and 0.01–0.06 ppm NO_2 on the surface (70). In another large survey carried out in the US, average levels of EC in personal samples were 41–405 μg EC/m^3 for underground and 1–39 μg EC/m^3 for above-ground miners (72). In other studies, average exposure levels of 27–637 μg EC/m^3, 2–9 ppm CO, 0.7–15 ppm NO and 0.2–5.5 ppm NO_2 have been reported for underground miners (Table 2).

In three studies conducted in Sweden and Norway in 1996–2004, average exposures of tunnel construction workers were in the range 132–314 μg EC/m^3 (inhalable particles), 5–9 ppm CO, 2.6 ppm NO and 0.2–0.9 ppm NO_2 (17, 213, 420). A recent study from Norway conducted in 2010–2011 indicated a decrease in exposure to diesel exhaust at tunnel construction sites; the average exposure was 56 μg EC/m^3 (inhalable particles) and 0.09 ppm NO_2 (19). For above-ground construction sites, average levels of 4–13 μg EC/m^3, 1 ppm CO, 0.2 ppm NO and 0.02–0.3 ppm NO_2 have been reported (Table 3).

For warehouse, dock and terminal workers average exposure levels of 4–122 μg EC/m^3, 2–5 ppm CO, 0.1 ppm NO and 0.1 ppm NO_2 were reported. For on-road vehicle mechanics, reported exposure levels were 4–39 μg EC/m^3 and 0.05–0.2 ppm NO_2 (Table 3). In two fire stations in the US, mean area concentrations of 6.1 and 16 μg EC/m^3 (inhalable) were detected. The levels were reduced to 1.5 μg EC/m^3 after installation of diesel particulate filters on the vehicles (354).

A large study concerning exposure of truck drivers to DEP was carried out in the US in 2001–2005 (81). The mean concentration in the cabins of the trucks was 1.1–1.6 μg EC/m^3. As expected, the concentration of EC in the cabin correlated

with the age of the truck engine. In earlier studies, mean EC concentrations of 5–22 μg EC/m^3 were reported. In two studies from the 1980s and early 2000s, truck drivers' exposure to NO and NO_2 was in the order of 0.3 ppm NO and 0.04 ppm NO_2, respectively. Corresponding exposure levels have been reported also for other professional drivers (Table 4).

In the railroad industry, average exposure levels of 4–39 μg EC/m^3, < 1–5 ppm CO, 0.2–1.1 ppm NO and 0.03–0.3 ppm NO_2 have been reported (Table 5).

As examples of European urban air concentrations, average values in the range of ~ 1.6–4.5 μg EC/m^3 measured in the early 2000s were reported from the UK, the Netherlands and Austria (180, 190, 345). Slightly higher values of 7.6–11.8 μg EC/m^3 were measured in 1999–2000 in Italy (12). Data from 2010 analysed by the European Environment Agency showed annual NO_2 averages of 0.05 ppm (96–98 μg/m^3) in London and Paris and 0.02 ppm (44–47 μg/m^3) in Stockholm and Zürich at the monitoring stations recording the highest averages (101).

Table 2. Occupational exposure measurements of diesel exhaust in the *mining industry* (personal monitoring) [adapted mainly from Pronk *et al.* (336)].

Job description/agent	Sampling duration (h) [a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Underground							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Production	-	6 [b]	148 (136)	85 (3.5)	UK	2004 [c]	(209)
Production	> 4	343	202 (32–144) [d]	111 (1.4–4.8) [d]	US	2002 [c]	(72)
Production	> 4	4	241 [e]	202 (1.8)	Estonia	2002 [c]	(44)
Production	> 4	15	637 (75–508) [d]	-	US	1999	(249)
Maintenance	> 4	269	144 (17–462) [d]	66 (1.7–4.6) [d]	US	2002 [c]	(72)
Mining [f]	> 4	779	40–384 [h]	27–347 [h]	US	1998–2001	(70)
Mining [f]	-	7 [b]	66 (28)	62 (1.5)	UK	2004 [c]	(209)
Elemental carbon, submicron			µg/m^3	µg/m^3			
Production	> 4	38	219 (65–193) [d]	-	US	1997 [c]	(388)
Maintenance	> 4	8	53 (46)	-	US	1997 [c]	(388)
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Production	< 1–4	12	538 (512)	-	US	2007 [c]	(56)
Elemental carbon, sampling fraction not given			µg/m^3	µg/m^3			
Mining [f]	-	27	27	-	Sweden	2006 [c]	(477)
Carbon monoxide			ppm	ppm			
Production	1– > 4	5	2.0 (0.6)	1.9 (1.4)	US	1991	(275-277)
Mining [f]	-	≥ 5 [b, g]	8.9	-	US	1976–1977	(9)
Mining [f]	-	≥ 5 [b, g]	6.1	-	US	1976–1977	(9)

Table 2. Occupational exposure measurements of diesel exhaust in the *mining industry* (personal monitoring) [adapted mainly from Pronk *et al.* (336)].

Job description/agent	Sampling duration (h) [a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Nitrogen monoxide			ppm	ppm			
Production	> 4	9	14.7 (2.8)	14.5 (1.2)	US	1991	(275-277)
Production	> 4	7	4.2 (1.7)	3.9 (1.5)	US	1991	(275-277)
Production	> 4	6	4.7 (1.0)	4.6 (1.2)	US	1991	(280)
Mining [f]	> 4	54 [b]	11.0 (5.7)	-	US	1988	(278)
Mining [f]	> 4	25	0.7 (0.6)	-	US	1988	(275-278)
Mining [f]	> 4	666	0.20–1.5 [h]	0.11–1.0 [h]	US	1998	(70)
Nitrogen dioxide			ppm	ppm			
Production	> 4	9	2.9 (0.5)	2.9 (1.2)	US	1991	(275-277)
Production	> 4	7	0.8 (0.4)	0.7 (1.6)	US	1991	(275-277)
Production	> 4	6	0.7 (0.1)	0.7 (1.1)	US	1991	(280)
Production	-	183	1.9 (1.6)	-	US	1978 [c]	(110)
Production	> 4	41	0.2 [e]	0.1 (1.5–2.8) [d]	US	1976–1980	(444)
Production	> 4	76	0.2 (0.1–0.1) [d]	-	US	1982 [c]	(344)
Production	-	29	0.2	-	Sweden	2006 [c]	(477)
Production	> 4	54 [b]	1.5 (0.9)	-	US	1988	(278)
Production	> 4	25	5.5 (3.9)	-	US	1988	(275-277)
Mining [f]	> 4	60	0.2 (0.1)	-	US	1982 [c]	(2)
Mining [f]	> 4	689	0.10–0.60 [h]	0.12–0.52 [h]	US	1998	(70)

Table 2. Occupational exposure measurements of diesel exhaust in the *mining industry* (personal monitoring) [adapted mainly from Pronk *et al.* (336)].

Job description/agent	Sampling duration (h) [a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Above ground							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Production/maintenance	> 4	164	13 (2–89) [d]	2 (1.8–6.2) [d]	US	2002 [c]	(72)
Production/maintenance	> 4	265	3.5	1–4 [h]	US	1998	(70)
Elemental carbon, submicron			µg/m^3	µg/m^3			
Production/maintenance	> 4	23	23 (15–54) [d]	-	US	1997 [c]	(388)
Nitrogen monoxide			ppm	ppm			
Production/maintenance	> 4	12	0.3 (0.2)	-	US	1988	(278)
Production/maintenance	> 4	225	0.02–0.11 [h]	0.01–0.05 [h]	US	1998	(70)
Nitrogen dioxide			ppm	ppm			
Production/maintenance	> 4	12	0.04 (0.03)	-	US	1988	(278)
Production/maintenance	> 4	233	0.01–0.06 [h]	0.01–0.03 [h]	US	1998	(70)

[a] > 4: sample collection/measurement for more than 4 hours (representative of a work day).
[b] Area sample representative of personal exposure.
[c] Publication year (sampling year not available).
[d] Range of SDs/GSDs.
[e] AM estimated from GM and GSD or from range.
[f] Job not specified.
[g] n ≥ 5: at least 5 samples for all jobs combined in the study.
[h] Range of AMs/GMs in six or seven facilities.
AM: arithmetic mean, GM: geometric mean, GSD: geometric standard deviation, SD: standard deviation, UK: United Kingdom, US: United States.

Table 3. Occupational exposure measurements of diesel exhaust from *off-road vehicles* (indoors and outdoors) and *on-road vehicles* (indoors) (personal monitoring) [adapted from Pronk *et al*. (336)].

Job description/agent	Sampling duration (h) [a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Tunnel construction							
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Tunnel	> 4	10	220	160 (2.2)	Norway	1996–1999	(17)
Tunnel	> 4	12	132 [b]	87 (2.5)	Sweden	2002–2004	(213)
Tunnel	> 4	149	56	35 (2.6)	Norway	2010–2011	(19)
Carbon monoxide			ppm	ppm			
Tunnel	> 4	52	5 (3.7)	-	Sweden	1991 [d]	(420)
Nitrogen monoxide			ppm	ppm			
Tunnel	> 4	53	2.6 (1.5)		Sweden	1991 [d]	(420)
Nitrogen dioxide			ppm	ppm			
Tunnel	> 4	18	0.22 [b]	0.19 (0.58)	Sweden	2002–2004	(213)
Tunnel	> 4	82	0.8	0.6 (1.5–4.5) [c]	Norway	1996–1999	(17)
Tunnel	> 4	53	0.88 (0.68)	-	Sweden	1991 [d]	(420)
Tunnel	> 4	163	0.09	0.06 (0.002)	Norway	2010–2011	(19)
Other construction							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Heavy (highway)	> 4	261	13	8 (2.7)	US	1994–1999	(464)
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Above ground	> 4	22	13 [b]	8 (2.8)	Sweden	2002–2004	(213)
Electric utility installation	> 4	120	4	-	US	1996–1997	(447)
Carbon monoxide			ppm	ppm			
Electric utility installation	> 4	27	1 (0.6–0.6) [c]	-	US	1996–1997	(447)

Table 3. Occupational exposure measurements of diesel exhaust from *off-road vehicles* (indoors and outdoors) and *on-road vehicles* (indoors) (personal monitoring) [adapted from Pronk *et al*. (336)].

Job description/agent	Sampling duration (h) [a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Nitrogen monoxide			ppm	ppm			
Electric utility installation	> 4	27	0.2 (0.2–0.4) [c]	-	US	1996–1997	(447)
Nitrogen dioxide			ppm	ppm			
Above ground	> 4	33	0.02 [b]	0.02 (1.06)	Sweden	2002–2004	(213)
Electric utility (outdoors)	> 4	24	0.32 (0.2–0.2) [c]	-	US	1996–1997	(447)
Dock/warehouse							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Fork-lift truck	> 4	39 [e]	36 [b]	27	UK	2004 [d]	(443)
Dockworkers	> 4	27	122	66 (3.3)	UK	2000 [d]	(124)
Dockworkers	> 4	12	9 [b]	7 (2)	Georgia	1999	(117)
Elemental carbon, submicron			µg/m^3	µg/m^3			
Dockworkers	> 4	54	24 (0.4–2.5) [c]	2 (1.3–27.2) [c]	US	1991 [d]	(475)
Dockworkers	> 4	≥ 5 [f]	- [g]	7	US	1990	(474)
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Dockworkers	> 4	5	4 (1.8)	4 (1.5)	Georgia	1992	(279)
Nitrogen dioxide			ppm	ppm			
Dockworkers	> 4	≥ 5 [f]	- [g]	0.18	US	1990	(474)
Airport							
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Baggage and screening	> 4	72	11 (5.4)		US	2004	(286)
Carbon monoxide			ppm	ppm			
Baggage and screening	> 4	61	2.4 [b]	-	US	2004	(286)
Mechanics and refuelers	> 4	10	5 (1.5)	4.7 (1.3)	US	1992	(281)

Table 3. Occupational exposure measurements of diesel exhaust from *off-road vehicles* (indoors and outdoors) and *on-road vehicles* (indoors) (personal monitoring) [adapted from Pronk *et al*. (336)].

Job description/agent	Sampling duration (h) [a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Nitrogen monoxide			ppm	ppm			
Baggage and screening	> 4	40	0.13 (0.07)	-	US	2004	(286)
Nitrogen dioxide			ppm	ppm			
Baggage and screening	> 4	40	0.12 (0.07)	-	US	2004	(286)
Marine terminal/ferry							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Ferry	> 4	20	49	37 (2.5)	UK	2000 [c]	(124)
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Marine terminal	> 4	168	6 (0.9–9.0) [c]	-	US	2003–2005	(287)
Carbon monoxide			ppm	ppm			
Marine terminal	> 4	60	2.5	-	US	2003–2005	(287)
Elemental carbon, respirable			µg/m^3	µg/m^3			
Truck repair	> 4	10	4 [b]	4 (1.6)	US	1999	(117)
Ambulance depot	> 4	3	31	29 (1.6)	UK	2000 [d]	(124)
Bus repair	> 4	53	39	31 (2.1)	UK	2000 [d]	(124)
Bus repair	> 4	15	39 [b]	38 (1.3)	Estonia	2002 [d]	(44)
Vehicle testing	> 4	11	11	11 (1.8)	UK	2000 [d]	(124)
Elemental carbon, submicron			µg/m^3	µg/m^3			
Truck repair	> 4	80	27 (4.1)	4 (12.1)	US	1980s	(475)
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Truck/bus repair + inspection	> 4	40	21 [b]	11 (3.2)	Sweden	2002–2004	(213)
Bus repair	> 4	4	ND	ND	US	1998	(283)

Table 3. Occupational exposure measurements of diesel exhaust from *off-road vehicles* (indoors and outdoors) and *on-road vehicles* (indoors) (personal monitoring) [adapted from Pronk *et al*. (336)].

Job description/agent	Sampling duration (h) [a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Nitrogen dioxide			ppm	ppm			
Truck/bus + inspection	> 4	60	0.05 [b]	0.05 (0.9)	Sweden	2002–2004	(213)
Bus	-	232	0.24 (0.26)	-	US	1987 [d]	(111)
Firefighter							
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Firefighter	> 4	27	24 (max)	-	US	2002 [d]	(354)
Firefighter	> 4	18	40 (20.3)	35 (1.7)	US	1995 [d]	(95)
Firefighter	> 4	12	10 (max)	-	US	1997	(282)
Firefighter	< 1	8	ND	ND	US	1998	(284)
Parking attendants							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Parking attendants	> 4	34 [e]	1.1 (0.6)	1.1 (1.8)	US	2002 [d]	(341)

[a] > 4: sample collection/measurement for more than 4 hours (representative of a work day).
[b] AM estimated from GM and GSD or from range.
[c] Range of SDs/GSDs.
[d] Publication year (sampling year not available).
[e] Area sample representative of personal exposure.
[f] n ≥ 5: at least 5 samples for all jobs combined in the study.
[g] AM could not be calculated.
AM: arithmetic mean, GM: geometric mean, GSD: geometric standard deviation, ND: not detected, SD: standard deviation, UK: United Kingdom, US: United States.

Table 4. Occupational exposure measurements of diesel exhaust from *on-road vehicles* (personal monitoring) [adapted from Pronk *et al.* (336)].

Job description/agent	Sampling duration (h)[a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Professional drivers							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Truck-local	> 4	5	7[b]	6 (1.6)	US	1999	(117)
Truck-long haul	> 4	5	5[b]	4 (2.0)	US	1999	(117)
Bus	> 4	5	10[b]	9 (1.3)	Estonia	2002[c]	(44)
Bus	> 4	39	2.0 (1.3)	1.4 (3.3)	US	2002[c]	(341)
Elemental carbon, submicron			µg/m^3	µg/m^3			
Truck-local	> 4	56	5 (0.9)	0.9 (4.0)	US	1980s	(475)
Truck-local	> 4	576[d]	2 (2.3)	1 (2.8)	US	2001–2005	(81)
Truck-long haul	> 4	72	5 (0.4)	0.4 (3.8)	US	1980s	(475)
Truck-long haul	> 4	349[d]	1 (0.8)	1 (2.3)	US	2001–2005	(81)
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Truck	1– > 4	3	10 (6.0)	9 (1.8)	US	1992	(279)
Bus and truck[e]	> 4	20	11[b]	6 (2.9)	Sweden	2002–2004	(213)
Taxi[e]	> 4	8	8[b]	7 (1.6)	Sweden	2002–2004	(213)
Elemental carbon, sampling fraction not given			µg/m^3	µg/m^3			
Truck-local	> 4	4[d]	5 (0.1)	5 (1.0)	US	1985	(274)
Truck-long haul	> 4	4[d]	22 (13.2)	19 (2.0)	US	1985	(274)
Nitrogen monoxide			ppm	ppm			
Truck-local	> 4	4[d]	0.23 (0.05)	0.22 (1.3)	US	1985	(274)
Truck-long haul	> 4	4[d]	0.27 (0.10)	0.25 (1.5)	US	1985	(274)

Table 4. Occupational exposure measurements of diesel exhaust from *on-road vehicles* (personal monitoring) [adapted from Pronk *et al.* (336)].

Job description/agent	Sampling duration (h)[a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Nitrogen dioxide			ppm	ppm			
Taxi[e]	> 4	12	0.03[b]	0.02 (0.7)	Sweden	2002–2004	(213)
Bus and truck[e]	> 4	30	0.03[b]	0.03 (0.7)	Sweden	2002–2004	(213)
Truck	> 4	40	0.04 (0.02)	-	Sweden	1997–1999	(213)
Taxi	> 4	20	0.03 (0.01)	-	Sweden	1997–1999	(213)
Bus	> 4	42	0.03 (0.01)	-	Sweden	1997–1999	(213)

[a] > 4: sample collection/measurement for more than 4 hours (representative of a work day).
[b] AM estimated from GM and GSD or from range.
[c] Publication year (sampling year not available).
[d] Area sample representative of personal exposure.
[e] Mostly diesel powered vehicles.
AM: arithmetic mean, GM: geometric mean, GSD: geometric standard deviation, SD: standard deviation, US: United States.

Table 5. Occupational exposure measurements of diesel exhaust in the *railroad industry* (personal monitoring) [adapted from Pronk *et al*. (336)].

Job description/agent	Sampling duration (h)[a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Railroad workers							
Elemental carbon, respirable			µg/m^3	µg/m^3			
Driver, assistant, shunter driver	> 4	19	20 (18.7)	16 (2.0)	Russia	2002 [b]	(44)
Maintenance, rolling stock	> 4	64	39	17 (1.9)	UK	2000 [b]	(124)
Elemental carbon, respirable/inhalable			µg/m^3	µg/m^3			
Hostler	> 4	5	4 (1.3)	3 (1.5)	Canada	1999–2000	(436)
Engineer/driver, conductor/trainmen	> 4	76 [c]	5 (1.1–15.8) [d]	3 (1.5–3.5) [d]	Canada	1999–2000	(436)
Maintenance, rolling stock	> 4	48	5 (4.9–8.8) [d]	3 (2.4–2.7) [d]	Canada	1999–2000	(436)
Elemental carbon, inhalable			µg/m^3	µg/m^3			
Non-operating crew trailing locomotive	> 4	47 [c]	10 (12)	6	Canada	2003	(376)
Engineer's operating console	1– > 4	49 [c]	6	4 (3)	US	1996–1998	(226)
Carbon monoxide			ppm	ppm			
Non-operating crew trailing locomotive	> 4	280 [c]	4.50 (max)	-	Canada	2003	(376)
Locomotive/caboose	> 4	16 [c]	< 1	-	US	1974–1976	(157)
Nitrogen monoxide			ppm	ppm			
Non-operating crew trailing locomotive	> 4	46 [c]	1.13 (0.87)	0.82	Canada	2003	(376)
Maintenance, locomotive	> 4	9 [c]	0.55	-	Canada	1996	(437)
Locomotive/caboose	> 4	16 [c]	0.23	-	US	1974–1976	(157)
Maintenance, rolling stock	> 4	18	0.26	-	Canada	1996	(437)

Table 5. Occupational exposure measurements of diesel exhaust in the *railroad industry* (personal monitoring) [adapted from Pronk *et al*. (336)].

Job description/agent	Sampling duration (h)[a]	No. of samples	Exposure level AM (SD)	Exposure level GM (GSD)	Location	Sampling year	Reference
Nitrogen dioxide			ppm	ppm			
Non-operating crew trailing locomotive	> 4	181[c]	0.3 (max)	-	Canada	2003	(376)
Maintenance, locomotive	> 4	9[c]	0.05	-	Canada	1996	(437)
Locomotive and caboose	> 4	16[c]	0.03	-	US	1974–1976	(157)
Maintenance, rolling stock	> 4	18	0.10	-	Canada	1996	(437)

[a] > 4: sample collection/measurement for more than 4 hours (representative of a work day).
[b] Publication year (sampling year not available).
[c] Area sample representative of personal exposure.
[d] Range of SDs/GSDs.
AM: arithmetic mean, GM: geometric mean, GSD: geometric standard deviation, SD: standard deviation, UK: United Kingdom, US: United States.

6. Toxicokinetics

6.1 Diesel exhaust particles

Upon inhalation of diesel exhaust, DEP deposition will occur throughout the respiratory tract, with a majority of the particles reaching the alveolar region (306, 423). In 9 healthy volunteers, the measured total deposited mass and number fraction of DEP [generated during both idling (60 µg DEP/m^3) and transient driving (300 µg DEP/m^3)] in the respiratory tract was ~ 30% and ~ 50–65%, respectively, at rest, with a high intra-individual variation. The mean total deposited respiratory dose was calculated to be 0.14 µg per µg DEP/m^3/hour (351).

Applying measurement data on DEP number-size distributions and the International Commission on Radiological Protection (ICRP) 66 lung deposition model, Oravisjärvi *et al*. estimated that ~ 60% of the deposited DEP particles are retained in the alveolar region. Heavy exercise was estimated to increase the total deposition by 4–5-fold, and the alveolar deposition by 5–6-fold (306).

From the tracheobronchial region, DEP is cleared by mucociliary clearance and removed into the gastrointestinal system within 24 hours (448). The main clearance mechanism for particles in the alveolar region is phagocytosis by alveolar macrophages, and subsequent movement within alveolar and bronchial lumen into the conducting airways followed by mucociliary clearance. There are also data suggesting that DEP, similarly to other types of fine particles, may, in particular at high exposure levels, translocate through the alveolar epithelium into the interstitium, lymph nodes and possibly end up into the systemic circulation (423).

The clearance rate is substantially lower from the alveolar region than from the tracheobronchial region; the alveolar retention half-time was 60–100 days in rats with a lung burden of ≤ 1 mg DEP/lung (448). At higher lung burdens, the retention half-time increases linearly due to an overwhelming of the alveolar macrophage mediated clearance ("lung overload"). In humans, the alveolar clearance rate is even lower than in rats; retention half-times of several hundred days have been reported for insoluble particles (423).

The metabolism of PAHs and other DEP-adsorbed organics in the lungs may lead to the formation of reactive metabolites (448). The clearance rate of particle-associated PAHs from the lungs is lower than the clearance of the substances inhaled as such.

6.2 Gas phase constituents of diesel exhaust

The main components of the gas phase of diesel exhaust are nitrogen, carbon dioxide (CO_2), oxygen, water vapour, nitrogen oxides (NO_X) and carbon monoxide (CO) (423). Of these, NO_X and CO are considered in the following sections.

Nitrogen dioxide

In humans, 80–90% of inhaled NO_2 is taken up via the respiratory tract during normal breathing, and over 90% at maximum breathing (449).

Dosimetric model calculations show that NO_2 is absorbed mainly in the lower respiratory tract. Uptake of NO_2 by the upper respiratory tract further decreases with increasing ventilation rates, causing a greater proportion to be delivered to the lower respiratory tract. The site of maximal tissue dose ranges from the upper respiratory bronchioles in humans to the alveolar ducts in rats (424).

NO_2 uptake in the respiratory tract is suggested to be rate-limited by chemical reactions of NO_2 with the components of the epithelial lining fluid. It is assumed that NO_2 is absorbed by the lung epithelium into the systemic circulation mainly in the form of nitrites and/or nitrates produced in these reactions. In the body, nitrite is converted to nitrate, which is released from the body in urine (424).

Nitrogen monoxide

Inhaled NO is absorbed through the epithelium of the respiratory tract into the circulation. Respiratory absorption of 64–93% of inhaled NO has been reported in humans (449).

In the blood, NO readily reacts with haemoglobin, producing nitrosylhaemoglobin, which in the presence of oxygen leads to the formation of methaemoglobin. Further metabolism of nitrosylhaemoglobin results in the formation of nitrate which is released from the body in urine. Endogenous NO has an important function in mediating vasodilation, host defence reactions and neurotransmission (429).

Carbon monoxide

Inhaled CO is readily taken up by the lower respiratory tract. CO diffuses from the alveolar gas phase into the bloodstream where it binds to haemoglobin, producing carboxyhaemoglobin (COHb). CO may also bind to haem-containing proteins in other tissues. The absorbed CO is eliminated from the body mainly by exhalation (395).

7. Biological monitoring

PAHs and their oxygen and nitrogen derivatives comprise up to 1% of the particulate mass of untreated diesel exhaust (423). Therefore, markers of poorly evaporating, particulate PAHs have been used for biomonitoring of diesel exhaust exposure. The most commonly used marker is 1-hydroxypyrene in urine indicating exposure to pyrene, which usually correlates well with the amount of common carcinogenic PAHs (like benzo[a]pyrene) in PAH mixtures (50, 181).

Schoket *et al*. saw slightly elevated 1-hydroxypyrene levels among 48 garage workers occupationally exposed to diesel exhaust (371). Similarly, slightly elevated levels of urinary hydroxy-metabolites of naphthalene, phenanthrene and pyrene were seen in a Finnish study among diesel exhaust exposed garage workers when compared to a non-exposed control group (205). According to the Finnish Institute of Occupational Health (FIOH) biomonitoring statistics from the years 2005–2007, 1-hydroxypyrene levels in diesel/gasoline exhaust exposed

workers remained low; the mean being 1.3 nmol/l, with a maximum of 8.6 nmol/l and a 90th percentile of 3.1 nmol/l (n = 29). The Finnish reference value for the occupationally non-exposed population is 3.0 nmol/l (104). Especially, when taking into account the decreased particulate and PAH levels with new technology diesel engines, biomarkers of PAHs are not considered very sensitive markers of exposure to diesel exhaust.

DNA adducts measured by ^{32}P post-labelling have been detected in the lungs of animals exposed to diesel exhaust via inhalation. Gallagher *et al*. exposed rats to diesel exhaust at 7 500 µg DEP/m^3 for 2, 6 and 24 months and detected a modest increase in nitro-PAH derived adducts, whereas PAH-derived adducts were not increased (109). Increases in total DNA adduct levels in the lungs after inhalation exposure of diesel exhaust have been seen also by other researchers (5, 49, 93, 174). These exposures represent exhausts of pre-2000 diesel engines.

In humans, increased incidences of DNA adducts in peripheral blood lymphocytes have been seen. Hemminki *et al*., Hou *et al*. and Nielsen *et al*. noticed an increased adduct frequency in lymphocytes among diesel exposed bus and truck terminal workers (152), bus maintenance workers (160) and garage workers (270), respectively. Increased DNA adduct levels have also been reported among bus drivers and traffic police exposed to ambient air pollution partly derived from diesel exhaust (271, 314, 410).

Only in a few studies have PAH-derived haemoglobin adducts been measured among diesel exhaust exposed workers. In the study by Nielsen *et al*., 1-hydroxypyrene levels correlated with hydroxyethylvaline haemoglobin adducts but not with DNA adducts (270). Zwirner-Baier and Neumann developed a method to measure five nitroarene haemoglobin adducts (1-nitropyrene, 2-nitrofluorene, 3-nitrofluoranthene, 9-nitrophenanthrene and 6-nitrochrysene) and measured the levels of these adducts in the blood of 29 bus garage workers, 20 urban hospital workers and 14 rural council workers. The bus garage workers did not differ from the other groups with respect to their adduct levels. A significant difference between people from urban and rural areas was found when all five adducts were added together (476).

Both DNA and haemoglobin adduct analyses are labour-intensive and expensive, and are therefore rarely used for routine biomonitoring of exposure. In addition, like in the case of measurement of PAH metabolites in urine, the decreased particulate and PAH levels with new technology diesel engines have decreased the usefulness of adducts in the assessment of exposure to diesel exhaust.

There are some molecular epidemiological studies available on the ability of diesel exhaust to cause micronuclei, chromosomal aberrations, sister chromatid exchanges (SCEs) and DNA damage in peripheral blood lymphocytes of exposed workers. These studies are summarised in Section 10.4. Most of the studies showing increased incidences of genotoxic effects in humans represent, however mixed exposure to gasoline or diesel exhaust present in urban air. Since these markers of genotoxicity are non-specific, they are applicable only for scientifically

controlled studies in which exposed group of workers is compared to an unexposed but otherwise matched control group.

Occupational exposure to CO can be biomonitored by measuring COHb levels in blood (395). Although diesel exhaust contains CO, the measured air CO levels are usually low, even in mining and tunnel construction work. Thus, COHb is not a good biomarker for exposure to diesel exhaust.

Overall, these biomonitoring methods are mainly useful for research purposes and there is no suitable method for routine biomonitoring of workers occupationally exposed to diesel exhaust.

8. Mechanisms of toxicity

8.1 Pulmonary effects

A major mechanism postulated for the respiratory effects of DEP is induction of reactive oxygen species (ROS), mainly superoxide (O_2^-) and hydroxyl (OH^-) radicals, and a subsequent inflammatory response in the lungs.

DEP contain constituents such as PAHs, quinones and transition metals which may be capable of producing ROS through redox chemistry both outside and inside the lung cells (218). ROS may also be produced by alveolar macrophages during particle phagocytosis (233).

Low levels of oxidative stress lead to activation of antioxidant and detoxification enzymes, e.g. haem oxygenase 1 (HO-1) and glutathione-*S*-transferases (GSTs), which protect the cells from the oxidative damage (218). At higher levels, the protective response may fail to provide adequate protection, leading to inflammatory and cytotoxic effects.

The inflammatory effects of DEP are mediated by redox-sensitive mitogen-activated protein (MAP) kinase and nuclear factor kappa B (NF-κB) cascades which are responsible for the production of inflammatory cytokines, chemokines and adhesion molecules (218). Cytokines and chemokines, including tumour necrosis factor alpha (TNF-α) and interleukins (ILs), together with the adhesion molecules are involved in the recruitment and activation of inflammatory cells in the lungs (92). Activated leukocytes produce large quantities of ROS causing further oxidative damage to the surrounding cells (7). The inflammatory cascade also includes activation of phospholipase A2, leading to an increase in local vasodilation and vasopermeability to enhance the accumulation of inflammatory cells. Besides the inflammatory effects, oxidative stress inside a cell may also lead to cytotoxicity through mitochondrial release of pro-apoptotic factors (218).

Of the gas phase constituents of diesel exhaust, NO_2 is a strong oxidant that reacts with antioxidants and lipids and proteins on cell membranes in the lower respiratory tract, causing the formation of further reactive products, e.g. peroxides (449). As discussed above, the oxidative stress may cause an inflammatory response in the lungs and increase the lung epithelial permeability. NO_2 has also

been found to reduce the airway ciliary activity, impairing mucociliary clearance (424). NO may increase local vasodilation in the lungs (429).

Mechanisms related to (pulmonary) genotoxicity and lung cancer are discussed in the following section.

8.2 Genotoxicity and cancer

The mechanisms of diesel exhaust caused lung cancer are likely to be multi-factorial. In animal studies, cancer has been shown to be related primarily to DEP (Section 9.4.5). Although the gaseous phase of the exhaust is known to contain small amounts of carcinogenic substances and has caused positive responses in bacterial mutagenicity tests (Section 9.3), filtered (particle-free) diesel exhaust has not caused cancer in the animal studies.

Diesel exhaust related genotoxicity may be caused by genotoxic compounds directly reacting with DNA or by generation of ROS with subsequent oxidative DNA damage. DEP contain several genotoxic compounds, including PAHs and their oxygen and nitrogen derivatives. These (or their reactive metabolites) can bind directly to DNA resulting in DNA damage. DEP/DEP extracts have caused positive responses both *in vitro* and *in vivo* genotoxicity assays (Section 9.3) including DNA adduct formation (5, 49, 80, 93, 94, 174, 204, 332, 368). Some studies have also shown elevated levels of urinary 1-hydroxypyrene and DNA adduct in lymphocytes of humans exposed to diesel exhaust, suggesting that the genotoxic and carcinogenic components of DEP are bioavailable also *in vivo* (Chapter 7). However, in a 2-year nose-only inhalation study in rats by Stinn *et al*., diesel exhaust caused a dose-related and persistent inflammatory response and an increase in the tumour incidence, but no increase in the levels of DNA adducts (394).

Diesel exhaust related genotoxicity and cancer may also be caused by reactive oxygen/nitrogen species (ROS/RNS) in the lung tissue. ROS/RNS are generated by inflammatory cells during particle-elicited inflammation but PAH-derived redox-cycling semiquinones may also contribute to the generation of ROS (370). Oxidative DNA damage has been reported in animals after intratracheal and inhalation exposure to diesel exhaust and DEP (93, 168, 169, 174, 267, 363).

Tokiwa *et al*. studied the formation of 8-hydroxydeoxyguanosine (8-OHdG) in mice after intratracheal injections of DEP. 8-OHdG formation in the lungs was elevated when purified carbonaceous particles were administered, but not when polyaromatic compounds were applied. These results suggest that carbonaceous particles, but not organic components of DEP, promote the formation of 8-OHdG (408). The authors proposed that the DEP related oxidative damage is caused by indirect secondary mechanisms involving the generation of hydroxyl radicals during phagocytosis of DEP by alveolar macrophages and neutrophils (407, 408).

Current evidence supports the role of lung overloading in oxidative DNA damage and cancers seen in rat bioassays after high-level exposure to diesel exhaust. Particle overload is caused by the deposition of high levels of particles

in the lungs resulting in an impairment of alveolar macrophage-mediated lung clearance (155, 299). This results in accumulation of excessive particulate lung burdens, influx of leukocytes, chronic pulmonary inflammation and generation of ROS. Generation of ROS results in DNA damage and eventually in cancer. The rat has been shown to be an especially sensitive species to this cascade of events. Because of this fact, rat studies using high exposure levels may not be suitable to elucidate the mechanisms of human carcinogenicity of diesel exhaust.

In humans, Perezt *et al*. and Pettit *et al*. demonstrated changes in gene expression connected to oxidative stress after controlled human exposure situations (326, 329). In addition, recruitment of inflammatory cells and inflammatory reactions resulting in increased airway neutrophil and lymphocyte levels and increased IL-8 levels in bronchoalveolar lavage (BAL) fluid have been seen in controlled human exposure studies at occupationally relevant exposure levels (365, 391). It can be hypothesised that in addition to the genotoxicity caused by mutagens bound to DEP or present in the gas phase, induction of chronic inflammation and ROS by diesel exhaust contributes to genotoxicity, cell proliferation and eventually to carcinogenesis in humans.

IARC recently concluded that there is strong mechanistic evidence that diesel engine exhaust can induce lung cancer in humans through genotoxic mechanisms including DNA damage, gene and chromosomal mutations, changes in relevant gene expression, production of ROS and inflammatory responses. In addition, the co-carcinogenic, cell proliferative and/or tumour-promoting mechanisms probably contribute to the lung carcinogenesis induced by diesel engine exhaust (167).

8.3 Cardiovascular effects

Several pathways have been suggested for the mechanisms of cardiovascular effects of fine particulate matter, including DEP (53, 254, 361). Firstly, inflammatory mediators, e.g. cytokines, acute phase proteins or activated inflammatory cells, released from the lungs may end up in the systemic circulation. The inflammatory mediators may affect the vascular system directly or e.g. by increasing the liver production of coagulation factors (53) or by affecting lipoprotein function (361). Recent data emphasise the role of particle-induced pulmonary acute phase response as an initiator of the process. Evidence from epidemiological, animal and *in vitro* studies indicates that the pulmonary acute phase response is related to the risk of cardiovascular diseases (361).

As an alternative pathway, particulates may disturb the autonomic nervous system balance or heart rhythm by interacting with lung receptors or nerves (53). Also, fine particles or particle constituents have been suggested to potentially translocate from the lungs into the systemic circulation, and to act directly on the vascular system. At a molecular level, a central role of ROS and oxidative stress is suggested at multiple stages, e.g. in promoting systemic inflammation, stimulating vasoconstriction and promoting atherosclerotic plaque instability (254).

Of the gas phase constituents, CO is well known for its ability to bind to haemoglobin and other haem-containing proteins, which at sufficiently high exposure

levels may lead to hypoxia, cardiac dysfunction and myocardial ischaemia (395). NO is a pulmonary vasodilator (434). NO that enters the systemic circulation is bound to haemoglobin, leading to the formation of methaemoglobin. Although methaemoglobin formation may lead to tissue hypoxia at high exposure levels (> 100 ppm NO) (434), this is not expected at the diesel exhaust related workplace levels of NO (≤ 15 ppm NO).

8.4 Immunological effects

Several mechanistic pathways have been suggested for an association of DEP and development or exacerbation of asthma or allergic diseases. In addition to the oxidative stress and inflammatory pathways discussed in Section 8.1 which may contribute to the pulmonary inflammation associated with allergic asthma, there are studies indicating that in conjunction with allergens, DEP may stimulate the T-helper cell type 2 (Th2) immune response and enhance the production of allergen-specific immunoglobulin (Ig)E and IgG (309). Oxidative stress is indicated to play a role both in the inflammatory and in the adjuvant effects of DEP (218, 347).

Of the gas phase constituents, NO_2 is suggested to exacerbate asthma through its inflammatory effects on the lung and effects on the lung permeability (424).

9. Effects in animals and in vitro studies

9.1 Irritation and sensitisation

The gas phase of diesel exhaust contains several irritating constituents such as NO_2 and aldehydes. No animal studies on the irritative or sensitising effects of diesel exhaust were, however, identified. Studies on immunological effects are reviewed in Section 9.2.5.

9.2 Effects of single, short-term and subchronic exposure

This chapter includes studies with exposure periods up to 13 weeks (90 days). Longer-term studies are presented in Section 9.4.

9.2.1 Acute toxicity

Pattle *et al*. exposed groups of mice, guinea pigs and rabbits by inhalation to undiluted diesel exhaust for 5 hours under different engine operating conditions, all leading to very high exposure levels. Mortality was followed up to 7 days. Exposure to NO_2 and CO was postulated to be the main cause of death (Table 6) (312). For comparison, the lethal concentration for 50% of the exposed animals at single administration (LC_{50}) is 88 ppm for pure NO_2 and 1 800 ppm for CO (4 hours inhalation, rats) (288).

Table 6. Effects of 5 hours of inhalation exposure of mice (40/dose), guinea pigs (10/dose) and rabbits (4/dose) to diesel exhaust under different engine operating conditions (312).

DEP ($\mu g/m^3$) [a]	CO (ppm)	NO_2 (ppm)	Alde-hydes (ppm) [b]	Mortality (%) [c]	Histopathology and COHb% [d]	Irritative potential [e]	Postulated main cause of death
74 000	560	23	16	0 (mouse) 0 (guinea pig) 0 (rabbit)	Mild tracheal and lung damage, "low" COHb	Highly irritating	–
53 000	380	43	6.4	48 (mouse) 90 (guinea pig) 0 (rabbit)	No tracheal damage, moderate lung damage, "low" COHb	Mildly irritating	NO_2
122 000	418	51	6.0	3 (mouse) 60 (guinea pig) 0 (rabbit)	No tracheal damage, severe lung damage, "low" COHb	Irritating	NO_2
1 070 000	1 700	12	154	100 (mouse) 100 (guinea pig) 100 (rabbit)	Mild to severe tracheal damage, mild lung damage, COHb 50–60%	Highly irritating	CO, "irritants"

[a] Determined as total particulate mass.
[b] Total aldehyde concentration determined by a titrimetric method.
[c] Total mortality up to 7 days.
[d] Applicable to all three species.
[e] Subjectively assessed by an observer applying his eye to a port in the chamber wall.
CO: carbon monoxide, COHb: carboxyhaemoglobin, DEP: diesel exhaust particles, NO_2: nitrogen dioxide.

9.2.2 Pulmonary effects

Table 7 lists studies on pulmonary effects of single, short-term and subchronic inhalation exposure to diesel exhaust in animals at non-lethal concentrations.

In the "Advanced Collaborative Emissions Study" (ACES), rats were exposed for 13 weeks (16 hours/day, 5 days/week) to diesel exhaust from a heavy-duty diesel engine with an exhaust after-treatment system fulfilling the US 2007 emission standards. At 13 µg DEP/m^3 (3.6 ppm NO_2), an increase in inflammatory markers in BAL, mild epithelial hyperplasia in terminal bronchioles, alveolar ducts and alveoli, accumulation of alveolar macrophages and occasional mild fibrotic lesions in alveolar ducts were observed. A slight reduction of pulmonary function was also indicated. No pulmonary effects were seen at levels ≤ 4 µg DEP/m^3 (≤ 1.0 ppm NO_2). In mice, a corresponding exposure resulted only in an increase in the number of neutrophils in BAL (246).

Particle-laden alveolar macrophages and alveolar type II cell hyperplasia were observed in rats after exposure to diesel exhaust at 101 or 952 µg DEP/m^3 (0.05 or 0.31 ppm NO_2) for 7 days (6 hours/day). No pulmonary effects were observed at 59 µg DEP/m^3 (0.02 ppm NO_2). When the same exposure protocol was carried out applying an exhaust after-treatment system (urea selective catalytic reduction), septal cell hyperplasia was observed at the highest exposure level only (36 µg DEP/m^3, 0.78 ppm NO_2) (413).

Mice exposed to diesel exhaust from an engine without exhaust after-treatment at 234 µg DEP/m^3 (~ 0.04 ppm NO_2) for 7 days (6 hours/day) showed increased

levels of lung inflammatory markers (e.g. IL-6 and TNF-α) and a decreased clearance of respiratory syncytial virus. No significant effects on inflammatory markers or viral clearance were observed after a corresponding exposure applying catalysed particle trap and low sulphur diesel fuel (7 µg DEP/m^3, ~ 0.04 ppm NO_2) (248).

Campen *et al*. detected increased numbers of polymorphonuclear leukocytes (PMNs) in BAL of mice exposed to diesel exhaust at 3 634 µg DEP/m^3 for 3 days (6 hours/day). No effect on PMN count was observed for a corresponding filtered exhaust or for total exhaust at 512 µg DEP/m^3 (59).

McDonald *et al*. studied the impact of different engine loads on the composition of diesel exhaust and effects on lung inflammation and viral clearance in mice. At the same particle mass concentration (~ 250 µg DEP/m^3), high engine load (100% of the engine capacity) resulted in a significantly higher proportion of EC (> 80% vs < 20%) and a lower proportion of organic carbon (< 20% vs > 80%) in the particles than did partial engine load (55% of the capacity). For the gas phase, partial engine load resulted in higher concentrations of non-methane hydrocarbons (1.5 vs 0.2 mg/m^3) and CO (5.5 vs 2.0 ppm CO). Mice exposed to high load diesel engine exhaust for 7 days (6 hours/day) showed increased levels of HO-1 and the cytokines TNF-α and interferon gamma (IFN-γ) in lung homogenate. In addition, decreased clearance of respiratory syncytial virus with increased inflammatory changes in the lungs was observed at high load. These effects were significantly less evident at partial load (245).

Sunil *et al*. (398) compared pulmonary effects of short-term diesel exhaust exposure in young (2 months) and old (18 months) mice. Animals were exposed to diesel exhaust at 300 or 1 000 µg DEP/m^3 for 3 hours or 3 days (3 hours/day). Dose-related histopathological changes, including thickening of alveolar septa, were observed in the lungs of older mice. In contrast, increased total cell and protein count and decreased antioxidant expression in BAL, without histopathological changes, were observed in younger mice. The results indicate increasing sensitivity to pulmonary effects of diesel exhaust with age (398).

Table 7. Pulmonary effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
2 4 13	0.1 1.0 3.6	13 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Rat, Wistar Han 10/sex/group	Decreased antioxidant capacity (TEAC) of BAL at all levels (no dose-response). At 13 $\mu g/m^3$: epithelial hyperplasia, accumulation of AM and occasional fibrotic lesions (mild in severity); increase in total protein and albumin in BAL and HO-1 in lung tissue; increase in IL-α and IL-1β in the lungs of females; indications of slight effects on pulmonary function.	(246)
2 3 9	0.1 0.8 4.3	13 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Mouse, C57BL/6 10/sex/group	Increase in neutrophils in BAL at 9 $\mu g/m^3$; no histo-pathological lesions.	(246)
3 6 36	0.04 0.08 0.78	1–7 d (6 h/d)	9.2 L diesel engine with urea SCR system	Rat, F344 6/group (males)	At 36 $\mu g/m^3$: decrease in macrophages and increase in lymphocytes in BAL at day 3, recovering by day 7; mild type II cell hyperplasia at day 7 (3/6 rats).	(413)
7	~ 0.04	7 d (6 h/d)	5 kW diesel generator with catalysed particle trap (low sulphur fuel)	Mouse, C57BL/6 6–8/group (sex not stated)	No significant effects on inflammatory markers or respiratory viral clearance.	(248)
59 101 952	0.02 0.05 0.31	1–7 d (6 h/d)	9.2 L diesel engine	Rat, F344 6/group (males)	Decrease in macrophages and increase in lymphocytes in BAL at day 3 at 952 $\mu g/m^3$, recovering by day 7; particle-laden AM at ≥ 101 $\mu g/m^3$ at day 7 (6/6 rats); mild type II cell hyperplasia at 952 $\mu g/m^3$ at day 7 (3/6 rats).	(413)
100	nd	8 wk (7 h/d, 5 d/wk)		Mouse, C57BL/6 Nrf2-/- and Nrf2+/+ (females, no. not stated)	Increased airway reactivity to methacholine and mucus cell hyperplasia in Nrf2-/- mice; decrease in total cell and AM counts, and increase in lympho-cytes, eosinophils, IL-12 and IL-13 in BAL in Nrf2-/- mice; upregulation of several lung antioxidant genes in Nrf2+/+ mice.	(219)

Table 7. Pulmonary effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
174	0.5	4 wk (6 h/d, 5 d/wk)	28 kW diesel generator	Rat, F344 15/group; with ozone-induced mild lung inflammation (males)	No additional effect on inflammatory markers in BAL; no distinct effect on lung pathology.	(118)
200	nd	7 wk (6 h/d, 5 d/wk)		Mouse, ApoE-/- 12/group (males)	Significant increase in total and particle-laden AM.	(15)
234	~ 0.04	7 d (6 h/d)	5.0 kW diesel generator	Mouse, C57BL/6 6–8/group (sex not stated)	Increased levels of HO-1, TNF-α, IL-6 and IFN-γ in lung homogenate; decreased lung viral clearance with increased inflammatory changes.	(248)
250	nd	7 d (6 h/d)	5.0 kW diesel generator, engine load: partial 55% and high 100%	Mouse, C57BL/6 6–8/group (males)	Increased levels of HO-1, TNF-α and IFN-γ in lung homogenate (high engine load only); decreased lung viral clearance with increased inflammatory changes (high load only).	(245)
300 1 000	nd	3 h	5.5 kW diesel generator	Mouse, CB6F1; young (2 mo) and old (18 mo) (males, no. not stated)	Dose-related histopathological changes (e.g. thickening of alveolar septal walls) in older mice; no distinct effect on histology in younger mice; increased total cell count in BAL in younger mice; upregulated IL-6 mRNA in both, and lipocalin 24p3 and IL-8 mRNAs in older mice.	(398)
300 1 000	nd	3 d (3 h/d)	5.5 kW diesel generator	Mouse, CB6F1; young (2 mo) and old (18 mo) (males, no. not stated)	As above and in addition increased protein content in BAL in younger mice.	(398)
500 2 000	< 0.25 1.2	4 wk (4 h/d, 5 d/wk)	30 kW diesel generator	Rat, Wistar Kyota and SH 6–9/strain/group (males)	Increase in neutrophils in BAL (significant at 2 000 $\mu g/m^3$); small increase in GGT activity in BAL at 2 000 $\mu g/m^3$.	(121)

Table 7. Pulmonary effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
512 3 634 filtered: 6	nd	3 d (6 h/d)	5.5 kW diesel generator	Mouse, C57BL/6J and ApoE-/- 4–6/strain/group (males)	Increased PMN levels in BAL at 3 634 $\mu g/m^3$ (unfiltered only).	(59)
1 500	0.49	10–13 wk (20 h/d, 7 d/wk)	Diesel engine	Rat, F344, 168/group Mouse, A/J, 672/group Hamster, Syrian, 236/group (all males)	Increase in lung weight; inflammatory changes, increase in thickness of alveolar walls; minimal species differences.	(183)
1 900	0.94	2 h	28 kW diesel generator, 2011	Rat, F344 16/group (sex not stated)	Increase in alkaline phosphatase (marker of damage of type II cells) in BAL at 18 h post-exposure; no other effects on markers of inflammation, cyto-toxicity or oxidative stress; upregulation of iNOS and CYP1A1 mRNA in the lung at 4 h post-exposure (recovered by 18 h), and HO-1 mRNA at 18 h post-exposure.	(428)
1 900	0.94	2 h	28 kW diesel generator, 2011	Rat, F344 10/group (males)	Transient increase in IL-6, TNF-α, HO-1 and alkaline phosphatase in BAL at 18–72 h post-exposure; transient increase in anti-oxidant enzymes in lung homogenate at 4–18 h.	(201)
5 000 20 000	nd	4 d (1.5 h/d)	Resuspended diesel particles (SRM 1650)	Mouse, BALB/cJ and Muta™ 4–5/strain/group (females)	Increase in IL-6 expression in lung tissue, and in neutrophils and AM in BAL at 1 h post-exposure at 20 000 $\mu g/m^3$ (increase in neutrophils persisted at 22 h).	(93, 349)
6 000	nd	4 wk (20 h/d, 5.5 d/wk)	Diesel engine	Rat, F344 16/group (males)	Macrophage aggregation, increased PMN count, type II cell proliferation, thickened alveolar walls.	(446)

Table 7. Pulmonary effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
6 300	nd	8 wk (20 h/d, 7 d/wk)	Diesel engine	Guinea pig, Hartley (both sexes, no. not available)	Increase in relative lung weight; AM aggregation; hypertrophy of goblet cells; focal hyperplasia of alveolar epithelium.	(453)
6 400	2.1	4 wk (20 h/d, 7 d/wk)	Diesel engine	Cat, inbred 15/group (males)	Focal pneumonitis or alveolitis; few effects on lung function.	(319)
6 400 6 800	2.5 2.8	4 wk (20 h/d, 7 d/wk)	Diesel engine	Rat, Sprague Dawley 15/group (males)	Decreased body weight, increased vital capacity and total lung capacity.	(316)
6 800	2.9	4 wk (20 h/d, 7 d/wk)	Diesel engine	Guinea pig, Hartley 41–51/group (both sexes)	Increase in pulmonary flow.	(453)
20 000 80 000	nd	1.5 h	Resuspended diesel particles (SRM 1650)	Mouse, BALB/cJ and Muta™ 4–5/strain/group (females)	Dose-dependent increase in IL-6 expression in lung tissue at 1 h post-exposure (persisted at 22 h at 80 000 $\mu g/m^3$).	(93, 349)
20 000	nd	4 d (1.5 h/d)	Resuspended diesel particles (SRM 2975), nose-only	Mouse, C57BL (TNF+/+) and B6 (TNF-/-) 4/strain (females)	Increased fraction of neutrophils in BAL (both strains); increased expression of IL-6 mRNA (higher in TNF-/- mice); increased expression of TNF mRNA (TNF+/+ mice only).	(360)
72 000	nd	4 d (1.5 h/d)	Resuspended diesel particles (SRM 2975)	Mouse, C57BL 10/group (females)	No effect on inflammatory markers in BAL at 56 days post-exposure.	(162)

AM: alveolar macrophage(s), ApoE-/-: apolipoprotein E deficient (ApoE is involved in lipoprotein metabolism), BAL: bronchoalveolar lavage, CYP1A1: cytochrome P4501A1, DEP: diesel exhaust particles, GGT: γ-glutamyl transferase, HO-1: haem oxygenase 1, IFN-γ: interferon gamma, IL: interleukin, iNOS: inducible nitric oxide synthase, nd: no data, NO_2: nitrogen dioxide, Nrf2-/-: nuclear factor erythroid derived 2 deficient (Nrf is a regulator of cellular resistance to oxidants), PMN: polymorphonuclear leukocyte, SCR: selective catalytic reduction, SH: spontaneously hypersensitive (a heart failure prone animal model), SRM: standard reference material, TEAC: trolox equivalent antioxidant capacity, TNF-α: tumour necrosis factor alpha, US: United States.

9.2.3 Haematological and cardiovascular effects

Identified studies on haematological and cardiovascular effects of single, short-term or subchronic inhalation exposure to diesel exhaust are summarised in Table 8.

In the ACES programme, no significant changes in haematology, serum chemistry, clotting indicators or plasma markers of vascular or systemic inflammation, immune function or general toxicity were observed in rats and mice exposed for 13 weeks (16 hours/day, 5 days/week) to diesel exhaust from a heavy duty engine fulfilling the US 2007 emission standards (highest concentration: 9–13 μg DEP/m^3, 3.6–4.3 ppm NO_2, 7.1–11 ppm CO) (74, 246).

Transient changes in heart rate and heart rate variability have been reported in healthy animals after 1–4 hours exposure to whole diesel exhaust at ~ 500 μg DEP/m^3 (4, 52, 208). Kooter *et al*. reported a transient increase in platelets in blood and thrombogenicity in lung homogenate and plasma in healthy rats after 2 hours exposure to diesel exhaust at 1 900 μg DEP/m^3 (0.94 ppm NO_2, 14 ppm CO) (201).

Alterations in cardiovascular function have been observed in spontaneously hypertensive, heart failure prone rats and atherosclerotic mice during and after short-term exposure to total and particle-free diesel exhausts (59, 61, 62, 208). Carll *et al*. exposed hypertensive rats to whole (472 μg DEP/m^3, 0.3 ppm NO_2, 9.5 ppm CO) and filtered (3 μg DEP/m^3, 0.4 ppm NO_2, 9.7 ppm CO) diesel exhaust for 4 hours. More pronounced changes in electrocardiography and heart rate variability during the exposure and increase in post-exposure arrhythmias were observed with the filtered exhaust, indicating either an inhibiting effect or a competing impact of the particles (62).

Bai *et al*. and Campen *et al*. reported changes in atherosclerotic plaque composition characteristic of unstable plaques in atherosclerotic mice exposed to diesel exhaust at ≥ 200 μg DEP/m^3 for 7 weeks (16, 60). In addition to these inhalation studies, Miller *et al*. reported more frequent, larger and more severe atherosclerotic plaques in atherosclerotic mice exposed by oropharyngeal aspiration to 35 μg of DEP (SRM 2975) twice a week for 4 weeks. The changes were concomitant with pulmonary inflammation and increased antioxidant gene expression in the liver (an indication of oxidative stress) (253).

Mice exposed by inhalation to high levels of DEP (20 000 μg DEP/m^3, 4 days, 1.5 hours/day) responded with increased pulmonary serum amyloid A expression, which indicates pulmonary acute phase response and is related to an increased risk of cardiovascular diseases (362).

McDonald *et al*. studied the effects of engine load on the cardiovascular effects of diesel exhaust. Atherosclerotic mice were exposed to diesel exhaust at high and partial engine load (~ 3 500 μg DEP/m^3) for 3 days (6 hours/day). A significant reduction of heart rate was seen at both loads, instantly at partial load and after 4 hours of exposure at high load. At partial load, characterised by a higher concentration of CO and non-methane hydrocarbons in the gas phase, T-wave alterations were also observed (245).

Table 8. Haematological and cardiovascular effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
2 4 13	0.1 1.0 3.6	~1 ~2 11	13 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Rat, Wistar Han 10/group/sex	No significant effects on haematology, serum chemistry or coagulation indicators.	(246)
2 4 13	0.1 1.0 3.6	~1 ~2 11	13 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Rat, Wistar Han 10/group/sex	Transient dose-dependent increase in total and HDL cholesterol in males after 4 weeks of exposure; no significant effects on 29 plasma markers of vascular or systemic inflammation, immune function or general toxicity.	(74)
2 3 9	0.1 0.8 4.3	~1 ~2 7.1	13 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Mouse, C57BL/6 10/group/sex	No significant effects on haematology, serum chemistry or coagulation indicators.	(246)
2 3 9	0.1 0.8 4.2	nd nd 7.1	13 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Mouse, C57BL/6 10/group/sex	No significant effects on 29 plasma markers of vascular or systemic inflammation, immune function or general toxicity.	(74)
2 107 306 972	0.8 0.4 0.7 4.1	0.9 2.9 9.6 29	7 d (6 h/d)	5.9 L turbo engine, 2000	Rat, SH 10–12/group (6 males, 4–6 females)	Increased heart rate in all exposed groups; indication of a dose-related prolongation of the PQ interval.	(61)
109 305 1 012 filtered: 28	nd	3.6 10 31 filtered: 31	7 wk (6 h/d, 7 d/wk)	5.9 L turbo engine, 2000	Mouse, ApoE-/- 10/group (males)	Increase in aortic lipid peroxides and macrophage accumulation in the plaques at ≥ 305 $\mu g/m^3$ (increase in lipid peroxidation observed also with filtered exhaust); no significant effect on athero-sclerotic plaque area.	(60)

Table 8. Haematological and cardiovascular effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
150 500 filtered: 15 21	< 0.5 < 0.5 filtered: < 0.5 < 0.5	6 19 filtered: 6 18	4 h	4.8 kW diesel generator, HEPA filtering	Rat, SH, 65 (males) and Wistar Kyota, 12 (males)	Decrease in heart rate, immediate ST depression, PR prolongation and bradycardia in SH rats (filtered exhaust only; no dose-response); post-exposure ST depression in SH rats (unfiltered exhaust only; no dose-response); decrease in heart rate in Wistar rats (unfiltered 500 $\mu g/m^3$ only).	(208)
174	0.5	2.3	4 wk (6 h/d, 5 d/wk)	28 kW diesel generator	Rat, F344 with ozone-induced mild lung inflammation 15/group (males)	Decrease in leukocytes, lymphocytes, basophils and von Willebrand factor in the blood; no effect on other haematological parameters; no effect on responses to vasodilators.	(118)
200	nd	nd	7 wk (6 h/d, 5 d/wk)	5.9 L turbo engine, 2002, 75% load	Mouse, ApoE-/- 10/group (males)	Increase in lipid content, cellularity, foam cell formation and smooth muscle cell content of the plaques; enhanced iNOS expression in the thoracic aorta and the heart; attenuated vasoconstriction to phenylephrine; no significant effect on athero-sclerotic plaque volume.	(15, 16)
200 400	nd	nd	3 d (6 h/d)	Diesel generator	Mouse, ApoE-/- 10/group (males)	Attenuated vasoconstriction to phenylephrine (no dose-response); no effect on vasodilatation to acetylcholine.	(193)
200 400	nd	nd	7 wk (6 h/d, 5 d/wk)	Diesel generator	Mouse, ApoE-/- (males, no. not stated)	Increased lung and plasma HSP70 levels; attenuated vasoconstriction to phenylephrine (no dose-response); no effect on vasodilatation to acetylcholine.	(193)
300	nd	~3	5 h	5.5 kW diesel generator	Rat, Sprague Dawley 9 exposed, 5 controls (males)	Attenuated vasodilatation to acetylcholine.	(69)

Table 8. Haematological and cardiovascular effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
472 filtered: 3	0.3 filtered: 0.4	9.5 filtered: 9.7	4 h	4.8 kW diesel generator, HEPA filtering	Rat, SH 20 (males)	Increased heart rate variably and T-wave flattening during exposure; post-exposure QT prolongation and arrhythmias; more pronounced effects with filtered exhaust.	(62)
500	1.1	4.3	3 h	Diesel engine	Rat, Wistar 10 healthy, 10 with CHF (all males)	Decrease in heart rate variability in both groups (returned to baseline in 2.5 h); increase in ventricular premature beats in CHF group (persisted 5 h).	(4)
500 2 000	< 0.25 1.2	1.3 4.8	4 wk (4 h/d, 5 d/wk)	30 kW diesel generator	Rat, Wistar Kyota and SH 6–9/strain/group (males)	Decrease in cardiac mitochondrial aconitase activity (significant at 2 000 $\mu g/m^3$); down-regulation of genes which gave a hypertensive-like cardiac gene expression pattern in healthy rats; no distinct effect on haematological parameters.	(121)
512 3 634 filtered: 6	nd	nd	3 d (6 h/day)	5.5 kW diesel generator, filtering by ceramic particle trap	Mouse, ApoE-/- 4–6/group (males)	Decrease in heart rate, T-wave depression and bradycardia at 3 634 $\mu g/m^3$ (both unfiltered and filtered exhaust).	(59)
556	nd	9.8	1 h	Diesel generator	Mouse, BALB/c 24/group (males)	Increase in heart rate variability at 30 and 60 min post-exposure.	(52)
1 900	0.94	14	2 h	28 kW diesel generator, 2011	Rat, F344 10/group (males)	Transient increase in mean platelet volume and component in blood at 24 h post-exposure; transient increase in thrombogenicity in lung homogenate and plasma at 4–48 h.	(201)
3 500	nd	~24 (high) ~77 (partial)	3 d (6 h/d)	5.5 kW diesel generator; engine load: partial 55% and high 100%	Mouse, ApoE-/- 5/group (males)	Decrease in heart rate (at both loads, immediate and enhanced at partial load); increase in T-wave area (partial load only).	(245)

Table 8. Haematological and cardiovascular effects in animals after single, short-term and subchronic inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
6 300 6 800	2.3 2.9	~17	8 wk (20 h/d, 7 d/wk)	Diesel engine	Guinea pig, Hartley 41–51/group (both sexes)	Small decrease in heart rate at 6 800 $\mu g/m^3$; no effect on heart mass or ECG.	(453)
6 400 6 800	2.5 2.8	~17	4 wk (20 h/d, 7 d/wk)	Diesel engine	Rat, Sprague Dawley 15/group (males)	Decreased body weight, reduced arterial blood pH.	(316)
6 800	2.9	~17	4 wk (20 h/d, 7 d/wk)	Diesel engine	Guinea pig, Hartley 41–51/group (both sexes)	Bradycardia.	(453)
20 000	nd	nd	4 d (1.5 h/d)	Resuspended diesel particles (SRM 2975), nose-only	Mouse, C57BL/cJ females (no. not stated)	Increased serum amyloid A (Saa3) mRNA expression in lung tissue.	(362)

ApoE-/-: apolipoprotein E deficient (ApoE is involved in lipoprotein metabolism), CHF: chronic ischaemic heart failure, CO: carbon monoxide, DEP: diesel exhaust particles, ECG: electrocardiogram, HDL: high density lipoprotein, HEPA: high-efficiency particulate arrestance, HSP70: heat shock protein 70, iNOS: inducible nitric oxide synthase, nd: no data, NO_2: nitrogen dioxide, SH: spontaneously hypertensive (a heart failure prone animal model), SRM: standard reference material, US: United States.

9.2.4 Neurological effects

Table 9 lists all studies on neurological effects of diesel exhaust exposure on animals. Long-term studies (> 13 weeks) presented in the table are discussed separately in Section 9.4.3.

In male rats, increased levels of pro-inflammatory cytokines and/or markers of oxidative stress have been identified in different regions of the brain after 2 hours of exposure to diesel exhaust at 1 900 µg DEP/m^3 (428) and 4 weeks of exposure at ≥ 174 µg DEP/m^3 (119, 211).

Female mice exposed to nanoparticle-rich diesel exhaust at 122 µg DEP/m^3 for 13 weeks (5 hours/day, 5 days/week) were reported to have a reduced learning performance in the Morris water maze test. A non-significant reduction of performance was observed at 35 µg DEP/m^3. Mice exposed to particle-free exhaust showed practically no difference compared to a control group (459). No effect on learning performance was observed in male mice exposed for 4 weeks (5 hours/day, 5 days/week) to 149 µg DEP/m^3 (458). Decreased locomotive activity in the open field test was reported in female mice exposed 4 days (1.5 hours/day) to resuspended diesel particulates at 72 000 µg DEP/m^3 (162).

9.2.5 Immunological effects

Studies on immunological effects of inhalation exposure to diesel exhaust are summarised in Table 10 (including the only long-term study available, which is described in Section 9.4.4).

Female mice exposed to 169 µg DEP/m^3 (0.5 ppm NO_2) or a corresponding particle-free exhaust for 8 weeks (5 hours/day, 5 days/week), showed an increase in ovalbumin-induced eosinophilic lung inflammation and an increase in Th2-related cytokines and chemokines in lung homogenate. No impact on the ovalbumin-induced response was observed at 36 µg DEP/m^3 (0.2 ppm NO_2) (405).

In a study of Stevens *et al*., female mice exposed to diesel exhaust at 560 or 2 100 µg DEP/m^3 for 5 days (4 hours/day) showed a dose-related increase in the lung inflammatory response to ovalbumin (392). No effect on ovalbumin-induced lung inflammation was observed in female mice exposed to 103 µg DEP/m^3 for 12 weeks (7 hours/day, 5 days/week). A transient increase in ovalbumin-induced airway responsiveness to methacholine at 2–4 weeks post-exposure was, however, reported (237).

In guinea pigs, enhanced responses to ovalbumin and histamine have been observed after 3 hours and 8 weeks of inhalation exposure to diesel exhaust at high levels (≥ 3 000 µg DEP/m^3) (144, 198). An increase in ovalbumin-induced IgE and IgG levels in serum was also reported for rats exposed 5 days (4 hours/day) to resuspended diesel particulates at 21 000–23 000 µg DEP/m^3 (90, 91).

Table 9. Neurological and brain effects in animals after single, short- and long-term inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
3 5 12	0.1 0.9 4.2	1.1 1.9 6.4	104 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Rat, Wistar Han 5/sex/group	No exposure-related effects on markers of oxidative stress or lipid peroxidation in tissue samples from the hippocampus region (measured by TBARS assay).	(136)
35 122 filtered: 5.5	0.16 0.48 filtered: 0.47	1.1 2.8 filtered: 2.8	13 wk (5 h/d, 5 d/wk)	7.8 L diesel engine; conditions adjusted to achieve particles < 100 nm	Mouse, BALB/c 6/group (females)	Dose-related increase in escape latency in the Morris water maze behavioural test (significant at 122 $\mu g/m^3$; unfiltered exhaust only); up-regulation of NR2A mRNA in hippocampus at 122 $\mu g/m^3$.	(459)
35 100 311 992	0.3 0.7 1.3 6.9	1.5 3.6 10 31	26 wk (6 h/d, 7 d/wk)	5.9 L turbo diesel engine, 2000	Rat, F344 8/group (males)	Increase in TNF-α in the midbrain at ≥ 100 $\mu g/m^3$, and in the frontal and temporal lobes and olfactory bulb at 992 $\mu g/m^3$; elevated levels of IL-1β in the midbrain at 992 $\mu g/m^3$; increase in biomarkers for Alzheimer's disease in the frontal and temporal lobes and for Parkinson's disease in the midbrain at ≥ 311 $\mu g/m^3$.	(210)
149	0.53	3.3	4 wk (5 h/d, 5 d/wk)	7.8 L diesel engine; conditions adjusted to achieve particles < 100 nm	Mouse, BALB/c 6/group (males)	No effect on escape latency in the Morris water maze behavioural test; upregulation of NR1, NR2A, NR2B and CaMKIV mRNAs in the olfactory bulb.	(457, 458)
174	0.5	2.6	4 wk (6 h/d, 5 d/wk)	28 kW diesel generator	Rat, F344 with ozone-induced mild lung inflammation 10/group (males)	Increase in TNF-α and IL-1α in striatum; no effects on other parts of the brain.	(119)

Table 9. Neurological and brain effects in animals after single, short- and long-term inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
500 2 000	nd	nd	4 wk (4 h/d, 5 d/wk)	30 kW diesel generator	Rat, Sprague Dawley and Wistar Kyota 4/group (males)	Increase in TNF-α, IL-1β, IL-6 and MIP-1α in the midbrain, olfactory bulb and cortex, and RAGE and in IBA-1 in the midbrain at both exposure levels.	(211)
1 900	0.9	14	2 h	28 kW diesel generator, 2011, nose-only exposure	Rat, F344 16/group (males)	Increased expression of HO-1 in the olfactory bulb and CYP1A1 in the pituitary gland at 4 h post-exposure (recovered by 18 h), and HO-1 in the cerebral cortex and COX-2 in the cerebellum at 18 h.	(428)
72 000	nd	nd	4 d (1.5 h/d)	Resuspended diesel particles (SRM 2975)	Mouse, C57BL 10/group (females)	Decreased locomotive activity in open field test; no effect on escape latency in the Morris water maze test.	(162)

CaMKIV: calcium-calmodulin-dependent protein kinase, CO: carbon monoxide, COX-2: cyclooxygenase-2, CYP1A1: cytochrome P4501A1, DEP: diesel exhaust particles, HO-1: haem oxygenase 1, IBA-1: ionised calcium-binding adaptor molecule 1, IL: interleukin, MIP-1α: macrophage inflammatory protein 1α, nd: no data, NO_2: nitrogen dioxide, NR: *N*-methyl-*D*-aspartate receptor subunit, RAGE: receptor for advanced glycation end products, SRM: standard reference material, TBARS: thiobarbituric acid reactive substances, TNF-α: tumour necrosis factor alpha, US: United States.

Table 10. Immunological effects in animals after single, short- and long-term inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
36 169 filtered: < LOD	0.2 0.5 filtered: 0.5	8 wk (5 h/d, 5 d/wk)	8.0 L diesel engine; conditions adjusted to achieve particles < 100 nm; ULPA filtering	Mouse, IRC 4–12/group (females)	Increase in OVA-induced eosinophils in BAL, inflammatory cell infiltration and mucus hyperplasia in the lungs, and Th2 related cytokines, MCP-1 and myeloperoxidase in lung homogenate (high concentration unfiltered, and filtered exhaust only); increase in OVA-specific IgE in serum (filtered exhaust only).	(405)
103	2.2	12 wk (7 h/d, 5 d/wk)	2.3 L diesel engine, load 80%	Mouse, BALB/c 46/group (females)	Transient increase in OVA-induced airway responsiveness to methacholine after 2–4 weeks of exposure; transient increase in the gene expression of several pro-asthmatic cytokines/chemokines; no effect on OVA-induced inflammatory markers in BAL.	(237)
560 2 100	< 0.25 1.1	5 d (4 h/d)	30 kW diesel generator	Mouse, BALB/c 6–8/group (females)	Dose-depended increase in OVA-induced eosinophils, neutrophils and lymphocytes in BAL (significant at both levels).	(392)
1 000 3 200	1.4 4.4	3 h	7.4 L heavy-duty diesel engine	Guinea pig, Hartley 8/group (males)	Increase in histamine-induced nasal secretion and sneezing at 3 200 $\mu g/m^3$; no effect on nasal secretion or sneezing without histamine induction.	(198)
1 280	nd	3 wk (5 h/d, 7 d/wk)	5.5 kW diesel generator	Mouse, BALB/c 5/group (females)	Increase in *Aspergillus fumigatus*-induced IgE levels in serum; altered methylation of IFN-γ and IL-4 gene promoters.	(222)
3 000	nd	8 wk (12 h/d, 7 d/wk)		Guinea pig, Hartley 8/group (females)	Enhanced OVA-induced decrease in specific airway conductance; increased mucus secretion and eosinophilic inflammation; shortened cilia.	(144)
3 240 filtered: 10	1.0 filtered: 1.1	24 wk (16 h/d, 5 d/wk)	6.9 L diesel engine	Mouse, BDF 60/group (females)	Significant increase in the number of animals expressing JCP-specific IgE in serum (both groups); non-significant increase of JCP-induced IgE level in serum (both groups) as compared to JCP exposed only.	(234)

Table 10. Immunological effects in animals after single, short- and long-term inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
21 000	nd	5 d (4 h/d)	Resuspended diesel particles (SRM 2975)	Rat, BN/CrlBR 5/group (males)	Increase in OVA-induced IgE and IgG levels in serum; decrease in OVA-induced inflammatory markers in BAL (sensitisation after exposure).	(90)
23 000	nd	5 d (4 h/d)	Resuspended diesel particles (SRM 2975)	Rat, BN/CrlBR 5/group (males)	Increase in OVA-induced IgE and IgG levels in serum; increase in OVA-induced inflammatory markers in BAL (sensitisation prior to exposure).	(91)

BAL: bronchoalveolar lavage, DEP: diesel exhaust particles, IFN-γ: interferon gamma, Ig: immunoglobulin, IL: interleukin, JCP: Japanese cedar pollen, LOD: limit of detection, MCP-1: monocyte chemoattractant protein 1, nd: no data, NO_2: nitrogen dioxide, OVA: ovalbumin, SRM: standard reference material, Th2: T-helper cell type 2, ULPA: ultra low particulate air.

9.3 Genotoxicity

9.3.1 Bacterial mutagenicity tests

Studies from the 1970s to the 1990s evaluating the mutagenicity of older technology diesel engine exhaust have been summarised in earlier reviews (166, 423). Most of these studies were performed with DEP extracts.

Bacterial mutagenicity tests summarised in the IARC monograph (1989) showed positive responses in several *Salmonella* strains with and without metabolic activation after exposure to the extracted organic fraction of diesel exhaust particulates (166). Especially strain TA98 has been sensitive to the DEP extracts (166, 423). Using chemical fractioning, the mutagenicity of DEP extracts was specifically linked to nitroarenes (268, 289, 353, 364, 372, 406). These observations are supported by studies using different diesel fuels (383, 440). Also the sulphur content of diesel fuel has been shown to contribute to the mutagenicity of diesel exhaust in bacterial mutagenicity tests (58, 383).

There are fewer studies on the mutagenicity of whole DEP than of solvent extracted DEP. Wallace *et al*. tested DEP from exhaust pipe scrapings of two trucks, extracted by dichloromethane or dispersed by dipalmitoyl lecithin (an artificial pulmonary surfactant), in the Ames mutagenicity assay. Positive mutagenic responses were obtained with both dichloromethane extraction and dipalmitoyl lecithin dispersion suggesting that possible mutagens associated with inhaled particles may be dispersed or solubilised also *in vivo* in lungs (426).

In addition to DEP extracts, gaseous emissions from diesel exhaust have also shown positive responses in bacterial mutagenicity tests mainly in the absence of exogenous metabolic activation (166, 423). The same is true also for semi-volatile organic compounds (SVOCs), which have shown positive responses especially in strain TA98. The mutagenic potency of SVOCs is, however, significantly lower than that of DEP extracts (14, 441).

There are some studies available on the effects of diesel exhaust after-treatment systems on the mutagenicity of DEP extracts. The use of diesel oxidation catalyst or continuously regenerating particle trap have even caused increased mutagenicity of DEP extracts under some conditions (57, 66, 298, 311). It has been suggested that the increased mutagenicity is due to the ability of oxidation catalysts to increase the formation of direct acting mutagens by the reaction of NO_X with PAHs resulting in the formation of nitrated-PAHs (57, 298). On the other hand, Shi *et al*. saw decreased mutagenicity after the use of a diesel oxidation catalyst (379). In a study by Krahl *et al*., a selective catalytic reduction after-treatment system was effective in reducing the mutagenicity of DEP extracts (202).

Westphal *et al*. studied the effects of a diesel oxidation catalyst on the mutagenicity of both DEP extracts and the gas phase. The use of the catalyst reduced the mutagenicity of the gas phase and to a lesser extent of the DEP extracts in the Ames test (442). The mutagenicity of DEP extracts, DEP suspensions and whole diesel exhaust was decreased also in the study by Andre *et al*. after the use of diesel oxidation catalyst and diesel particulate filter on a Euro 3 compliant engine. Based on the mutagenic pattern, nitroaromatics seemed to play a significant role

in the mutagenicity of DEP. However, the remaining mutagenicity of whole diesel exhaust after diesel oxidation catalyst/diesel particulate filter after-treatment was suggested to be attributable to the gas phase (3).

9.3.2 Mammalian cell tests

Genotoxic responses in mammalian cells of DEP and DEP extracts of older technology diesel engines have been summarised in earlier reviews (166, 423).

Direct exposure of human peripheral lymphocyte cultures to diesel exhaust for 3 hours resulted in an increased SCE frequency in 2 out of 4 cultures (417). DEP extracts caused hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations in Chinese hamster ovary (CHO) cells (54, 67, 214) with or without metabolic activation (S9 mix) and thymidine kinase mutations in human lymphoblast cells with S9 and in mouse lymphoma L15178Y cells with and without metabolic activation (220, 258). In addition, several studies have demonstrated an increase in SCEs in cultured human peripheral lymphocytes or CHO cells after exposure to DEP extracts (22, 54, 227, 264).

There are also several newer studies (published after the year 2000) on the *in vitro* genotoxicity of DEP extracts in mammalian cells. The comet assay has been the most popular test system employed. A significant induction of DNA strand breaks has been observed in different cell lines after exposure to DEP extracts from heavy-duty diesel trucks (89, 224, 300, 384). Also SRMs 1650 and 2975 have shown positive responses in the comet assay (89, 93, 179). Oh and Chung noted also an induction of micronuclei after treatment of CHO cells with crude DEP extracts. When the CHO cells were exposed to different fractions of the DEP extracts genotoxic effects were confined to the aromatic and slightly polar fraction (300). Micronucleus induction was observed in Chinese hamster V79 lung cells after exposure to DEP extracts from three diesel engines from 1998–2000 (224). Jalava *et al*. studied the effect of a combined diesel oxidation catalyst-particle oxidation catalyst after-treatment system on the induction of DNA strand breaks in the comet assay in a mouse macrophage cell line. The catalyst did not affect the genotoxicity of DEP extracts from an engine operated with conventional diesel fuels (178).

Gene mutations have been observed in the CD59 locus of human-hamster hybrid (A_L) cells and FE1 MutaMouse pulmonary epithelial cells after exposure to DEP (SRMs 1650b and 2975) (21, 176). Modest increases in DNA strand breaks were seen when human alveolar adenocarcinoma cells were exposed to DEP collected from the filter of a Euro 4 compliant diesel engine (151). However, in a study by Totlandsdal *et al*., DEP collected from light-duty diesel engines induced DNA strand breaks in human bronchial epithelial BEAS-2B cells only at high, clearly cytotoxic doses (412).

DEP collected from exhaust pipes of two diesel engines and either extracted by dichloromethane or dispersed in dipalmitoyl lecithin (an artificial pulmonary surfactant) were tested for their ability to cause SCEs in Chinese hamster V79 lung cells (188). Dichloromethane extracts and dipalmitoyl lecithin dispersions

caused similar increases in SCEs. When the samples were fractionated, the mutagenic activity of the solvent-extracted samples was shown to reside exclusively in the supernatant fraction and the mutagenic activity of surfactant dispersed samples was exclusively in the sedimented fraction. This was interpreted to suggest that DEP caused genotoxicity may also be caused by dispersion of particles in the pulmonary surfactant. Similar conclusions were made by Gu *et al*. (125, 126) when DEP extracted by dichloromethane or dispersed in dipalmitoyl lecithin were tested in the micronucleus assay in Chinese hamster lung (V79) and ovary (CHO) cells (126) and in an assay for unscheduled DNA synthesis (125).

9.3.3 In vivo studies

In vivo genotoxicity studies conducted before the year 2000 are summarised in earlier reviews (166, 423). Studies have been performed by exposing animals to whole diesel exhaust emissions or to DEP extracts administered via different routes. The most relevant studies are summarised below.

Inhalation of diesel exhaust did not cause any significant increases in bone marrow micronuclei or chromosomal aberrations in mice after exposure up to 7 weeks (8 hours/day, 5 days/week) at 6 000–7 000 µg DEP/m^3 (322). Neither were SCEs induced in bone marrow in mice after 1 month of exposure to diesel exhaust at 12 000 µg DEP/m^3 nor in the peripheral lymphocytes of rats after 3 months of exposure to 1 900 µg DEP/m^3 (321). However, a 6-month exposure of Chinese hamsters to diesel exhaust at 6 000 µg DEP/m^3 resulted in an increased number of bone marrow micronuclei but not SCEs (324). Negative results were reported also by Morimoto *et al*. in the mouse bone marrow micronucleus test after exposure to diesel exhaust from a light-duty diesel engine at 400 and 2 000 µg DEP/m^3 for up to 18 months (264) and by Ong *et al*. in mice and rats after 6 and 24 months of exposure, respectively, at 1 900 µg DEP/m^3 (303). Similarly, no increase in chromosomal aberrations in circulating lymphocytes was seen after a 2-year exposure of rats to diesel exhaust at 6 200–6 500 µg DEP/m^3 (342). The dominant lethal test and the heritable translocation test conducted in mice after inhalation exposure did not show positive responses either (423).

Oxidative DNA damage (measured as 8-OHdG) has been seen in the lungs of rats exposed by inhalation to 3 500 µg DEP/m^3 for 1–12 months (108, 174). Also DNA adducts have been observed in the lungs of rats and mice after short- and long-term inhalation exposure to diesel exhaust at ≥ 3 000 µg DEP/m^3 (5, 49, 93, 109, 174). Induction of mutations in the lungs in the *gpt* (guanine phosphoribosyl transferase) and the *lacI* (lactose-inducible lac operon transcriptional repressor) loci of transgenic Big Blue® rats and in the *gpt* locus of *gpt* delta mice was seen after 4 weeks of inhalation exposure to diesel exhaust at 6 000 µg DEP/m^3 (367), after 4–24 weeks exposure at 3 000 µg DEP/m^3 and after 12 weeks of exposure at 1 000 µg DEP/m^3 (143). No increase in mutant frequency was seen after 4 weeks of exposure at 1 000 µg DEP/m^3 (367).

Tsukue *et al*. compared the oxidative damage caused by exposure of rats to diesel exhaust from either a conventional heavy-duty diesel engine or an engine

with selective catalytic reduction (SCR) after-treatment system for 1–7 days (6 hours/day). Exposure to exhaust from the SCR engine did not result in any clear increase in serum 8-OHdG whereas some increase was seen with exhaust from the conventional engine (413).

As a part of the ACES programme, the induction of DNA damage (comet assay) in lungs, 8-OHdG in serum and micronuclei in peripheral blood were evaluated after short- and long-term diesel exhaust exposure. Mice and rats were exposed to diesel exhaust derived from heavy-duty diesel engines that fulfilled the US 2007 emission standards at exposure levels of 0.1, 0.9 and 4.2 ppm NO_2 (3, 5 and 12 μg DEP/m^3). The exposure duration was 1 and 3 months in the case of mice and 1, 3, 12 and 24 months in the case of rats (16 hours/day, 5 days/week). The comet assay showed no significant exposure-related increases in DNA damage after short- or long-term exposure to diesel exhaust. Neither did the 8-OHdG assay show any clear exposure-related increases in serum 8-OHdG levels in rats or mice. Examination of the mean frequencies of micronucleated reticulocytes or normochromic erythrocytes across the exposure groups and durations of exposure did not show any significant exposure-related induction of micronuclei in either species (31, 32, 136, 137).

Intraperitoneal and intratracheal injections of DEP or DEP extracts have increased SCEs in lung cells of exposed Syrian hamsters as well as in embryonic liver cells after exposure of pregnant hamsters (129, 323). Moreover, induction of micronuclei in polychromatic erythrocytes has been seen after intraperitoneal administration of DEP extracts (229, 385). Intratracheal administration of DEP resulted also in oxidative damage (168, 169, 267, 363).

In several recent *in vivo* studies, SRM 2975 has been used as test material. In general, these studies have shown induction of DNA strand breaks (comet assay) and oxidative damage in lungs/BAL cells after inhalation or in the lung, colon and liver after oral exposure (93, 94, 266, 349, 350, 360). SRM 2975 caused increased oxidative damage and DNA adducts in different tissues after oral exposure (80) and an increase in mutation rate at the ESTR (expanded simple tandem repeat) locus and a non-significant increase in DNA strand breaks in the offspring of female mice exposed to 19 000 μg DEP/m^3 for 1 hour/day at gestation days 7–19 (161, 352).

9.3.4 Conclusion on genotoxicity

DEP and DEP extracts have shown genotoxic responses *in vitro*. Bacterial mutagenicity studies with the gaseous phase of diesel exhaust have also shown positive responses, although the data are much more limited.

Inhalation studies with diesel exhaust in rodents have shown increases in the levels of DNA strand breaks, DNA adduct levels, oxidative DNA damage and in *gpt* and *lacI* mutations in the lungs of transgenic mice, whereas bone marrow and peripheral blood cell micronucleus, SCE and chromosomal aberration tests have been mostly negative. Oral, intraperitoneal and intratracheal administration of

diesel exhaust particulates or DEP extracts have produced genotoxic responses in several organs.

No *in vitro* genotoxicity studies on new technology diesel engines were located. However, recent inhalation studies with diesel exhaust from a heavy-duty diesel engine fulfilling the US 2007 emission standards did not show local or systemic genotoxicity or oxidative DNA damage in rodents. This suggests that new diesel engine and after-treatment technologies may decrease the genotoxic potency of diesel exhaust when expressed per unit of engine work (per kWh). This decrease can be mostly attributed to the significant reduction of particulate matter in the exhaust.

9.4 Effects of long-term exposure and carcinogenicity

This chapter includes studies with exposure durations exceeding 13 weeks (90 days). Shorter-term studies are presented in Section 9.2.

9.4.1 Pulmonary effects

Studies on pulmonary effects of long-term inhalation exposure to diesel exhaust are listed in Table 11. Carcinogenicity is discussed separately in Section 9.4.5.

McDonald *et al*. (the ACES programme) observed mild epithelial hyperplasia in terminal bronchioles, alveolar ducts and adjacent alveoli, mild periacinar fibrotic lesions and occasional accumulation of alveolar macrophages in rats exposed to diesel exhaust from a US 2007 compliant heavy-duty diesel engine for 121–130 weeks (16 hours/day, 5 days/week) at 12 µg DEP/m^3 (4.2 ppm NO_2). There was also a mild progressive decrease in pulmonary function (forced expiratory flows), which was more consistent in females than in males. According to the authors, the pulmonary function data suggest that the smallest airways were more affected than the larger airways, which is in agreement with the morphological changes observed in the smallest airways. Biochemical changes in lung tissue and BAL indicated mild inflammation and oxidative stress. The observed lung lesions progressed slightly from 3 to 12 months, without further progression from 12 months onwards. No pulmonary effects were observed at ≤ 5 µg DEP/m^3 (≤ 0.9 ppm NO_2). A slight degeneration of the olfactory epithelium was observed in some animals, primarily in the high exposure group (246, 247).

Kato *et al*. (186) reported a slight increase in particle-laden alveolar macrophages and mild alveolar type II cell hyperplasia in rats exposed to 210 µg DEP/m^3 (0.2 ppm NO_2) for 104 weeks (16 hours/day, 6 days/week). The effects were more pronounced at concentrations ≥ 1 100 µg DEP/m^3 (1.0 ppm NO_2). The morphological changes in the alveoli and the inflammatory changes in BAL observed at 1 100 µg DEP/m^3 were nearly absent in the group exposed to a corresponding particle-free exhaust (10 µg DEP/m^3, 1.1 ppm NO_2). Shortening of tracheal and bronchial cilia was observed with both filtered and unfiltered diesel exhaust (171, 186).

Several studies have demonstrated inflammation and fibrosis in the lungs of rats exposed to diesel exhaust for 104–130 weeks at exposure levels exceeding

800 µg DEP/m^3 (147, 172, 173, 241, 273, 394). Only a slight increase in particle-laden alveolar macrophages and minor changes in BAL were observed in rats exposed to diesel exhaust at 1 000 µg DEP/m^3 (4.2 and 6.9 ppm NO_2) for 6 months (6 hours/day, 7 days/week). No adverse effects were observed at concentrations ≤ 300 µg DEP/m^3 (≤ 1.3 ppm NO_2) (343, 374).

In mice, a dose-related increase in particle-laden alveolar macrophages was observed after exposure to diesel exhaust at 350, 3 500 or 7 000 µg DEP/m^3 for 104 weeks. Soot accumulation was often accompanied by thickened alveolar walls and in some cases also fibrotic lesions, in particular at the highest level. In contrast to rats, no pronounced exposure-related alveolar hyperplasia was observed in mice (238).

Lewis *et al*. compared the pulmonary effects of diesel exhaust in rats and cynomolgus monkeys exposed for 104 weeks to 2 000 µg DEP/m^3. Accumulation of particle-laden alveolar macrophages, alveolar septal hyperplasia and occasional fibrotic lesions were observed in rats. Except for particle-laden macrophages, no histopathological lesions were reported for the monkeys (212).

Changes in lung function have been observed in rats at concentrations exceeding 3 500 µg DEP/m^3 for 104–130 weeks (51, 240, 242).

Table 11. Pulmonary effects in animals after long-term inhalation exposure to diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
3 5 12	0.1 0.9 4.2	121–130 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Rat, Wistar Han 10/sex/group	Mild epithelial hyperplasia in terminal bronchioles, alveolar ducts and adjacent alveoli, mild periacinar fibrotic lesions, slight increase in markers of oxidative stress and inflammation in BAL and lung tissue, and slight decrease in lung function at 12 $\mu g/m^3$ (4.2 ppm NO_2); slight degeneration of olfactory epithelium in some animals (primarily in the high-exposure group).	(247)
30 100 300 1 000	0.2 0.4 0.8, 1.3 4.0, 6.9	26 wk (6 h/d, 7 d/wk)	5.9 L turbo engine, 2000	Rat, F344 12/sex/group	Increase in lactate dehydrogenase and decrease in TNF-α in BAL in females (significant at 1 000 $\mu g/m^3$); slight increase in particle-laden AM in lungs at 1 000 $\mu g/m^3$; no other histopathological changes.	(343, 374)
110 410 1 080 2 310	0.1 0.3 0.7 1.4	130 wk (16 h/d, 6 d/wk)	Light-duty diesel engine	Rat, F344/Jcl 215/group (120 males, 95 females)	Inflammatory changes, accumulation of particle-laden AM, type II cell hyperplasia and fibrotic lesions at ≥ 1 080 $\mu g/m^3$.	(172, 173)
210 1 000 4 400	nd	19 wk (7 h/d, 5 d/wk)	Diesel engine	Rat, F344; Mouse, CD-1 (both sexes, no. not available)	Increase in PMNs, proteases and AM aggregation in both species at 4 400 $\mu g/m^3$; no effects on pulmonary function in rats (not tested in mice).	(239)
210 1 100 3 100 filtered: 10	0.2 1.0 3.0 filtered: 1.1	104 wk (16 h/d, 6 d/wk)	7.4 L diesel engine, 1991	Rat, Wistar 60/group (males)	Increase in total cell count, PMNs and fucose and decrease in AM in BAL at ≥ 1 100 $\mu g/m^3$; increase in total protein at 3 100 $\mu g/m^3$; dose-dependent shortening of tracheal and bronchial cilia, and Clara-cell hyperplasia at ≥ 1 100 $\mu g/m^3$ (both unfiltered and filtered); dose-dependent type II cell hyperplasia at ≥ 210 $\mu g/m^3$ (only mild at 210 $\mu g/m^3$).	(171, 186)

Table 11. Pulmonary effects in animals after long-term inhalation exposure to diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
220 1 140 2 940 filtered: 10	0.2 1.1 2.9 filtered: 1.0	104 wk (16 h/d, 6 d/wk)	7.4 L diesel engine, 1991	Guinea pig, Hartley 150 (sex not specified)	Dose-related increase in eosinophils, total protein, lactate dehydrogenase, fucose, sialic acid and phospholipid in BAL at 1 140 and 2 940 $\mu g/m^3$ (from week 52; unfiltered exhaust only).	(170)
250 750 1 500 6 000	nd	65 wk (20 h/d, 7 d/wk)	Diesel engine	Rat, F344: 120 Mouse, A/J: 450 Hamster, Syrian: 120 (all males)	Accumulation of particle-laden AM; increase in connective tissue in alveolar walls, type II cell proliferation and mild fibrosis at $\geq$ 750 $\mu g/m^3$.	(183)
250 750 1 500 6 000	nd	104 wk (20 h/d, 5.5 d/wk)	Diesel engine	Guinea pig, Hartley 9/group (males)	Minimal response at 250 $\mu g/m^3$; morphological changes at $\geq$ 750 $\mu g/m^3$; increase in PMNs at 750 and 1 500 $\mu g/m^3$; thickened alveolar membranes, cell proliferation and fibrosis at 6 000 $\mu g/m^3$.	(24, 425, 465)
350 3 500 7 100	0.1 0.3 0.7	104 wk (7 h/d, 5 d/wk)	5.7 L diesel engine, 1980	Mouse, CD-1 59–82 males + 88–104 females/group	Exposure-related increase in lung soot, particle-laden AM and lung lesions (not reported in detail); alveolar bronchiolisation at 7 100 $\mu g/m^3$.	(238)
350 3 500 7 100	0.1 0.3 0.7	130 wk (7 h/d, 5 d/wk)	5.7 L diesel engine, 1980	Rat, F344 183/sex	Diffusing capacity and lung compliance reduced at $\geq$ 3 500 $\mu g/m^3$.	(240, 242)
350 3 500 7 100	0.1 0.3 0.7	130 wk (7 h/d, 5 d/wk)	5.7 L diesel engine, 1980	Rat, F344 Mouse, CD-1 183/sex/species	Inflammatory changes at $\geq$ 3 500 $\mu g/m^3$; alveolar and bronchiolar epithelial metaplasia in rats at $\geq$ 3 500 $\mu g/m^3$; fibrosis in rats and mice at 7 100 $\mu g/m^3$.	(153, 241)
430 filtered: < LOD	nd	22 wk (5.2 h/d, 4 d/wk)	5.5 kW diesel generator	Mouse, ApoE-/- 20/group (males)	No significant changes in BAL.	(337)

Table 11. Pulmonary effects in animals after long-term inhalation exposure to diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
460 960 1 840 3 720	0.3 0.7 1.4 3.0	130 wk (16 h/d, 6 d/wk)	Heavy-duty diesel engine	Rat, F344/Jcl 215/group (120 males, 95 females)	Inflammatory changes, accumulation of particle-laden AM, type II cell hyperplasia and fibrotic lesions at $\geq$ 960 $\mu g/m^3$.	(172, 173)
700 2 200 6 600	nd	104 wk (19 h/d, 5 d/wk)	Diesel engine	Rat, F344; Hamster, Syrian (both sexes, no. not available)	Pulmonary function changes consistent with obstructive and restrictive airway diseases at 6 600 $\mu g/m^3$ (no specific data provided).	(51)
800 2 500 6 980	0.3 1.2 3.8	104 wk (18 h/d, 5 d/wk)	1.6 L diesel engine	Rat, Wistar 1 780 (females)	Reduced lung clearance rate, bronchoalveolar hyperplasia and interstitial fibrosis in all groups (severity and incidence increased with concentration); changes in BAL (not specified), increased incidence of lung tumours and decreased body weight at $\geq$ 2 500 $\mu g/m^3$.	(147)
1 500	0.5	87 wk (20 h/d, 5.5 d/wk)	Diesel engine	Rat, F344 (males, no. not available)	Increased functional residual capacity, expiratory volume and flow.	(123)
2 000	1.5	104 wk (7 h/d, 5 d/wk)	Diesel engine	Rat, F344 72/sex	Accumulation of particle-laden AM; alveolar septal hyperplasia; fibrotic lesions; no effects on pulmonary function.	(212)
2 000	1.5	104 wk (7 h/d, 5 d/wk)	Diesel engine	Monkey, Cynomolgus 15/group (males)	AM aggregation; no fibrosis, inflammation or emphysema; decreased expiratory flow; no effect on vital or diffusing capacities.	(212)
2 000	1.5	104 wk (7 h/d, 5 d/wk)	Diesel engine	Rat, F344 (both sexes, no. not available)	Multifocal histiocytosis, inflammatory changes, type II cell proliferation, fibrosis.	(38, 316)
2 440 6 300	0.73 3.8	104 wk (7 h/d, 5 d/wk)	6.2 L diesel engine, 1988	Rat, F344 114–115/sex/group)	AM hyperplasia, epithelial hyperplasia, inflammation, broncho-alveolar metaplasia, septal fibrosis and lung tumours at both levels.	(273)
3 000 10 000	~ 1.6 ~ 5.6	104 wk (6 h/d, 7 d/wk) + 26 wk follow-up	1.6 L diesel engine, 1994	Rat, Wistar 100/group (both sexes)	Exposure-related histopathological changes (e.g. alveolar metaplasia, chronic inflammation, septal fibrosis, lung tumours); at 3 000 $\mu g/m^3$ apparent only after the follow-up.	(394)
3 900	1.2	104 wk (7–8 h/d, 5 d/wk)	Diesel engine	Rat, Wistar (females, no. not available)	Inflammatory changes, 60% adenomatous cell proliferation; no effect on minute volume, compliance or resistance.	(149)

Table 11. Pulmonary effects in animals after long-term inhalation exposure to diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
4 240	1.5	140 wk (19 h/d, 5 d/wk)	Diesel engine	Rat, Wistar (females, no. not available)	Thickened alveolar septa; AM aggregation; inflammatory changes; hyperplasia; lung tumours; decrease in dynamic lung compliance; increase in airway resistance.	(148)
4 240	1.5	120 wk (19 h/d, 5 d/wk)	Diesel engine	Mouse, NMRI (females, no. not available)	Inflammatory changes, bronchioloalveolar hyperplasia, alveolar lipoproteinosis and fibrosis.	(148)
4 240	1.5	120 wk (19 h/d, 5 d/wk)	Diesel engine	Hamster, Syrian (96/sex)	Significant increase in airway resistance.	(148)
4 900	1.8	104 wk (8 h/d, 7 d/wk)	Diesel engine	Rat, F344 (males, no. not available)	Type II cell proliferation, inflammatory changes, bronchial hyperplasia and fibrosis.	(175)
6 000	nd	39 wk (8 h/d, 7 d/wk)	Diesel engine	Rat, Sprague Dawley, Mouse, A/HEJ (males, no. not available)	Increase in lung protein content and collagen synthesis but a decrease in overall lung protein synthesis in both species.	(38, 316)
6 000 12 000	nd	26 wk (8 h/d, 7 d/wk)	Diesel engine	Hamster, Chinese 10/group (males)	Decrease in vital capacity, residual volume and diffusing capacity; increase in static deflation lung volume.	(460, 461)
6 000 12 000	nd	26 wk (8 h/d, 5 d/wk)	Diesel engine	Hamster, Chinese (males, no. not available)	Inflammatory changes, AM accumulation, thickened alveolar lining, type II cell hyperplasia, oedema and increase in collagen.	(317)
6 000– 12 000	2.7– 4.4	124 wk (8 h/d, 7 d/wk)	Diesel engine	Cat, inbred 25/group (males)	Decrease in vital capacity, total lung capacity and diffusing capacity; no effect on expiratory flow.	(262, 318, 319)
6 000– 12 000	2.7– 4.4	124 wk (8 h/d, 7 d/wk)	Diesel engine	Cat, inbred (males, no. not available)	Inflammatory changes, AM aggregation, bronchiolar epithelial metaplasia, type II cell hyperplasia, peribronchiolar fibrosis.	(165, 331)
8 300	4.0– 6.0	87 wk (6 h/d, 5 d/wk)	Diesel engine	Rat, Wistar (females, no. not available)	Inflammatory changes, AM aggregation, alveolar cell hypertrophy, interstitial fibrosis, emphysema (diagnostic methodology not given).	(184)

AM: alveolar macrophage(s), ApoE-/-: apolipoprotein E deficient (ApoE is involved in lipoprotein metabolism), BAL: bronchoalveolar lavage, DEP: diesel exhaust particles, LOD: limit of detection, NO_2: nitrogen dioxide, PMN: polymorphonuclear leukocyte, TNF-α: tumour necrosis factor alpha, US: United States.

9.4.2 Haematological and cardiovascular effects

Table 12 lists identified studies on haematological and cardiovascular effects of long-term inhalation exposure to diesel exhaust.

Conklin and Kong (the ACES programme) observed exposure-related increasing trends in the plasma soluble intercellular adhesion molecule 1 (sICAM-1) and in IL-6 levels, and decreasing trends in total and non-high-density lipoprotein (non-HDL) cholesterol levels in female rats after 104 weeks of exposure (16 hours/day, 5 days/week) to diesel exhaust from a heavy-duty diesel engine, fulfilling the US 2007 emission standards, at 3, 5 or 12 µg DEP/m^3 (0.1, 0.9 or 4.2 ppm NO_2, and 1.1, 1.9 or 6.4 ppm CO). These changes were not observed in male rats. No changes were observed in other plasma markers or in cardiovascular histopathology (73).

Reed *et al*. observed a dose-related decrease in clotting factor VII in rats exposed to diesel exhaust at 300 or 1 000 µg DEP/m^3 (0.8 or 4.0 ppm NO_2) for 26 weeks (6 hours/day, 7 days/week). No changes in red or white blood cell count, haemoglobin, haematocrit, platelet count, other clotting factors or other haematological parameters were detected. No change in clotting factor VII occurred at ≤ 100 µg DEP/m^3 (≤ 0.4 ppm NO_2, ≤ 3.6 ppm CO) (343).

A slight increase in red blood cell count and a slight decrease in white blood cell count were reported for rats exposed to ≥ 1 080 µg DEP/m^3 (≥ 0.7 ppm NO_2, ≥ 4.0 ppm CO) for 130 weeks (16 hours/day, 6 days/week). No haematological effects were observed at ≤ 410 µg DEP/m^3 (≤ 0.3 ppm NO_2, ≤ 2.1 ppm CO) (173).

In a study of Quan *et al*., atherosclerotic mice showed increased serum vascular cell adhesion molecule 1 (VCAM-1) levels and enhanced phenylephrine-induced vasoconstriction after exposure to whole (430 µg DEP/m^3, 5 ppm CO) and particle-free diesel exhaust for 22 weeks (5.2 hours/day, 4 days/week). No significant effect on constriction or relaxation response to serotonin, acetylcholine or sodium nitroprusside was observed. After 22 weeks of exposure, animals exposed to whole diesel exhaust showed a slight but statistically significant increase in plaque area in the brachiocephalic artery. The effect was not seen in animals exposed to particle-free exhaust or in either group after 13 weeks of exposure (337).

Table 12. Haematological and cardiovascular effects in animals after long-term inhalation exposure to diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
3 5 12	0.1 0.9 4.2	1.1 1.9 6.4	104 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Rat, Wistar Han 10/sex/group	Exposure-related increase in plasma sICAM-1 and IL-6, and decrease in total and non-HDL cholesterol in females at 104 weeks; no effects on cardiovascular histopathology.	(73)
30 100 300 1 000	0.2 0.4 0.8 4.0	1.5 3.6 10 31	26 wk (6 h/d, 7 d/wk)	5.9 L turbo engine, 2000	Rat, F344 12/sex/group	Dose-related decrease in clotting factor VII (significant at ≥ 300 $\mu g/m^3$); no other effects on haematological parameters.	(343)
110 410 1 080 2 310 3 720	0.1 0.3 0.7 1.4 3.0	1.2 2.1 4.0 7.1 13	130 wk (16 h/d, 6 d/wk)	Light-duty diesel engine	Rat, F344/Jcl 215/group (120 males, 95 females)	Slight increase in haemoglobin, haematocrit and erythrocyte counts, and decrease in mean corpuscular volume and mean corpuscular haemoglobin at $\geq 1\,080$ $\mu g/m^3$.	(173)
210 1 100 3 100 filtered: 10	0.2 1.0 3.0 filtered: 1.1	1.9 5.7 13 filtered: 5.8	104 wk (16 h/d, 6 d/wk)	7.4 L diesel engine, 1991	Rat, Wistar 60/group (males)	No effects on haematological parameters at any exposure level.	(171)
220 1 140 2 940 filtered: 10	0.2 1.1 2.9 filtered: 1.0	1.9 5.7 14 filtered: 5.7	104 wk (16 h/d, 6 d/wk)	7.4 L diesel engine, 1991	Guinea pig, Hartley 150 (sex not specified)	Increase in plasma leukotriene C4 at $\geq 1\,140$ $\mu g/m^3$ at week 104; no other effects on haematological parameters.	(170)
250 750 1 500	0.1 0.3 0.5	3.0 4.8 6.9	78 wk (20 h/d, 5.5 d/wk)	Diesel engine	Rat, F344; Guinea pig, Hartley (males, no. not available)	No changes in heart mass or haematology at any exposure level in either species.	(315)

Table 12. Haematological and cardiovascular effects in animals after long-term inhalation exposure to diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Effects	Reference
430 filtered: < LOD	nd	5.0 filtered: 5.0	22 wk (5.2 h/d, 4 d/wk)	5.5 kW diesel generator	Mouse, ApoE-/- 20/group (males)	Increase in serum VCAM-1 levels; enhanced vasoconstriction to phenylephrine; slight increase in plaque area (unfiltered exhaust only).	(337)
700 2 200 6 600	nd	nd nd 32	104 wk (16 h/d, 5 d/wk)	Diesel engine	Rat, F344 (both sexes, no. not available)	Increase in haemoglobin, haematocrit, erythrocyte and leukocyte counts and banded (immature) neutrophils; suggestion of an increase in prothrombin time; increased heart/body weight and right ventricular/heart ratios and decreased left ventricular contractility at 6 600 $\mu g/m^3$.	(51)
2 000	1.5	12	104 wk (7 h/d, 5 d/wk)	Diesel engine	Rat, F344 72/sex/group	Increase in banded (immature) neutrophils; no effect on heart or pulmonary arteries.	(212, 427)
2 000	1.5	12	104 wk (7 h/d, 5 d/wk)	Diesel engine	Monkey, Cynomolgus 15/group (males)	Increase in mean corpuscular volume.	(212)
3 000 10 000	~ 1.6 ~ 5.6	10 37	104 wk (6 h/d, 7 d/wk) + 26 wk follow-up	1.6 L diesel engine, 1994	Rat, Wistar 99/group (both sexes)	Increase in erythrocyte and leukocyte counts at 10 000 $\mu g/m^3$.	(394)
3 900	1.2	19	75 wk (7–8 h/d, 5 d/wk)	Diesel engine	Hamster, Syrian (both sexes, no. not available)	Increase in mean corpuscular volume at 29 weeks; decrease in erythrocyte and leukocyte counts.	(149)
6 000– 12 000	2.7–4.4	20–33	124 wk (8 h/d, 7 d/wk)	Diesel engine	Cat, inbred (males, no. not available)	Increase in banded neutrophils (significant at 12 months, but not at 24 months).	(320)
8 300	4.0–6.0	50	78 wk (6 h/d, 5 d/wk)	Diesel engine	Rat, Wistar (males, no. not available)	3% increase in carboxyhaemoglobin (COHb).	(184)

ApoE-/-: apolipoprotein E deficient (ApoE is involved in lipoprotein metabolism), CO: carbon monoxide, DEP: diesel exhaust particles, HDL: high-density lipoprotein, IL: interleukin, LOD: limit of detection, nd: no data, NO_2: nitrogen dioxide, sICAM-1: soluble intercellular adhesion molecule 1, US: United States, VCAM-1: vascular cell adhesion molecule 1.

9.4.3 Neurological effects

In a study focusing on neuroinflammatory and neuropathological effects of diesel exhaust, male rats (8/group) were exposed by inhalation at 35, 100, 311 or 992 µg DEP/m^3 (0.3, 0.7, 1.3 or 6.9 ppm NO_2) for 26 weeks (Table 9). Elevated levels of TNF-α were observed in the midbrain of the rats at ≥ 100 µg DEP/m^3, and in the frontal and temporal lobe and the olfactory bulb at 992 µg DEP/m^3. Increased levels of IL-1β were detected in the midbrain at 992 µg DEP/m^3. In addition to the cytokines, elevated levels of biomarkers for Alzheimer's disease [A β42 and tau (pS199)] in the frontal and temporal lobe, and increased levels of a biomarker for Parkinson's disease (α-synuclein) in the midbrain were seen at ≥ 311 µg/m^3 (210).

Within the ACES programme, no exposure-related changes in the markers of lipid peroxidation were detected in the hippocampus of rats (5 sex/group) exposed for 104 weeks (16 hours/day, 5 days/week) to diesel exhaust from a US 2007 compliant heavy-duty diesel engine at exposure levels up to 4.2 ppm NO_2 (12 µg DEP/m^3) (136) (Table 9).

9.4.4 Immunological effects

Maejima *et al*. studied the allergic immune response in mice. Female mice (60 per group) were exposed by inhalation to untreated (3 240 µg DEP/m^3, 1.0 ppm NO_2) or filtered (10 µg DEP/m^3, 1.1 ppm NO_2) diesel exhaust for 24 weeks, as well as to high levels (500 000 grains/m^3) of Japanese cedar pollen (JCP) for 2 days/week during the same exposure period (Table 10). At the end of the exposure, the number of animals expressing JCP-specific IgE in serum was significantly higher in both groups exposed to diesel exhaust (73% and 67%, respectively) than in the group exposed to JCP alone (33%). A similar response (63%) was observed in animals exposed to dust of volcanic ash instead of diesel exhaust (234).

9.4.5 Carcinogenicity

Table 13 presents the identified animal studies on carcinogenic effects of inhalation exposure to diesel exhaust. A statistically significant increase in lung tumour incidence has been observed in several studies in rats exposed to whole diesel exhaust at concentrations of ≥ 2 200 µg DEP/m^3 for 104–130 weeks (51, 147, 173, 241, 273, 394). No indication of carcinogenicity in other organs was detected in the studies. The studies applied diesel engines from the mid-1990s or earlier.

No effect on lung tumour incidence was observed in rats exposed to filtered (particle-free) diesel exhaust or to whole diesel exhaust at ≤ 800 µg DEP/m^3 for 104–152 weeks (51, 147, 148, 241, 260). Correspondingly, no indication of tumour development was detected in a 121–130-week inhalation study in rats exposed to exhaust from a US 2007 compliant heavy-duty diesel engine at concentrations up to 4.2 ppm NO_2 (12 µg DEP/m^3) (ACES programme) (247).

No clear evidence of carcinogenicity of diesel exhaust in mice or hamsters has been observed even at high particle loads (51, 147, 148).

Table 13. Animal inhalation carcinogenicity studies with diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Lung tumour type and incidence; number of animals with tumours/number examined (% of animals with tumours)	Reference
3 5 12	0.1 0.9 4.2	121–130 wk (16 h/d, 5 d/wk)	US 2007 compliant heavy-duty engine	Rat, Wistar Han 100/sex/group	None.	(247)
30 100 300 1 000	0.2 0.4 0.8 4.0	26 wk (6 h/d, 7 d/wk) + 26 wk follow-up	5.9 L turbo engine, 2000	Mouse, A/J 20/sex/group	Adenomas: 25/42 (60%), 26/43 (61%), 23/38 (63%), 18/34 (53%); control: 20/43 (47%).	(343)
100 1 100	0.1 0.7	52 wk (16 h/d, 6 d/wk) + 26–78 wk follow-up	Light-duty diesel engine	Rat, F344 24–25/group (sex not specified)	None.	(173)
100 400 1 100 2 300	nd	130 wk (16 h/d, 6 d/wk)	Light-duty diesel engine	Rat, F344 123–125/group (both sexes)	Adenomas, adenosquamous carcinomas, squamous cell carcinomas: 3/123 (2.4%), 1/125 (0.8%), 5/123 (4.1%), 3/124 (2.4%); control: 4/123 (3.3%).	(403)
250 750 1 500	nd	35 wk (20 h/d, 7 d/wk)	Diesel engine	Mouse, A/J 388–399/group (males)	Adenomas: 131/388 (34%), 109/399 (27%), 99/396 (25%); control: 130/388 (34%).	(445)
250 750 1 500	nd	65 wk (20 h/d, 7 d/wk) + 35 wk follow-up	Diesel engine	Rat, F344 30/group (males)	Carcinomas: 1/30 (3.3%), 3/30 (10%), 1/30 (3.3%); control: 0/30 (0%).	(445)
350 3 500 7 100	0.1 0.3 0.7	104 wk (7 h/d, 5 d/wk)	5.7 L diesel engine, 1980	Mouse, CD-1 155–186/group (both sexes)	Adenomas, carcinomas: 25/171 (15%), 15/155 (9.7%), 14/186 (7.2%); control: 21/157 (13%).	(238)

Table 13. Animal inhalation carcinogenicity studies with diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Lung tumour type and incidence; number of animals with tumours/number examined (% of animals with tumours)	Reference
350 3 500 7 100	nd	130 wk (7 h/day, 5 d/wk)	5.7 L diesel engine, 1980	Rat, F344 221–230/group (both sexes)	Adenomas, adenocarcinomas, squamous cell carcinomas, squamous cysts: 1.3%, 3.6% [a], 13% [a]; control: 0.9%.	(241)
500 1 800	nd	52 wk (16 h/d, 6 d/wk) + 26–78 wk follow-up	Heavy-duty diesel engine	Rat, F344 24–25/group (sex not specified)	None.	(173)
500 1 000 1 800 3 700	nd	130 wk (16 h/d, 6 d/wk)	Heavy-duty diesel engine	Rat, F344 123–125/group (both sexes)	Adenomas, adenosquamous carcinomas, squamous cell carcinomas: 1/123 (0.8%), 0/125 (0%), 4/123 (3.3%), 8/124 (6.5%) [a]; control: 1/123 (0.8%).	(173)
700 2 200 6 600 filtered: ~ 0	nd	104 wk (16 h/d, 5 d/wk)	Diesel engine	Rat, F344 143–260/group (both sexes)	Adenomas, squamous cell carcinomas, adenocarcinomas; unfiltered: 1/143 (0.7%), 14/144 (9.7%) [a], 55/143 (39%) [a]; filtered, medium: 0/144 (0%); filtered, high: 0/143 (0%); control: 3/260 (1.2%).	(51)
800 2 500 7 000	0.3 1.2 3.8	104 wk (18 h/d, 5 d/wk) + 26 wk follow-up	1.6 L diesel engine	Rat, Wistar 100–220/group (females)	Adenomas, adenocarcinomas, squamous cell carcinomas, benign squamous cell tumours: 0/198 (0%), 11/200 (5%) [a], 22/100 (24%) [a]; control: 1/217 (0.5%).	(147)
1 500	nd	13 wk (20 h/d, 7 d/wk) + 26 wk follow-up	Diesel engine	Mouse, A/J 458–485/group (males)	Adenomas: 165/485 (34%); control: 144/458 (31%).	(183)
2 000	1.5	104 wk (7 h/d, 5 d/wk)	Diesel engine	Rat, F344 288 (both sexes)	None.	(212)

Table 13. Animal inhalation carcinogenicity studies with diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Lung tumour type and incidence; number of animals with tumours/number examined (% of animals with tumours)	Reference
2 440 6 300	0.7 3.8	104 wk (16 h/d 5 d/wk) + 6 wk follow-up	6.2 L diesel engine, 1988	Rat, F344 210–214/group (both sexes)	Adenomas, adenocarcinomas, adenosquamous carcinomas, squamous cell carcinomas: 13/210 (6.2%) [b], 38/212 (18%) [b]; control: 3/214 (1.4%).	(273)
2 000– 4 000	nd	78–104 wk (4 h/d, 4 d/wk)	Diesel engine	Rat, F344 12–15/group (females)	None.	(404)
2 000– 4 000	nd	82–121 wk (4 h/d, 4 d/wk)	Diesel engine	Mouse, IRC 45–69/group (both sexes)	Adenomas, adenocarcinomas: 9/69 (13%); control: 4/45 (8.9%).	(404)
2 000– 4 000	nd	82–121 wk (4 h/d, 4 d/wk)	Diesel engine	Mouse, C57BL 12–38/group (both sexes)	Adenomas, adenocarcinomas: 11/38 (29%); control: 1/12 (8.3%).	(404)
3 000 10 000	~ 1.6 ~ 5.6	104 wk (6 h/d, 7 d/wk) + 26 wk follow-up	1.6 L diesel engine, 1994	Rat, Wistar 100/group (both sexes)	Adenomas, carcinomas, squamous cell carcinomas, benign keratinising cystic cell tumours: 23/100 (23%) [a], 46/100 (46%) [a]; control: 2/101 (2.0%).	(394)
4 000 filtered: ~ 0	nd	140–152 wk (19 h/d, 5 d/wk)	Diesel engine	Rat, Wistar 92–96/group (females)	Adenomas, squamous cell tumours: unfiltered: 17/95 (18%) [a]; filtered: 0/92 (0%); control: 0/96 (0%).	(148, 260)
4 000 filtered: ~ 0	nd	130 wk (19 h/d, 5 d/wk)	Diesel engine	Mouse, NMRI 76–93/group (both sexes)	Adenomas, adenocarcinomas: unfiltered: 24/76 (32%) [a]; filtered: 29/93 (31%) [a]; control: 11/84 (13%); note: unusually low tumour rate in the control group.	(148)
4 000 filtered: ~ 0	nd	130 wk (19 h/d, 5 d/wk)	Diesel engine	Hamster, Syrian 96/group (both sexes)	None.	(148)

Table 13. Animal inhalation carcinogenicity studies with diesel exhaust [adapted mainly from US EPA (423)].

DEP ($\mu g/m^3$)	NO_2 (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals	Lung tumour type and incidence; number of animals with tumours/number examined (% of animals with tumours)	Reference
4 500 filtered: 10	2.3 filtered: 2.9	104 wk (18 h/d, 5 d/wk) + 26 wk follow-up	1.6 L diesel engine	Mouse, C57BL/6N 120/group (females)	Adenomas, adenocarcinomas, squamous cell carcinomas, cystic keratinising squamous cell tumours: unfiltered: 8.5%; filtered: 3.5%; control: 5.1%.	(147)
4 500 filtered: 10	2.3 filtered: 2.9	100 wk (18 h/d, 5 d/wk)	1.6 L diesel engine	Mouse, NMRI 120/group (females)	Adenomas, adenocarcinomas: unfiltered: 23%; filtered: 47%; control 30%.	(147)
4 900 filtered: ~ 0	nd	104 wk (8 h/d, 7 d/wk)	Diesel engine	Rat, F344 24/group (females)	Adenomas, adenocarcinomas, large cell and squamous cell carcinomas: unfiltered: 8/19 (42%); filtered: 0/16 (0%); control: 1/22 (4.5%).	(175)
6 600 filtered: ~ 0	nd	104 wk (19 h/d, 5 d/wk)	Diesel engine	Hamster, Syrian Golden, 202–204/ group (both sexes)	None.	(51)
6 000–12 000	2.7–4.4	65 wk (24 h/d, 7 d/wk)	Diesel engine	Mouse, Sencar 260 (both sexes)	Adenomas, carcinomas: 11%[a]; control: 5.6%.	(320)
7 000	3.8	59 wk (18 h/d, 5 d/wk) + 42 wk follow-up	1.6 L diesel engine	Mouse, NMRI 120/group (females)	Adenomas, adenocarcinomas: 32%; control: 30%.	(147)
8 300	4–6	87 wk (6 h/d, 5 d/wk)	Diesel engine	Rat, Wistar 6/group (males)	Adenomas: 1/6 (17%); control: 0/6 (0%).	(184)

[a] Significantly different from the control group.
[b] Significantly different coefficient of slope for the neoplastic response to exposure for both sexes.
DEP: diesel exhaust particles, nd: no data, NO_2: nitrogen dioxide, US: United States.

9.5 Reproductive and developmental effects

Tables 14 and 15 list identified studies on reproductive and developmental effects in animals exposed to diesel exhaust by inhalation.

Dose-dependent decreases in daily sperm production and degenerative changes in seminiferous tubules were reported in adult mice exposed to diesel exhaust at 300, 1 000 or 3 000 μg DEP/m^3 for 26 weeks. The reduction of daily sperm production was still significant in the two high-exposure groups one month after cessation of the exposure (472). No morphological changes in testis or effects on sperm head count were, however, observed in adult rats exposed to similar concentrations for 35 weeks. Testosterone levels were increased in the highest exposure group (414). Degenerative changes in seminiferous tubules and effects on testosterone levels of male rats and mice were also suggested in two scantly reported studies with nanoparticle-rich diesel exhaust. Loss of spermatozoa was suggested in rats based on testicular histology, but sperm counts were not studied. In mice, no changes in sperm count were observed (215, 216).

Alterations in the development of male reproductive function in the offspring of rodents exposed to diesel exhaust during gestation have also been reported. Ono *et al*. reported reduced daily sperm production and histopathological changes in seminiferous tubules in mice exposed *in utero* to unfiltered (1 000 μg DEP/m^3, 4.6 ppm NO_2) or filtered (4.1 ppm NO_2) diesel exhaust (304, 305). Watanabe observed a decrease in daily sperm production, Sertoli cells and spermatids in rats exposed *in utero* to filtered or unfiltered diesel exhaust at 170 or 1 710 μg DEP/m^3 (0.1 or 0.8 ppm NO_2), without a clear dose-response (430). Kubo-Irie *et al*. reported degenerative changes in seminiferous tubules and a decreased number of Sertoli cells and normal spermatozoa, without changes in daily sperm production, in mice exposed to diesel exhaust at 170 μg DEP/m^3 (0.04 ppm NO_2) *in utero* and 12 weeks after birth (203).

Decreased foetal weight and pup weight gain have been reported in mice after *in utero* exposure to unfiltered exhaust at levels exceeding ≥ 1 000 μg DEP/m^3 (107, 304). Hougaard *et al*. reported decreased pups weight gain during lactation in mice exposed to resuspended diesel particulates (19 000 μg DEP/m^3) during gestation. No signs of maternal toxicity were indicated (161).

Suzuki *et al*. and Yokota *et al*. reported decreased spontaneous activity in male mice exposed *in utero* to 170 μg DEP/m^3 (0.04 ppm NO_2) (400) or to 1 000 μg DEP/m^3 (0.2 ppm NO_2) (468), respectively. Alterations in motor coordination and impulsive behaviour were reported at 1 000 μg DEP/m^3 (0.2 ppm NO_2) (469). No data on maternal toxicity or other reproductive outcomes were given in these studies. No effect on cognitive function was observed in mice exposed *in utero* to resuspended diesel particles at 19 000 μg DEP/m^3 (161). In a scantly reported study, apoptotic changes in the cerebella of mice exposed *in utero* to 300, 1 000 or 3 000 μg DEP/m^3 were reported (396, 397).

Auten *et al*. reported an increase in pro-inflammatory cytokines in the placenta and foetal lung of mice exposed *in utero* to whole diesel exhaust at 2 000 μg DEP/m^3 (1.2 ppm NO_2) or to aspirated DEP (6 × 50 μg). Also, worsening of

ozone-induced lung inflammation and airway hyperactivity were observed in the pups (11). Offspring of mice exposed intranasally to a single dose of 50 µg of DEP at gestation day 14 showed increased airway hyperresponsiveness, increased eosinophil count in BAL, and increased pulmonary infiltration after sensitisation and challenge with ovalbumin. A similar response was seen with titanium dioxide and carbon black particles (103). Increased allergic response to Japanese cedar pollen was reported in rats exposed *in utero* and 3 days postnatally to diesel exhaust at 1 730 µg DEP/m^3 (0.8 ppm NO_2) or to a corresponding concentration of particle-free diesel exhaust (432). Slight gender-specific changes in inflammatory markers in BAL were observed in mice exposed *in utero* to 800 or 3 100 µg DEP/m^3 (0.4 or 1.2 ppm NO_2). No consistent effect on ovalbumin, bovine serum albumin or sheep red blood cell-induced immune response or severity of allergic lung inflammation was detected (378). A decrease in mould-fungus-induced allergic response (IgE production) was observed in mice exposed *in utero* to diesel exhaust at 1 000 µg DEP/m^3 (75).

In summary, animal studies suggest that diesel exhaust may have effects on male fertility when exposed *in utero* or during the adult life. Effects on testicular histology and sperm production have been seen in rats and mice mainly at exposure concentrations ≥ 170–300 µg DEP/m^3. Changes in sexual hormone levels have also been reported but the data are inconsistent. The evidence for other effects on foetal development is scattered. Impaired motor coordination and activity, and enhanced allergic response have been reported in some of the studies.

The primary cause and toxicological mechanisms behind the observed effects on male reproductive function are not fully resolved. No significant differences have been reported between the effects of unfiltered (total) and filtered (gas phase) diesel exhaust (217, 304, 305, 430, 433), indicating the presence of effective compounds in the gas phase. On the other hand, prenatal exposure to DEP alone has also been reported to affect sperm production (150). Whole-body exposure was applied in all of the studies. Excessive exposure to DEP constituents through the gastrointestinal tract may therefore also have contributed to the observed effects.

Table 14. Reproductive effects in male animals after inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals and/or studied pups	Effects	Reference
15 36 169	0.06 0.15 0.51	0.7 1.1 3.3	4, 8 or 12 wk (5 h/d, 5 d/wk)	8.0 L diesel engine; conditions adjusted to achieve particles < 100 nm	Rat, F344 (mature) 8/group (males)	Degenerative changes in seminiferous tubules and loss of spermatozoa (no dose-response data given); sporadic changes in testosterone and progesterone (no dose-response); decreased body weight at 169 $\mu g/m^3$ at 12 weeks.	(216)
42 152 filtered: 1	0.16 0.54 filtered: 0.53	1.2 3.3 filtered: 3.3	8 wk (5 h/d, 5 d/wk)	8.0 L diesel engine; conditions adjusted to achieve particles < 100 nm	Mouse, C57BL/Jcl (mature) 8–9/group (males)	Degenerative changes and loss of germ cells in seminiferous tubules in all groups (no dose-response data provided); increased serum testosterone at 152 $\mu g/m^3$ (unfiltered only); no effect on epididymal sperm head count or morphology.	(215)
300 1 000 3 000	nd	3.8 8.5 15	26 wk (12 h/d, 7 d/wk)	2.7 L diesel engine	Mouse, ICR (mature) 20/group (males)	Degenerative changes in seminiferous tubules ($\geq$ 300 $\mu g/m^3$; dose-response); decrease in daily sperm production ($\geq$ 300 $\mu g/m^3$, dose-response), still significant at $\geq$ 1 000 $\mu g/m^3$ at 1 month post-exposure; increased lung weight ($\geq$ 300 $\mu g/m^3$).	(472)
300 1 000 3 000	0.59 1.7 5.2	1.5 4.6 14	35 wk (12 h/d, 7 d/wk)	2.7 L diesel engine	Rat, F344 (mature) 25/group (males)	Increase in prostate, seminal vesicle and coagulating gland weights and testicular testosterone at 3 000 $\mu g/m^3$; increase in serum luteinising hormone at 300 and 1 000 $\mu g/m^3$, no effect on testicular or body weights; no effect on testicular morphology, sperm head count or follicle-stimulating hormone.	(414)
5 630 filtered: ~ 0	4.1 filtered: ~ 4.1	nd	13 wk (6 h/d, 5 d/wk)	0.3 L diesel generator, HEPA filtering	Rat, F344 (newborn) 6/group (males)	Decreased daily sperm production and decreased number of spermatids (both groups); increase in serum testosterone and oestradiol, decrease in follicle-stimulating hormone (both groups) and luteinising hormone (unfiltered).	(433)

CO: carbon monoxide, DEP: diesel exhaust particles, nd: no data, HEPA: high-efficiency particulate arrestance, NO_2: nitrogen dioxide.

Table 15. Reproductive effects in female animals and developmental effects after inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals and/or studied pups	Effects	Reference
100	nd	nd	GD 2–13	2.3 L diesel engine, 80% load	Mouse, ICR, ddY and C57BL/6J 2–4 pups/group (males)	Indications of strain-related differences in sensitivity to diesel exhaust related to gene expression regulating male gonadal differentiation at GD 14 (ICR > ddY > C57BL/6J).	(473)
100 3 000	0.04 0.7	1.3 8.0	GD 2–3, 6–11 and 13 (8 h/d)	2.3 L diesel engine, 80% load	Mouse, ICR 10 dams/group, 15–25 pups/group (males)	Decreased expression of genes regulating male gonadal differentiation (≥ 100 $\mu g/m^3$; dose-response); no effect on litter size, sex ratio or implantation loss.	(470)
149 filtered: 3	0.53 filtered: 0.51	3.4 filtered: 3.3	GD 1–19 (5 h/d)	8.0 L diesel engine; conditions adjusted to achieve particles < 100 nm	Rat, F344 5 dams/group, 5–7 pups/group (males)	Decreased seminal vesicle and prostate weight to body weight at day 28; loss of germ cells in seminiferous tubules; changes in serum hormone concentrations and expression of related genes (both groups).	(217)
170	0.04	nd	GD 2–19 + 12 wk postnatally (8 h/d, 5 d/wk)	2.8 L diesel engine	Mouse, ICR 12–14 dams/group, 8 pups/group (males)	Degenerative changes in seminiferous tubules; decreased number of Sertoli cells; decreased percentage of normal spermatozoa; no effect on body weight, testis weight, daily sperm production or sperm motility; no effect on litter size, sex ratio or implantation loss; no signs of maternal toxicity.	(203)
170	0.04	1.3	GD 2–16 (5 h/d)	3 L diesel engine, 80% load	Mouse, ICR 12–14 dams/group, 10 pups/group (males)	Indication of decreased spontaneous locomotor activity; increase in dopamine and noradrenaline in prefrontal cortex.	(400)
170 1 710 filtered: ~ 0	0.1 0.8 filtered: ~ 0.1 ~ 0.8	nd	GD 7–20 (6 h/d)	0.3 L diesel generator, 45% load	Rat, F344 7–8 dams/group, 7–14 pups/group (males)	Reduced daily sperm production in adulthood (14 weeks); decreased number of spermatids and Sertoli cells in seminiferous tubules; increase in serum follicle-stimulating hormone (all groups; no clear dose-response).	(430)

Table 15. Reproductive effects in female animals and developmental effects after inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals and/or studied pups	Effects	Reference
300 1 000 3 000	nd	nd	GD 2–16 (12 h/d)	2.7 L diesel engine	Mouse, ICR 20 dams/group, 7–14 pups/group (males)	Increased body weights and relative testis and accessory gland weights at ≥ 1 000 $\mu g/m^3$ at day 28; increased serum testosterone (significant only at 1 000 $\mu g/m^3$); no effect on litter size or sex ratio.	(471)
300 1 000 3 000	1.3 4.6 11	0.9 8.9 24	GD 1.5–16 (12 h/d)	3.1 L diesel engine	Mouse, ICR 10–15 dams/group, 11–15 pups/group (both sexes)	Sporadic changes in expression of steroid and thyroid hormone related genes in male and female pups (no clear trend); no effect on litter size.	(416)
300 1 000 3 000	nd	nd	GD 2–16 (24 h/d)	Diesel engine	Mouse, ICR 10 dams/group, 20 pups/group (both sexes)	Increase in apoptotic cells in the brain (cerebellum) at 11 weeks (scantly reported results; no data on dose-response).	(396, 397)
300 1 000 3 000	0.53 1.7 4.2	1.6 6.1 14	17 wk (12 h/d, 7 d/wk) prior to mating	2.7 L diesel engine	Mouse, C57BL 24 dams/group	Impaired nest building in all exposed groups (significant at 3 000 $\mu g/m^3$); decreased weight gain of pups from week 4 onwards at 3 000 $\mu g/m^3$ (indications also at 1 000 $\mu g/m^3$); no effect on litter size or implantation loss.	(415)
300 1 000 3 000	1.3 4.6 11	nd	GD 2–13 (12 h/d)	2.3 L diesel engine, 80% load	Mouse, ICR 26–32 dams/group	Decreased foetal weight at 3 000 $\mu g/m^3$; increased number of absorbed placentas and altered expression of immune function related genes in placentas at 300 and 3 000 $\mu g/m^3$ (not at 1 000 $\mu g/m^3$); no effect on litter size, sex ratio or implantation loss.	(107)
500 2 000	< 1 1.2	11 37	GD 9–17 (4 h/d)	4.8 kW diesel generator	Mouse, C57BL 10–12 pups/group	Increased inflammatory markers in the placenta and foetal lung; increased ozone-induced inflammatory markers at 4 weeks (no data on dose-response); increased ozone-induced airway hyperreactivity at 2 000 $\mu g/m^3$; no effect on litter size.	(11)

Table 15. Reproductive effects in female animals and developmental effects after inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals and/or studied pups	Effects	Reference
800 3 100	0.4 1.2	5.4 18	GD 8–18 (4 h/d)	4.8 kW diesel generator	Mouse, BALB/c 20 dams/group, 12 pups/group/endpoint (both sexes)	Gender-specific changes in baseline levels of inflammatory markers in BAL; no effect on ovalbumin, bovine serum albumin or sheep red blood cell-induced immune response, or severity of allergic lung inflammation; non-significant increase in implantation loss and decrease in litter size at 3 100 $\mu g/m^3$.	(378)
1 000	0.23	2.7	GD 2–17 (8 h/d, 5 d/wk)	2.4 L diesel engine	Mouse, ICR 15 dams/group, 9–15 pups/group/endpoint (males)	Shorter retention time on a rotating rod and on an elevated platform (cliff avoidance); no difference in retention time on a narrow bar; decreased noradrenaline turnover in the cerebellum and increased in the hypothalamus; differing dopamine and serotonin levels in different parts of the brain.	(469)
1 000	0.23	2.7	GD 2–17 (8 h/d, 5 d/wk)	2.4 L diesel engine	Mouse, ICR 15 dams/group, 8–27 pups/group/endpoint (males)	Decreased spontaneous activity; decreased dopamine turnover in the striatum and nucleus accumbens.	(468)
1 000 filtered: 4	4.6 filtered: 4.1	nd	GD 2–19 (24 h/d)	3.1 L diesel engine	Mouse, ICR 10–14 dams/group, 7–10 pups/group/timepoint (males)	Multinucleated giant cells and partial vacuolation in seminiferous tubules (both groups); decreased daily sperm production and increased serum testosterone at 12 weeks (both groups); transient decrease in body weight and increase in accessory gland weight to body weight (unfiltered only).	(304, 305)

Table 15. Reproductive effects in female animals and developmental effects after inhalation exposure to diesel exhaust.

DEP ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Exposure duration	Other exposure conditions	Species, no. and sex of exposed animals and/or studied pups	Effects	Reference
1 000	nd	nd	GD 7–21 (5 h/d, 5 d/wk)	5.5 kW (0.4 L) diesel generator	Mouse, BALB/c 8–25 pups/group	Decreased *Aspergillus fumigatus*-induced IgE production and lung eosinophil count, and increased IgG1 production and macrophage count in offspring of diesel exhaust and/or *A. fumigatus* exposed dams at 10 weeks.	(75)
1 730 filtered: ~ 0	0.8 filtered: ~ 0.8	nd	GD 7 to day 3 after birth (6 h/d)	0.3 L diesel generator, HEPA filtering	Rat, F344 6 dams/group, 6–8 pups/group (males)	Increased Japanese cedar pollen-induced IgE production; decreased average spleen and thymus weights; non-significantly increased testosterone levels; no effect on the body weight of the pups (both groups).	(432)
5 630 filtered: ~ 0	4.1 filtered: ~ 4.1	nd	GD 7–20 (6 h/d)	0.3 L diesel generator, HEPA filtering	Rat, F344 24 dams/group, 6–11 pups/litter (both sexes)	Longer anogenital distance; delayed differentiation of testis, ovaries and thymus; higher maternal testosterone and lower progesterone levels; no effect on litter size, sex ratio or implantation loss (both groups).	(431)
19 000	nd	nd	GD 7–19 (1 h/d)	Resuspended diesel particles (SRM 2975)	Mouse, C57BL 20 dams/group, 8–9 pups/group (1 male/litter)	Reduced daily sperm production in adulthood (6 months); no effect on body weight, testis weight or anogenital distance; no indication of altered endocrine function.	(150)
19 000	nd	nd	GD 9–19 (1 h/d)	Resuspended diesel particles (SRM 2975)	Mouse, C57BL 20 dams/group, 12 pups/group (both sexes)	Decreased weight gain during lactation; non-significant effects on gene expression of inflammatory cytokines in the liver; no clear effect on cognitive function (slight improvement in the Morris water maze test; no effect in the open field test); no signs of DNA damage in the liver; no effect on litter size, sex ratio or implantation loss; no signs of maternal toxicity.	(161)

BAL: bronchoalveolar lavage, CO: carbon monoxide, DEP: diesel exhaust particles, GD: gestation day, HEPA: high-efficiency particulate arrestance, Ig: immunoglobulin, nd: no data, NO_2: nitrogen dioxide, SRM: standard reference material.

10. Observations in man

10.1 Irritation and sensitisation

The gas phase of diesel exhaust contains several irritating constituents such as NO_2 and aldehydes. Exposure to diesel exhaust may therefore cause acute irritation of the eyes and respiratory tract (423). Controlled studies on irritative effects are included in Table 16.

In a controlled human exposure chamber study, 25 healthy subjects exposed in a randomised, single-blinded fashion to filtered air and to diluted diesel exhaust (108 µg DEP/m^3, 0.2 ppm NO_2, 1.4 ppm hydrocarbons, 0.04 mg/m^3 formaldehyde) for 2 hours, with intermittent exercise, reported unpleasant smell (21/25), low-level nasal (14/25) and throat irritation (11/25) (ratings were 0–2 on a modified Borg scale of 0–11) and mild eye irritation (6/25) within the first hour of exposure (265). In a study of Carlsten *et al*., perceived exposure (blindly rated as none, medium, high or don't know), but not the true exposure, was significantly associated with self-reported nose and eye symptoms in 43 healthy subjects exposed to diesel exhaust at 100 or 200 µg DEP/m^3 ($\leq$ 0.05 ppm NO_2, $\leq$ 0.04 mg/m^3 formaldehyde) for 2 hours (65).

Wierzbicka *et al*. reported redness, secretion and swelling in the nose [odds ratio (OR) 6.4, 95% confidence interval (CI) 2.5–17] and in the eyes (OR 3.1, 95% CI 0.97–9.8) of 18 healthy, non-smoking volunteers after 2 hours of exposure to diesel exhaust (~ 300 µg DEP/m^3, 1.3 ppm NO_2, 0.4 mg/m^3 formaldehyde) (452). In a study by Rudell *et al*., 12 healthy subjects exposed to unfiltered (2.6×10^6/cm^3, 1.9 ppm NO_2, 0.4 mg/m^3 formaldehyde) and particle trap filtered (1.4×10^6/cm^3, 1.7 ppm NO_2, 0.4 mg/m^3 formaldehyde) diesel exhaust for 1 hour experienced unpleasant smell, eye and nasal irritation during the exposures (356).

No data related to sensitisation were identified in the literature. Studies related to immunological effects of diesel exhaust are reviewed in Section 10.2.4.

10.2 Effects of single and short-term exposure

10.2.1 Pulmonary effects

Several controlled human exposure studies focusing on pulmonary and cardiovascular effects of diesel exhaust have been published. Table 16 summarises studies examining lung inflammatory and lung functional effects in healthy and asthmatic volunteers.

Increased numbers of neutrophils and inflammatory cytokines (IL-6 and/or IL-8) in bronchial wash were observed in healthy volunteers exposed to diesel exhaust at ~ 100 µg DEP/m^3 (0.2–0.4 ppm NO_2) for 2 hours (28, 29, 391). Increases in neutrophil counts were also observed in sputum of healthy volunteers exposed to diesel exhaust or resuspended DEP at 200–300 µg DEP/m^3 for 1–2 hours (272, 295). In addition to an increased neutrophil count in bronchial wash, Salvi *et al*. reported an increase in lymphocyte count in BAL after a 1-hour exposure to 300 µg DEP/m^3 (1.6 ppm NO_2) (365). An increased lymphocyte count in BAL after

exposure to 100 µg DEP/m^3 (0.2 ppm NO_2) for 2 hours was also indicated by Stenfors *et al*. (391). No changes in BAL were, however, observed in three other studies applying similar exposure levels (28, 29, 265).

Increased airway resistance was observed in healthy volunteers exposed in a randomised, single-blinded manner to filtered air and to 108 µg DEP/m^3 (0.2 ppm NO_2, 1.4 ppm hydrocarbons, 0.04 mg/m^3 formaldehyde) for 2 hours (265, 391). Lung function responses were assessed immediately after exposure using a computerised whole-body plethysmograph. A transient decrease in peak expiratory flow (PEF) during the exposure was indicated at 276 µg DEP/m^3 (1.3 ppm NO_2) (466). No impact on PEF was detected in corresponding studies at 100–300 µg DEP/m^3 (0.4–1.6 ppm NO_2) (28, 365). No changes in forced vital capacity (FVC) or forced expiratory volume in one second (FEV_1) were observed in any of the studies (265, 272, 356, 357, 365, 391, 466).

In asthmatic volunteers, increased bronchial hyperresponsiveness was observed after 1-hour exposures to whole diesel exhaust at 300 µg DEP/m^3 (0.2–1.2 ppm NO_2) (164, 296). No effect on bronchial hyperresponsiveness was observed in asthmatic volunteers exposed for 2 hours at 100 µg DEP/m^3 (0.4 ppm NO_2) (28). Hussain *et al*. reported a progressive decline in FEV_1 at 0–24 hours post-exposure in asthmatic volunteers exposed 1 hour to diesel exhaust at 300 µg DEP/m^3 (0.2 ppm NO_2) (164). No impact on FEV_1 or FVC was, however, observed in asthmatics after 2 hours of exposure to 102 µg DEP/m^3 (0.4 ppm NO_2) (348).

Stenfors *et al*. exposed healthy (details above) and asthmatic subjects to diesel exhaust at 108 µg DEP/m^3 for 2 hours (0.2 ppm NO_2). A similar increase in airway resistance was observed in both groups. Airway neutrophilia and lymphocytosis observed in the healthy volunteers were not present in the asthmatics. Epithelial staining for the cytokine IL-10 was, however, increased in the asthmatic group (391). No lung inflammatory response was observed in asthmatic subjects exposed to ~ 100 µg DEP/m^3 (0.4 ppm NO_2) for 2 hours in the studies of Behndig *et al*. (28) and Riedl *et al*. (348).

Epidemiological studies and time series studies on air pollution episodes indicate an association between increased levels of air pollution, including traffic emissions, and exacerbation of pre-existing respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD) [reviewed in (189, 399)].

Table 16. Irritative and pulmonary effects in humans after controlled exposure to diesel exhaust.

DEP ($\mu g/m^3$, no. of particles/cm^3)	NO_2 (ppm)	Exposure duration and conditions	No. of subjects and gender	Effects	Reference
Healthy adults					
100	0.4	2 h, idling Volvo 1991 engine, light exercise (biking)	23 (10 females, 13 males)	Increase in neutrophils, IL-6 and MPO in BW (not in BAL) at 18 h post-exposure.	(28)
100	0.4	2 h, idling Volvo 1991 engine	15 (7 females, 8 males, never-smokers)	Increase in neutrophils, IL-8 and MPO in BW (not in BAL) at 18 h post-exposure; increase in GSH and urate in alveolar lumen.	(29)
108	0.2	2 h, idling Volvo 1991 engine, moderate exercise (biking)	25 (9 females, 16 males, never-smokers)	Increase in GSH in BW (not in BAL) at 6 h post-exposure; no change in neutrophils in BW or BAL (considerable inter-subject variation); increased airway resistance; no effect on dynamic lung function (FEV_1 or FVC); subjectively reported unpleasant smell, low-level nasal and throat irritation and mild eye irritation.	(265)
108	0.2	2 h, idling Volvo 1991 engine	25 (9 females, 16 males, non-smokers)	Increase in neutrophils, IL-6 and IL-8 in BW and lymphocytes in BAL at 6 h post-exposure; indications of inflammation in bronchial biopsy; increased airway resistance; no impact on dynamic lung function (FEV_1 or FVC).	(391)
200	nd	2 h, resuspended diesel particles (self-collected)	10 (7 females, 3 males, non-smokers)	Increase in neutrophils and MPO in sputum at 4 h post-exposure; no impact on dynamic lung function (FEV_1 or FVC).	(272)
270	0.4	1 h, running Volvo 1996 engine	15 (8 females, 7 males, never-smokers)	Increase in vascular endothelial adhesion molecules in bronchial tissue and eosinophils in BAL at 6 h post-exposure; no effect on neutrophils or lymphocytes in BW or BAL; no impact on dynamic lung function (FEV_1 or FVC).	(375)
276 ($3.9 \times 10^5/cm^3$)	1.3	2 h, idling Volkswagen 1998 engine	18 (9 females, 9 males, non-smokers)	Transient decrease in PEF during exposure; no impact on FEV_1 or FVC; no impact on inflammatory markers in nasal lavage.	(466)
300 ($4.3 \times 10^6/cm^3$)	1.6	1 h, idling Volvo 1991 engine, moderate exercise (biking)	15 (2 females, 13 males, non-smokers)	Increase in neutrophils and IL-6 in sputum at 6 h post-exposure.	(295)

Table 16. Irritative and pulmonary effects in humans after controlled exposure to diesel exhaust.

DEP ($\mu g/m^3$, no. of particles/cm^3)	NO_2 (ppm)	Exposure duration and conditions	No. of subjects and gender	Effects	Reference
300 ($4.3 \times 10^6/cm^3$)	1.6	1 h, idling Volvo 1991 engine, moderate exercise (biking)	15 (4 females, 11 males, non-smokers)	Increase in neutrophils in BW and lymphocytes in BAL at 6 h post-exposure; elevated mRNA expression for IL-8 in BW and bronchial tissue, and for IL-5 and GRO-α in bronchial tissue and epithelium, respectively; no impact on dynamic lung function (FEV_1, FVC or PEF).	(334, 365, 366)
$2.6 \times 10^6/cm^3$ filtered: $1.3 \times 10^6/cm^3$	1.3 filtered: 1.2	1 h, idling Volvo 1990 engine, with and without particle trap, light exercise (biking)	10 (2 females, 8 males, never-smokers)	Increase in neutrophils in BW (not in BAL) at 24 h post-exposure; similar effects with and without the particle trap.	(355)
$2.6 \times 10^6/cm^3$ filtered: $1.4 \times 10^6/cm^3$	1.9 filtered: 1.7	1 h, idling Volvo 1990 engine, with and without particle trap, light exercise (biking)	12 (4 females, 8 males, non-smokers)	Increased airway resistance at 30 min post-exposure; no impact on dynamic lung function (FEV_1 or FVC); subjectively reported unpleasant smell, nasal and eye irritation.	(356)
$4.3 \times 10^6/cm^3$	1.6	1 h, idling Scania 1968 engine	8 (gender not stated, non-smokers)	No impact on dynamic lung function (FEV_1 or FVC); subjectively reported unpleasant smell, nasal and eye irritation (only by few: throat irritation, headache, dizziness, nausea, tiredness, coughing).	(357)
Adults with asthma or allergic rhinitis					
93	0.9–1.3	2 h, Cummins engine (5.9 L), rest; intranasal dosing of attenuated influenza virus 2–3 h post-exposure	9 with allergic rhinitis + 8 healthy subjects; sham exposed controls: 7 allergic + 8 healthy (both genders)	Increased IFN-γ response in nasal lavage (both groups); no effect on other cytokines or chemokines measured; increased virus quantity and eosinophil cationic protein in nasal lavage (allergic group only).	(293)
102 ($1.2 \times 10^5/cm^3$)	0.4	2 h, idling Navistar 1999 engine (7.3 L), intermittent exercise (biking)	15 asthmatic (both genders)	No significant impact on cell counts or inflammatory or immunological markers in sputum; no impact on dynamic lung function (FVC, FEV_1) or on methacholine-induced bronchial reactivity; modest increase in airway resistance at 22 hours post-exposure.	(348)

Table 16. Irritative and pulmonary effects in humans after controlled exposure to diesel exhaust.

DEP ($\mu g/m^3$, no. of particles/cm^3)	NO_2 (ppm)	Exposure duration and conditions	No. of subjects and gender	Effects	Reference
104 ($1.2 \times 10^5/cm^3$)	0.3	2 h, idling Navistar 1999 engine (7.3 L), intermittent exercise (biking)	15 asthmatic, allergic to cat (both genders)	Near-significant increase in PMNs and eosinophils and decrease in macrophages in sputum; no significant effects on other inflammatory or immunological markers in sputum; no consistent impact on cat allergen induced bronchoconstrictive response or IgE in sputum.	(348)
100	0.4	2 h, idling Volvo 1991 engine, light exercise (biking)	32 asthmatic, (16/gender, never-smokers), 23 healthy subjects (10 females, 13 males)	No change in bronchial hyperresponsiveness at 40 h post-exposure; no impact on dynamic lung function (FEV_1, FVC or PEF); airway inflammatory responses in the healthy group (increase in neutrophils, IL-6 and MPO in BW at 18 h post-exposure) but not in the asthmatic subjects.	(28)
108	0.2	2 h, idling Volvo 1991 engine	15 asthmatic (10 females, 5 males, non-smokers), 25 healthy subjects (9 females, 16 males)	Increase in IL-10 in bronchial epithelium at 6 h post-exposure; increased airway resistance; no impact on dynamic lung function (FEV_1 or FVC); airway inflammatory response in the healthy group (increase in neutrophils, IL-6 and IL-8 in BW and lymphocytes in BAL at 6 h post-exposure) but not in the asthmatic subjects.	(391)
300	0.2	1 h, 5.5 kW diesel generator, intermittent exercise (biking)	16 asthmatic (9 females, 7 males)	Progressive reduction of FEV_1 at 0, 4 and 24 h post-exposure; increased methacholine-induced bronchial reactivity at 24 h post-exposure; no impact on exhaled NO; borderline to non-significant increase in nitrite concentration in exhaled breath condensate at 0, 4 and 24 hours post-exposure.	(164)
300	1.2	1 h, idling Volvo 1991 engine	14 asthmatic (7 females, 7 males, never-smokers)	Increase in IL-6 in sputum at 6 h post-exposure; increased bronchial hyperresponsiveness at 24 h post-exposure; increased airway resistance; no impact on dynamic lung function (FEV_1 or FVC).	(296)

BAL: bronchoalveolar lavage, BW: bronchial wash, DEP: diesel exhaust particles, FEV_1: forced expiratory volume in one second, FVC: forced vital capacity, GRO-α: growth-regulated oncogene alpha, GSH: reduced glutathione, IFN-γ: interferon gamma, Ig: immunoglobulin, IL: interleukin, MPO: myeloperoxidase, nd: no data, NO: nitrogen monoxide, NO_2: nitrogen dioxide, PEF: peak expiratory flow, PMN: polymorphonuclear leukocyte.

10.2.2 Haematological and cardiovascular effects

Controlled human exposure studies focusing on haematological and cardiovascular effects of diesel exhaust are listed in Table 17.

A reduced response to vasodilators was observed in healthy volunteers exposed to diesel exhaust at 250–350 µg DEP/m^3 (0.2–1.6 ppm NO_2) for 1–2 hours (23, 230, 255, 257, 418). Also, increased arterial stiffness and decrease in brachial artery diameter have been reported after 1–2 hours of exposure to 100–330 µg DEP/m^3 (0.02–0.6 ppm NO_2, 0.5–3.1 ppm CO) (232, 327). No effects on vasodilation were observed with a corresponding exposure to filtered exhaust (< 10 µg DEP/m^3, 0.2–3.4 ppm NO_2, ~ 3.2 ppm CO) (230, 255). Tong *et al*. observed a borderline significant decrease in brachial artery diameter in healthy volunteers at 300 µg DEP/m^3 (2.2 ppm NO_2, 6.9 ppm CO), which was absent at 100–214 µg DEP/m^3 (1.0–1.7 ppm NO_2, 3.1–5.4 ppm CO) (409).

Lucking *et al*. reported increased *ex vivo* thrombogenicity and *in vivo* platelet activation in healthy volunteers exposed to unfiltered diesel exhaust at 320–350 µg DEP/m^3 (0.2–0.7 ppm NO_2) for 1–2 hours (230, 231). No pro-thrombotic changes were, however, observed in three other studies with similar or lower exposures (63, 252, 348). In subjects with stable coronary artery disease, an increase in ST-segment depression and ischaemic burden was observed during 1 hour of exposure to diesel exhaust at ~ 300 µg DEP/m^3 (1.0 ppm NO_2, 2.9 ppm CO) (256).

Kipen *et al*. reported decreased leukocyte and erythrocyte proteasome activity in peripheral blood, possibly indicating oxidative stress at 193 µg DEP/m^3 (0.1 ppm NO_2, 4.5 ppm CO) for 2 hours (194). Increased neutrophil and platelet counts in the blood were reported in the study of Salvi *et al*. (300 µg DEP/m^3, 1.6 ppm NO_2, for 1 hour) (365). Xu *et al*. reported increased lymphocyte counts in the blood, without changes in other inflammatory markers or coagulation factors, after a 2-hour exposure at 276 µg DEP/m^3 (1.3 ppm NO_2) (466). No significant impact on neutrophil, leukocyte or platelet counts in the blood or on the levels of cytokines or coagulation factors were detected in other studies (23, 41, 63, 64, 255, 257, 272, 348, 418).

Tong *et al*. reported increased diastolic blood pressure in healthy volunteers exposed to diesel exhaust at 300 µg DEP/m^3 (2.2 ppm NO_2, 6.9 ppm CO) for 2 hours (409). Mills *et al*. observed increased systolic blood pressure in volunteers exposed for 2 hours to unfiltered diesel exhaust at 350 µg DEP/m^3 (0.2 ppm NO_2, 3.5 ppm CO) or a corresponding filtered exhaust (6 µg DEP/m^3, 0.2 ppm NO_2, 3.2 ppm CO) (255). No effect on blood pressure was observed in volunteers exposed to unfiltered diesel exhaust or to resuspended DEP at 200 µg DEP/m^3 for 2 hours or at 250–330 µg DEP/m^3 for 1 hour (23, 232, 272, 327, 409, 418).

Epidemiological studies indicate that short-term exposure to ambient air pollution, including traffic emissions, can trigger cardiovascular mortality and non-fatal cardiovascular events (8, 53). The risk is increased principally among susceptible individuals, including the elderly and those with pre-existing coronary artery disease. Although many studies have found a strong association between

metrics of traffic-related air pollution and elevated cardiovascular risk, it is still unclear whether this represents the harmful effects of DEP, (ultra)fine particles in general or other pollution components (53).

10.2.3 Neurological effects

Only one controlled human exposure study focusing on the effects of diesel exhaust on the brain was identified. In this study on 10 healthy men exposed for 1 hour to diluted diesel exhaust (~ 300 µg DEP/m^3, 1.6 ppm NO_2, 7.5 ppm CO), Crütz *et al*. observed a statistically significant increase in median power frequency in the frontal cortex within 30 minutes of exposure, measured by quantitative electroencephalography (QEEG). The median power frequency continued to rise during the 1-hour post-exposure observation period (77).

10.2.4 Immunological effects

In nasal challenge studies on healthy volunteers, 150 µg of DEP suspended in 200 µl saline and sprayed into each nostril (total dose of 300 µg DEP) induced an increase in nasal IgE at 96 hours after the challenge, and in related cytokines and chemokines at 6–24 hours after the challenge (84, 86, 87). The DEP was collected from the exhaust of a light-duty diesel passenger car (pre-1990). No increase in the IgE response was observed after exposure to 150 or 1 000 µg of the same material (84). In further studies applying the same material, nasal challenge with 300 µg of DEP prior to or concurrent with an allergen (keyhole limpet haemocyanin or ragweed antigen) was reported to enhance sensitisation and the allergic response (27, 85, 88).

In another nasal challenge study, no effect on IgE production or related cytokines was observed in healthy or asthmatic subjects at 4 or 96 hours after challenge with 600 µg of SRM 1650 or ozone-oxidised SRM 1650, sprayed in each nostril as 300 µg DEP suspended in 200 µl saline (200). Different compositions of the DEP materials applied are a plausible explanation for the different observations in comparison to the previous studies.

No significant impact on immunological markers in sputum at 23 hours after exposure, or on the response to an accompanying challenge with cat allergen, was observed in mildly asthmatic subjects exposed to diesel exhaust by inhalation at ~ 100 µg DEP/m^3 (0.4 ppm NO_2) for 2 hours (348). Respiratory responses related to inhalation exposure to diesel exhaust are further discussed in Sections 10.2.1 and 10.3.

Table 17. Haematological and cardiovascular effects in humans after controlled exposure to diesel exhaust.

DEP ($\mu g/m^3$, no. of particles/cm^3)	NO_2 (ppm)	CO (ppm)	Exposure duration and conditions	No. of subjects and gender	Effects	Reference
Healthy adults						
100 200	nd	nd	2 h, Cummins 2002 engine	13 (2 females, 11 males, never-smokers)	No impact on pro-thrombotic markers (D-dimer, PAI-1, vWF, CRP) in peripheral blood at 0, 3, 6 and 22 h post-exposure.	(63)
100 214 302	1.0 1.7 2.2	3.1 5.4 6.9	2 h, idling Cummins engine (5.9 L)	6 (2 females, 4 males, non-smokers, GSTM1 null genotype)	Decrease in brachial artery diameter, increase in diastolic blood pressure and changes in heart rate variability related parameters at 302 $\mu g/m^3$; increased neutrophil and platelet counts in blood at 214 $\mu g/m^3$.	(409)
102 205	0.02 0.02	0.5 0.9	2 h, Cummins 2002 engine	10 (2 females, 8 males, non-smokers)	Decrease in brachial artery diameter (indication of dose-response); increase in plasma levels of ET-1; no effect on blood pressure; no effect on plasma catecholamine levels or endothelium-dependent flow-mediated dilation.	(327)
106	0.8	2.8	2 h, Cummins engine, intermittent moderate exercise	7 (4 females, 3 males)	Increase in VCAM-1 expression in primary human coronary artery endothelial cells treated with plasma obtained 24 h post-exposure; plasma from subjects exposed to 0.5 ppm NO_2 increased the VCAM-1 expression both immediately and 24 h post-exposure.	(68)
193 ($0.7 \times 10^6/cm^3$)	0.1	4.5	2 h, diesel engine	38 (6 females, 32 males)	Decreased white and red blood cell proteasome activity in peripheral blood at 0 h post-exposure.	(194)
200	nd	nd	2 h, resuspended diesel particles (self-collected)	10 (7 females, 3 males, non-smokers)	No effect on heart rate or blood pressure; no effect on inflammatory markers (IL-6, P-selectin or TNF-α) in peripheral blood at 0 or 24 h post-exposure.	(272)
254 ($1.2 \times 10^5/cm^3$)	0.9	nd	1 h, running Volvo 1991 engine, moderate exercise (biking)	18 (males, non-smokers)	Attenuated vasodilation to bradykinin, acetylcholine, sodium nitroprusside and verapamil at 6 h post-exposure; no effect on heart rate or blood pressure; no effect on leukocyte, neutrophil or platelet counts, plasma TNF-a, IL-6, P-selectin or serum CRP at 2 or 6 h post-exposure.	(23)

Table 17. Haematological and cardiovascular effects in humans after controlled exposure to diesel exhaust.

DEP ($\mu g/m^3$, no. of particles/cm^3)	NO_2 (ppm)	CO (ppm)	Exposure duration and conditions	No. of subjects and gender	Effects	Reference
276 ($3.9 \times 10^5/cm^3$)	1.3	nd	2 h, idling Volkswagen 1998 engine	18 (9 females, 9 males, non-smokers)	Indication of increased lymphocyte count in the blood at 0 and 20 h post-exposure; no changes in other inflammatory markers or coagulation factors in the blood.	(466)
300 ($1.2 \times 10^6/cm^3$)	1.6	nd	1 h, idling Volvo 1991 engine, moderate exercise (biking)	15 (males, non-smokers)	Attenuated vasodilation to acetylcholine and bradykinin (not verapamil or sodium nitroprusside) at 24 h post-exposure; no effect on release of t-PA by bradykinin; no effect on heart rate or blood pressure; no effect on leukocyte, neutrophil or platelet counts, plasma IL-6, TNF-α or ET-1 or serum CRP at 24 h post-exposure.	(418)
300 ($1.2 \times 10^6/cm^3$)	1.6	7.5	1 h, idling Volvo 1991 engine, moderate exercise (biking)	30 (males, non-smokers)	Attenuated vasodilation to bradykinin, acetylcholine and sodium nitroprusside (not verapamil) at 2–4 and 6–8 h post-exposure; decreased release of t-PA by bradykinin at 6 h post-exposure; no effect on heart rate or blood pressure; no effect on leukocyte, neutrophil or platelet counts, plasma IL-6, TNF-α or ET-1 or serum CRP at 6 h post-exposure.	(257)
300 ($4.3 \times 10^6/cm^3$)	1.6	7.5	1 h, idling Volvo 1991 engine, moderate exercise (biking)	15 (4 females, 11 males, non-smokers)	Increase in neutrophils and platelets in peripheral blood at 6 h post-exposure.	(365)
320 ($2.0 \times 10^5/cm^3$) filtered: 7 ($3 \times 10^2/cm^3$)	0.7 filtered: 3.4	nd	1 h, Volvo TD40 GJE engine, with and without particle trap	19 (males, non-smokers)	Attenuated vasodilation to bradykinin, acetylcholine and verapamil (not sodium nitroprusside) at 6 and 8 h post-exposure (unfiltered exhaust only); increased thrombogenicity at 2 h post-exposure (Badimon *ex vivo* perfusion chamber) (unfiltered exhaust only).	(230)
330 ($1.2 \times 10^6/cm^3$)	0.6	3.1	1 h, idling Volvo 1991 engine	12 (males, non-smokers)	Increased arterial stiffness at 10 min post-exposure (transient); no effect on heart rate or blood pressure.	(232)

Table 17. Haematological and cardiovascular effects in humans after controlled exposure to diesel exhaust.

DEP ($\mu g/m^3$, no. of particles/cm^3)	NO_2 (ppm)	CO (ppm)	Exposure duration and conditions	No. of subjects and gender	Effects	Reference
348 ($1.2 \times 10^6/cm^3$) filtered: 6 ($2 \times 10^3/cm^3$)	0.2 filtered: 0.2	3.5 filtered: 3.2	2 h, unloaded Deutz engine, with and without Teflon filter, moderate exercise (biking)	16 (males, non-smokers)	Attenuated vasodilation to bradykinin, acetylcholine and sodium nitroprusside (not verapamil) at 6–8 h post-exposure (unfiltered exhaust only); increased systolic blood pressure (both filtered and unfiltered exhaust); no effect on leukocyte, neutrophil or platelet counts or plasma t-PA or PAI-1 at 0, 2 or 6 post-exposure.	(255)
348 ($1.2 \times 10^6/cm^3$)	0.2	3.5	2 h, unloaded Deutz engine, moderate exercise (biking)	20 (males, non-smokers)	Increased thrombogenicity at 2 and 6 h post-exposure (Badimon *ex vivo* perfusion chamber); increase in platelet-neutrophil and platelet-monocyte aggregates at 2 h post-exposure.	(231)
350	nd	nd	2 h, diesel engine, moderate exercise (biking)	16 (gender not stated)	No effect on clot permeability, maximum turbidity, lag time, fibre diameter, fibre density and fibrinogen level in blood at 2 or 6 h post-exposure.	(252)
Adults with metabolic or cardiovascular disease						
102 205	0.02 0.02	0.5 0.9	2 h, Cummins 2002 engine	17 with metabolic syndrome (6 females, 11 males), 10 healthy controls (2 females, 8 males) (all non-smokers)	Decrease in brachial artery diameter (indication of dose-response; milder effect than in healthy controls); increase in plasma levels of ET-1; no effect on blood pressure; no effect on plasma catecholamine levels or endothelium-dependent flow-mediated dilation.	(327)
102 206	0.02 0.03	0.5 0.7	2 h, Cummins 2002 engine	16 (13 with metabolic syndrome, 5 females, 11 males, non-smokers)	No consistent effect on heart rate variability at 1–22 h post-exposure; no effect on heart rate.	(325)
206	0.03	0.7	2 h, Cummins 2002 engine	16 with metabolic syndrome (6 females, 10 males, non-smokers)	No effect on pro-thrombotic markers (D-dimer, PAI-1, vWF) in peripheral blood at 3, 7 or 22 h post-exposure.	(64)

Table 17. Haematological and cardiovascular effects in humans after controlled exposure to diesel exhaust.

DEP ($\mu g/m^3$, no. of particles/cm^3)	NO_2 (ppm)	CO (ppm)	Exposure duration and conditions	No. of subjects and gender	Effects	Reference
$1.3 \times 10^6/cm^3$ (~ 300 $\mu g/m^3$)	1.0	2.9	1 h, idling Volvo 1991 engine, moderate exercise (biking)	20 with stable coronary artery disease (males, non-smokers)	Increased ST-segment depression and ischaemic burden during exposure; decreased release of t-PA by bradykinin at 6 h post-exposure; no effect on vasodilation to bradykinin or sodium nitroprusside at 6–8 h post-exposure; no effect on heart rate or blood pressure; no effect on plasma t-PA or PAI-1 or serum CRP at 6 or 24 h post-exposure.	(256)
Adults with asthma or other respiratory diseases						
102 ($1.2 \times 10^5/cm^3$)	0.4	1.7	2 h, idling Navistar 1999 engine (7.3 L), intermittent exercise (biking)	15 asthmatic (males and females, non-smokers)	No significant effects on coagulation factors (factor VII, fibrinogen, vWF) in serum.	(348)
104 ($1.2 \times 10^5/cm^3$)	0.3	1.7	2 h, idling Navistar 1999 engine (7.3 L), intermittent exercise (biking)	15 asthmatic (males and females, non-smokers)	No significant effects on inflammatory markers or coagulation factors (factor VII, fibrinogen, vWF) in serum.	(348)
300	nd	nd	1 h, idling Volvo 1991 engine, moderate exercise (biking)	15 with COPD (gender not stated, ex-smokers)	No effect on CRP, fibrinogen, vWF or D-dimer in peripheral blood at 6 or 24 h post-exposure.	(41)

CO: carbon monoxide, COPD: chronic obstructive pulmonary disease, CRP: C-reactive protein, DEP: diesel exhaust particles, ET-1: endothelin-1, GSTM1: glutathione-*S*-transferase Mu 1, IL: interleukin, nd: no data, NO_2: nitrogen dioxide, PAI-1: plasminogen activator inhibitor-1, TNF-α: tumour necrosis factor alpha, t-PA: tissue plasminogen activator, VCAM-1: vascular cell adhesion molecule 1, vWF: von Willebrand factor.

10.3 Effects of long-term exposure

Recent epidemiological studies related to non-cancer health effects of occupational exposure to diesel exhaust are presented in Table 18.

A US case-control study of Weinmann *et al*. focused on the association between lifetime occupational exposures and COPD, applying a job-exposure matrix for exposure assessment. The occupational exposures most strongly associated with COPD were diesel exhaust (OR 1.9, 95% CI 1.3–3.0), mineral dust (OR 1.7, 95% CI 1.1–2.7) and irritant gases and vapours (OR 1.6, 95% CI 1.2–2.2) (435). An increasing risk for COPD mortality with years in diesel exhaust exposed jobs was also indicated in a large cohort of US railroad workers (141, 142). A cohort study on Swedish construction workers indicated a small increase in COPD mortality among workers exposed to fumes, including diesel exhaust, (RR 1.2, 95% CI 1.1–1.4) or inorganic dust (RR 1.2, 95% CI 1.1–1.3) in comparison with non-exposed workers (36). A higher prevalence of COPD (OR 2.5, 95% CI 1.3–5.0) was also reported in a small cohort of Norwegian tunnel construction workers compared with other heavy construction workers (421). In contrast, large cohort studies on US non-metal miners or trucking industry workers did not reveal increased mortality for non-malignant respiratory diseases in comparison with the general population (10, 207).

In a study on German salt mine workers, a decline in lung function (FEV_1) over a 5-year period was associated with an estimated mean exposure to EC, NO_2, NO and inhalable and respirable dust. Because of the high correlation between diesel exhaust and dust exposure, it was not possible to separate their impact. The mean annual extra decrease of FEV_1 of 10–18 ml/year observed in the miners was related to a personal mean exposure of 90 µg EC/m^3, 0.4–0.5 ppm NO_2, 1.4–1.7 ppm NO, 7.1–13 mg/m^3 inhalable dust and 0.8–2.4 mg/m^3 respirable dust (228). A study on Norwegian tunnel construction workers associated the annual decrease in FEV_1 over a 6-year period with the cumulative exposure to NO_2, CO, total and respirable dust, oil mist and formaldehyde. A moderate to high correlation was observed between the exposure variables (18). By contrast, no association of lung function parameters with cumulative exposure to NO_2 (mean 0.007 ppm NO_2), NO (mean 0.6 ppm) or respirable dust (mean 1.9 mg/m^3) were indicated in German coal mine workers followed from the beginning of their career up to about 20 years (263).

Hoppin *et al*. (159) found a positive association between the frequency of driving diesel tractor and wheezing in US farmers. No such association was indicated in another study on US farmers (154).

In a cohort study on Swedish construction workers, slightly increased mortality from ischaemic heart disease was observed among workers exposed to diesel exhaust (RR 1.2, 95% CI 1.1–1.2) or inorganic dust (RR 1.1, 95% CI 1.1–1.2) in comparison with non-exposed workers (411). Increased mortality from ischaemic heart disease was also indicated among a cohort of US trucking industry workers (SMR 1.4, 95% CI 1.3–1.5) (207). In a follow-up, increasing years of employment were associated with increasing mortality from ischaemic heart disease in four job

categories, although these increases were not statistically significant (140). No increase of cardiovascular mortality was observed in the cohort of US non-metal miners (10).

Many epidemiological studies have associated long-term exposure to ambient air pollution, including traffic emissions, to increased cardiovascular mortality (53, 158, 333, 338). Among children, ambient air pollution, in particular traffic emissions, is associated with decreased lung function growth and increased risk of development of asthma (97, 122, 127). Some epidemiological studies have indicated associations between ambient air pollution and increased lung function decline and higher asthma incidence in adults. There are also studies indicating neurological effects of ambient air pollution, in particular among children and the elderly (76, 192).

Table 18. Epidemiological studies related to non-cancer health effects of occupational exposure to diesel exhaust.

Study population/controls	Exposure characterisation	Results	Comments	Reference
744 cases of COPD, 356 controls, US	Semi-quantitative (JEM)	Increased risk of COPD in subjects occupationally exposed to diesel exhaust (OR 1.9, 95% CI 1.3–3.0), irritant gases and vapours (OR 1.6, 95% CI 1.2–2.2) and mineral dust (OR 1.7, 95% CI 1.1–2.7); indication of dose-response (higher OR for high than moderate exposure; hampered by small number of subjects in the high exposure group).	Adjusted for smoking.	(435)
12 315 non-metal miners, US	Job title	Apart for pneumoconiosis associated with previous exposures of the subjects, no increase in SMRs of non-malignant respiratory diseases, cardiovascular diseases or diseases of the nervous system, in comparison with the US general population.		(10)
1 369 coal mine workers (2 mines), lung function data available from recruitment in 1974–79 until 1998, Germany	Cumulative and mean exposure based on job title, years of work and measurement data	No significant association between lung function parameters (FVC, FEV_1 or FEV_1%FVC) and cumulative exposure to NO_2, NO or respirable dust.		(263)
Salt mine workers (2 mines), 402 + 438, 1st survey 1995 290 + 278, 2nd survey 2000, Germany	Cumulative and mean exposure based on job title, years of work and measurement data	Decrease in lung function (FEV_1) associated with mean exposure to inhalable and respirable dust, EC, NO_2 and NO during the 5-year period; high correlation between diesel exhaust and dust exposure.	Adjusted for smoking.	(228)
143–286 salt mine workers, Germany	Exposure during the past 5 years assessed based on work history and measurement data	Increasing number of activated T-cells in blood with increasing exposure to inhalable and respirable dust, EC and NO_2; high correlation between diesel exhaust and dust exposure.	Adjusted for smoking, inflammatory diseases and BMI.	(13)

Table 18. Epidemiological studies related to non-cancer health effects of occupational exposure to diesel exhaust.

Study population/controls	Exposure characterisation	Results	Comments	Reference
22 underground iron ore miners (one mine), 21 white-collar workers (controls), Sweden	Job title (also exposure measurements carried out)	Increased macrophage and neutrophil counts in the sputum of the miners, both after ≥ 3 months of work and after ≥ 1 month of holiday; no difference in lung function (FVC, FEV_1) between the two groups.	Non-smokers with no history of respiratory diseases.	(477)
651 tunnel construction workers, Norway	Cumulative exposure based on job title, years of work and measurement data	Higher annual decrease in FEV_1 among worker groups with higher exposure than among groups with lower exposure during a 6-year period; decrease in FEV_1 associated with cumulative exposure to NO_2, CO, total and respirable dust, oil mist and formaldehyde (NO_2 showing the strongest association); moderate to high correlation between the exposure variables.	Non-smokers and ever-smokers assessed separately; the cohort included 191 workers from the cohort of Ulvestad *et al.* (421).	(18)
221 tunnel construction workers, 205 other heavy construction workers (controls), Norway	Job title and years of work	Decreased lung function (FVC, FEV_1) in relation to years of work in comparison to the control group; higher prevalence of COPD (OR 2.5, 95% CI 1.3–5.0); higher prevalence of self-reported respiratory symptoms.		(421)
389 tunnel construction workers, US	Job title and years of work	No significant difference in lung function in comparison to predicted values; tunnel workers reported more respiratory symptoms than operating engineers; no association with years of work.		(301)
248 087 construction workers, Sweden	Exposed vs non-exposed (JEM)	Increased mortality for ischaemic heart disease among workers exposed to diesel exhaust (RR 1.2, 95% CI 1.1–1.2), inorganic dust (RR 1.1, 95% CI 1.1–1.2), any particulate air pollutant (RR 1.1, 95% CI 1.1–1.1) or gases and irritants (RR 1.1, 95% CI 1.1–1.2) in comparison with non-exposed workers; in regression analysis, increased risk related to exposure to inorganic dust and fumes, including diesel exhaust, bitumen and metal fumes.	Adjusted for smoking.	(411)

Table 18. Epidemiological studies related to non-cancer health effects of occupational exposure to diesel exhaust.

Study population/controls	Exposure characterisation	Results	Comments	Reference
317 629 construction workers (of which 723 cases of COPD), Sweden	Exposed vs non-exposed (JEM)	Increased COPD mortality among workers exposed to inorganic dust (RR 1.2, 95% CI 1.1–1.3) or fumes, including diesel exhaust, bitumen and metal fumes (RR 1.2, 95% CI 1.04–1.4), in comparison with non-exposed workers; in regression analysis, increased risk related to exposure to inorganic dust among the whole cohort, and to fumes, gases and irritants and inorganic dust among never-smokers.		(36)
52 345 trucking industry workers (of which 1 357 cases of ischaemic heart disease), US	Job title and years of work	Increased mortality for ischaemic heart disease among long haul drivers (RR 1.4, 95% CI 1.2–1.7) and dockworkers (RR 1.3, 95% CI 1.1–1.5) in comparison with the whole cohort; no significant association with years of work.	Adjusted for healthy worker effect; indirectly adjusted for smoking.	(140)
54 319 trucking industry workers (of which 1 133 cases of ischaemic heart disease), US	Job title	Increased mortality for ischaemic heart disease among the whole cohort (SMR 1.4, 95% CI 1.3–1.5) and among drivers, dockworkers and shop workers assessed separately, in comparison with the US general population; no increase in mortality for non-malignant respiratory diseases.		(207)
93 692 transport workers, Canada	Job title	No increase in asthma, COPD, acute respiratory infections, ischaemic heart disease or other circulatory disorders among subjects occupationally exposed to traffic emissions in comparison with occupationally non-exposed subjects.	Adjusted for smoking.	(105)
30 671 railroad workers (of which 1 683 cases of COPD), 6 565 workers employed after dieselisation (332 cases), US	Job title and years of work	Linear association between years in diesel-exposed work and COPD mortality in workers hired after the introduction of diesel locomotives (2.1% increase in COPD mortality for each year).	Adjusted for smoking.	(141)
Railroad workers: 536 cases of COPD, 1 525 controls, US	Job title and years of work	Increasing COPD mortality with years in diesel-exposed jobs in workers hired after the introduction of diesel locomotives.	Adjusted for smoking.	(142)

Table 18. Epidemiological studies related to non-cancer health effects of occupational exposure to diesel exhaust.

Study population/controls	Exposure characterisation	Results	Comments	Reference
20 898 farmers (of which 3 922 with wheeze), US	Frequency of driving diesel tractors	Positive association between driving diesel tractor and wheeze (OR 1.3, 95% CI 1.1–1.5; increasing with increasing frequency of driving).	Adjusted for smoking, history of asthma and atopy.	(159)
926 asthmatic farmers (of which 202 with exacerbation), US	Frequency of driving diesel tractors	Inverse association between driving diesel tractor and exacerbations of asthma (OR 0.5, 95% CI 0.3–0.8).	Adjusted for smoking.	(154)
363 highway toll collectors, 147 office workers (controls), Taiwan	Job title	No significant differences in prevalence rates of self-reported chronic respiratory symptoms in comparison with the control group; increase in acute irritative symptoms (nose and throat irritation, nausea and headache).		(467)
58 highway toll collectors, 37 other workers from the same company (controls), Turkey	Job title	No significant differences in annual changes of lung function (FVC, FEV_1, MMF) or on self-reported respiratory symptoms between the groups over 4 years.		(6)
100 traffic policemen, 100 non-traffic policemen (controls), India	Job title	Decreased lung function (FVC, FEV_1) among traffic policemen in comparison to controls.	Non-smokers with no history of respiratory diseases.	(131)

BMI: body mass index, CI: confidence interval, CO: carbon monoxide, COPD: chronic obstructive pulmonary disease, EC: elemental carbon, FEV_1: forced expiratory volume in one second, FVC: forced vital capacity, JEM: job exposure matrix, OR: odds ratio, MMF: maximum mid-expiratory flow, NO: nitrogen monoxide, NO_2: nitrogen dioxide, RR: relative risk, SMR: standard mortality ratio, US: United States, VOC: volatile organic compounds.

10.4 Genotoxic effects

Two recent studies demonstrated effects of diesel exhaust exposure on the expression of genes related to oxidative stress in humans after controlled 1–2-hour exposures at 200 and 295 μg/m^3 of DEP, respectively (326, 329). Ulfvarson *et al.* performed a study in which volunteers were exposed for 3.7 hours to diesel exhaust resulting in NO_2 levels of 2 ppm. No increased mutagenicity was shown in urine specimens from the exposed persons. Similarly, no increased urinary mutagenicity was seen among crews of roll-on roll-off ships and car ferries and in bus garage staff occupationally exposed to gasoline and diesel exhaust (419).

There are few epidemiological studies on genotoxic effects among workers considered to be predominantly exposed to diesel exhaust. These studies include persons working as diesel drivers in mines, tunnel construction workers and train mechanists/railroad workers. Other studies, involving bus and taxi drivers, traffic police and airport personnel, represent exposure to mixed atmospheres derived from both diesel and gasoline exhaust.

Villarini *et al*. did not find elevated levels of SCEs or increased numbers of DNA strand breaks (measured by comet assay) in peripheral blood lymphocytes of 39 diesel exhaust exposed tunnel construction workers. The frequency of micronuclei, however, was significantly increased in the exposed subjects when compared to 34 matched controls (454). Knudsen *et al*. saw an increased number of DNA strand breaks in blood lymphocytes among 50 smoking underground miners when compared to 47 matched smoking surface workers (197). A slight increase in micronuclei in peripheral blood lymphocytes was seen also among garage mechanics (371). A study by Österholm *et al*. suggested an increased risk for HPRT-mutations in T-lymphocytes among bus maintenance workers exposed to diesel exhaust (478). In other studies involving railroad workers, car mechanics or bus maintenance workers, no clear association between exposure and mutagenic effects have been seen (160, 369, 455).

Several studies have been performed also among bus and taxi drivers, traffic police and airport personnel exposed to urban air polluted with diesel and gasoline exhaust. Many of these studies have shown positive responses in markers of genotoxicity including increased levels of chromosomal aberrations, SCEs, micronuclei and markers related to oxidative damage (167). Diesel exhaust may play a role in these changes although there are also other pollutants which may explain these effects, at least partly.

10.5 Carcinogenic effects

10.5.1 Lung cancer

10.5.1.1 Cohort and case-control studies

There is extensive epidemiological evidence for an association between occupational exposure to diesel exhaust and lung cancer. Tables 19 and 20 summarise the identified cohort and case-control studies related to lung cancer.

A large cohort study demonstrated increased lung cancer mortality among US non-metal miners in comparison with state-based mortality rates (SMR 1.3, 95%

CI 1.1–1.4), and an association of retrospective estimates of cumulative respirable EC exposure with lung cancer mortality (10). In a smoking-adjusted nested case-control study in the cohort, Silverman *et al*. observed an increase in lung cancer mortality with increasing cumulative exposure to respirable EC. The OR was 2.8 (95% CI 1.3–6.3) in the highest exposure quartile (≥ 536 µg EC/m^3-year) in comparison with the lowest quartile (< 3 µg EC/m^3-year) (381, 382). A small case-control study on German potash miners did not find an association between cumulative exposure to respirable EC and lung cancer risk (259). This study is, however, hampered by the high exposure level in the reference group and by other methodological concerns [reviewed in (439)].

In a cohort of US railroad workers, those in diesel-exposed jobs had increased lung cancer mortality (RR 1.4, 95% CI 1.3–1.5) in comparison with unexposed workers. For workers hired after the introduction of diesel engines, the risk was even higher (RR 1.8, 95% CI 1.5–2.1). There was, however, no relation between lung cancer risk and estimated exposure intensity based on locomotive emission data (206).

In a cohort study by Garshick *et al*. in the US trucking industry, the lung cancer risk was observed to increase with years of employment (113). An increase in lung cancer risk with retrospective estimates of cumulative respirable EC exposure was observed when mechanics were excluded from the cohort. The hazard ratios after adjusting for employment duration were 1.5 (95% CI 1.1–2.1) and 1.4 (95% CI 1.0–2.1) for the highest versus the lowest quartiles of 5- and 10-year lagged exposures, respectively (112). A case-control study in the trucking industry by Steenland *et al*. also demonstrated a positive and significant association between estimated cumulative EC exposure and lung cancer. The ORs in the highest quartile versus no exposure were 1.7 (95% CI 1.1–2.6) and 1.6 (95% CI 1.1–2.5) for no and 5-year lag, respectively (389).

Increasing lung cancer risks with increasing cumulative exposure to diesel exhaust are also indicated in recent population-based case-control studies (133, 330, 456).

10.5.1.2 Meta-analyses and pooled analyses

Olsson *et al*. pooled information on lifetime work histories and tobacco consumption of 13 304 cases and 16 282 controls from 11 case-control studies conducted in Europe and Canada. A job-exposure matrix assigning “no”, “low” or “high” exposure to diesel exhaust was applied to determine the level of exposure. Increasing cumulative exposure was associated with increasing lung cancer risk ($p < 0.01$). A statistically significant excess risk was observed for the highest quartile (OR 1.3, 95% CI 1.2–1.4). In addition, increasing lung cancer risk with years of exposure was observed in the exposed groups ($p < 0.01$). For > 30 years of high exposure, the OR was 1.5 (95% CI 1.1–2.0) and for > 30 years of low exposure it was 1.2 (1.1–1.3). The results were adjusted for age, sex, study, ever-employment in an occupation with established lung cancer risk, cigarette pack-years and time since quitting smoking (302).

Lipsett and Campleman conducted a meta-analysis of 30 cohort and case-control studies on the relationship between occupational exposure to diesel exhaust and lung cancer published 1975–1995. Inclusion criteria were adequate data reporting and case ascertainment, a latency period of ≥ 10 years and a unique study population (only one study per population was included). An increased pooled RR was obtained for all studies (RR 1.3, 95% CI 1.2–1.5), being even higher for studies adjusting for smoking (n = 20, RR 1.4, 95% CI 1.3–1.6). By occupation, the highest pooled risk estimates were obtained for truck drivers and other professional drivers (RR 1.5, 95% CI 1.3–1.6) and for railroad workers (RR 1.5, 95% CI 1.1–1.9) (221).

Bhatia *et al*. conducted a meta-analysis of 23 cohort and case-control studies on lung cancer risk related to diesel exhaust exposure. In their analysis, inclusion criteria were adequate data to confirm work with diesel equipment or engines, a latency period of ≥ 10 years, and a unique study population. Studies involving mining were excluded because of the possible influence of radon and silica exposures. The pooled RRs weighted by study precision were 1.3 (95% CI 1.2–1.4), and 1.4 (95% CI 1.2–1.5) for studies adjusting for smoking (37).

Vermeulen *et al*. (439) conducted a meta-regression of lung cancer mortality and cumulative exposure to EC based on the RR estimates reported in three occupational cohort studies differing with regard to the exposure lag time, two using a 5-year lag and the third a 15-year lag (112, 382, 389). The analysis focused on studies with quantitative exposure-response relationships. Figure 4 summarises the data applied for the meta-regression. The estimated excess of lung cancer deaths through age 80 for occupational exposures of 1, 10 and 25 µg EC/m^3

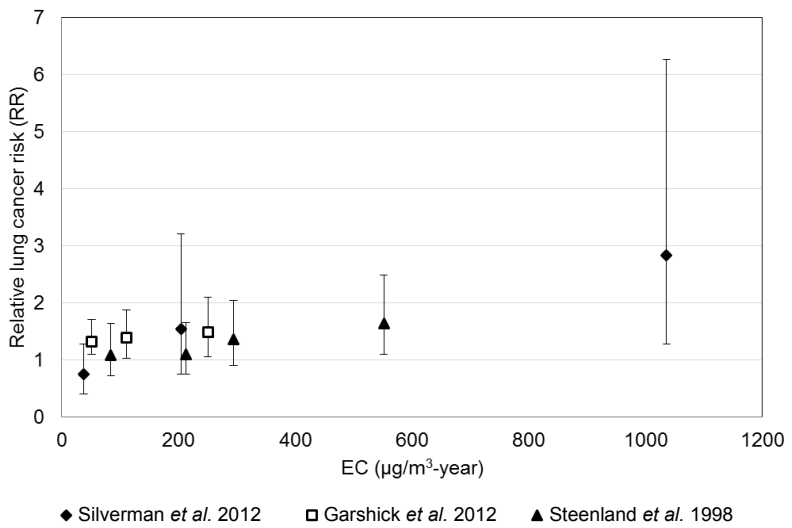

Figure 4. Relative risk estimates (with 95% confidence intervals) for lung cancer mortality calculated by Vermeulen *et al*. (439) based on hazard and odds ratios presented in three cohort studies (figure redrawn from Vermeulen *et al*.). EC: elemental carbon.

over 45 years was 17, 200 and 689 per 10 000 individuals, respectively. For a lifetime environmental exposure of 0.8 µg EC/m^3, the estimated excess was 21 per 10 000. The analysis was conducted with a log-linear model, assuming a 5-year lag and using age-specific lung cancer mortality rates from the US in 2009 as referent. A sensitivity analysis was conducted by applying different exposure lags on the data from the individual studies. Changes in exposure lag did not substantially affect the main estimate (438, 439).

10.5.2 Bladder cancer

There is some epidemiological evidence for an association between diesel exhaust exposure and bladder cancer. In their commentary in 2012, IARC concluded that an increased risk for bladder cancer was noted in many but not all case-control studies, but not in cohort studies. Many of the studies were hampered by low-quality exposure assessment, investigating mortality rather than incidence, and by not adjusting for smoking (33, 167).

Manju *et al*. carried out a meta-analysis of 30 cohort and case-control studies on the smoking-adjusted bladder cancer risk in professional drivers and railroad workers published in 1977–2008. Based on three cohort studies, the overall pooled risk ratio (RR) among motor vehicle and railroad workers was 1.1 (95% CI 1.0–1.2). A total pooled risk estimate for all 30 studies was not calculated because of large heterogeneity among the results of the individual studies. Based on case-control studies, the pooled odds ratio (OR) among bus drivers was 1.3 (95% CI 1.1–1.4) (10 studies), among truck drivers 1.2 (95% CI 1.1–1.3) (18 studies) and among railroad workers 1.2 (95% CI 1.0–1.4) (15 studies). Stratified analysis by year of publication indicated a reduced risk among bus and truck drivers in recent (1998–2008) publications compared to earlier publications (235).

Kogevinas *et al*. pooled data from 11 case-control studies on bladder cancer with detailed occupational and smoking information conducted in West and South Europe in 1976–1996. The studies comprised 4 101 male cases and 7 365 controls. A job-exposure matrix (FINJEM) was applied to evaluate particularly exposure to PAHs and diesel exhaust. A small increase in bladder cancer risk was seen for the highest exposure tertile for PAHs (OR 1.2, 95% CI 1.07–1.4), benzo[a]pyrene (OR 1.3, 95% CI 1.04–1.5), gasoline (OR 1.2, 95% CI 1.03–1.4) and diesel exhaust (OR 1.2, 95% CI 1.05–1.4). No statistically significant increase in cancer risk was observed for motor vehicle drivers, mechanics or railroad workers. A borderline significant increase was observed for transport equipment operators and miners (199).

Boffetta and Silverman conducted a meta-analysis of 29 cohort and case-control studies, and studies based on routinely collected data, on bladder cancer risk in relation to diesel exhaust exposure, allowing for ≥ 5 years latency time. An additional inclusion criterion was a definition of specific occupational groups or an adequate classification of diesel exhaust exposure. An increased pooled risk was observed for heavy equipment operators (RR 1.4, 95% CI 1.1–1.8) (5 studies), bus drivers (RR 1.3, 95% CI 1.2–1.5) (10 studies) and truck drivers (RR 1.2, 95% CI 1.1–1.3) (15 studies). For diesel exhaust exposure based studies, the pooled risk estimate

was close to unity (RR 1.1, 95% CI 1.0–1.3) (10 studies). A risk estimate for railroad workers or total pooled risk estimate for all studies were not calculated because of large heterogeneity among the results of the individual studies (47).

10.5.3 Other cancers

Although a few epidemiological studies indicate a positive association between occupational exposure to diesel exhaust and cancers on other sites than the lung and the bladder, the overall evidence for such an association is weak. Most of the studies are limited by rough exposure assessments, lack of adjustment for confounders and/or a limited number of cases.

In a hospital-based case-control study, 940 cases of laryngeal cancer were compared with 1 519 controls with other types of cancers (99). Occupational exposure to diesel exhaust was associated with an increased risk of laryngeal cancer (OR 1.5, 95% CI 1.3–2.0). Only supraglottic laryngeal cancer (137 cases) showed a trend with estimated exposure probability and intensity. Among never-smokers and never-drinkers, the association was not significant (OR 1.3, 95% CI 0.9–2.0, 55 cases) (98). No significant association between diesel exhaust exposure and laryngeal cancer was observed in six other case-control studies, reviewed in (307). A meta-analysis of 16 cohort and case-control studies addressing the association between laryngeal cancer and occupations with exposure to diesel exhaust showed a slightly elevated overall risk (RR) of 1.2 (95% CI 1.1–1.3) (307).

A population-based case-control study of 3 726 cancer patients in Canada showed a slightly elevated risk of colon cancer among subjects occupationally exposed to diesel exhaust (OR 1.3, 90% CI 1.1–1.6 for ever exposed; OR 1.7, 90% CI 1.2–2.5 for the highest exposure group). No increase in the risk of 11 other cancer types, including lung and bladder cancer, was observed (380). In another Canadian case-control study, 497 colon cancer cases were compared with 1 514 other cancer cases and 533 healthy controls. A borderline significant increase of colon cancer risk was observed among subjects exposed to diesel exhaust (OR 1.6, 95% CI 1.0–2.5) (120). A slightly elevated risk of colon cancer was also observed among Canadian taxi drivers (OR 1.5, 95% CI 1.01–2.5) but not among bus or truck drivers or locomotive operators (102).

A meta-analysis of 26 epidemiological studies on the risk of pancreatic cancer and occupational exposure to diesel exhaust showed no positive associations (43). Similarly, no consistent evidence was identified on an association between occupational exposure to diesel exhaust and leukaemia [reviewed in (42)].

10.6 Reproductive and developmental effects

No studies related to occupational exposure to diesel exhaust and reproductive or developmental outcomes were identified. Some of the epidemiological studies on ambient air pollution associate maternal exposure to ambient air pollution during pregnancy to preterm births, reduced birth weight and increased post-neonatal mortality (313, 335, 377, 387, 393). Data to conclude on the potential risk of diesel exhaust during pregnancy are, however, lacking.

Table 19. Cohort studies on lung cancer risk related to diesel exhaust exposure [adapted mainly from DFG (82)].

Study population	Exposure characterisation	Results	Comments	Reference
12 315 non-metal miners, 200 cases, US	Retrospective estimates of respirable EC	Increased lung cancer mortality (SMR 1.3, 95% CI 1.1–1.4); indication of dose-response when ever-underground and surface only workers were assessed separately (cumulative exposure; 15-year lag).		(10)
5 862 potash miners, 61 cases, Germany	Estimates of TC (based on current TC levels)	Non-significant increase in lung cancer mortality in the high-exposure groups in comparison with the group with lowest exposure (cumulative exposure).	Adjusted for smoking.	(269)
5 536 potash miners, 38 cases, Germany	Estimates of TC (based on current TC levels)	No increase in lung cancer risk (RR 0.78, 95% CI 0.55–1.1); non-significant increase with increasing exposure (RR 2.2, 95% CI 0.79–6.0) for the high-exposure group compared with the low-exposure group.	Few cases.	(402)
31 135 trucking industry workers, 779 cases, US	Retrospective estimates of respirable EC	No significant correlation with lung cancer mortality of the whole cohort; indication of dose-response when mechanics (n = 1 811) were excluded (cumulative exposure; 5–10-year lag).	Mechanics were exposed intermittently and mainly to aged diesel exhaust.	(112)
31 135 trucking industry workers, 779 cases, US	Job title and years of work	Increased lung cancer mortality with years of employment (not observed in all worker groups).	Adjusted for smoking.	(113)
54 319 trucking industry workers, 769 cases, US	Job title	Increased lung cancer mortality among drivers (SMR 1.1, 95% CI 1.02–1.2).		(207)
156 241 truck drivers, 557 cases, US	Membership in truck driver trade association	No significant increase in lung cancer mortality (SMR 1.0, 95% CI 0.92–1.1); lung cancer was, however, one of the three main causes of death (13% of deaths).		(40)
Truck drivers, 280 cases, UK	Job title	Increased lung cancer risk (SMR 1.6, 95% CI 1.4–1.8). [a]		(20)
Truck drivers, 161 cases, Sweden	Job title	Increased lung cancer risk (RR 1.3, 95% CI 1.1–1.6).		(1)
Truck drivers, 109 cases, US	Job title	Increased lung cancer risk (SMR 1.7, 95% CI 1.4–2.0).		(250)
14 225 truck drivers, 76 cases, Denmark	Job title	Increased lung cancer risk (SMR 1.6, 95% CI 1.3–2.0).		(138)

Table 19. Cohort studies on lung cancer risk related to diesel exhaust exposure [adapted mainly from DFG (82)].

Study population	Exposure characterisation	Results	Comments	Reference
868 truck drivers, 24 cases, Iceland	Job title and years of work	Non-significant increase in lung cancer mortality; no relation to years of exposure.	Few cases.	(340)
9 267 bus drivers/ maintenance workers, 386 cases, Italy	Job title and years of work	Increased lung cancer mortality (SMR 1.2, 95% CI 1.1–1.3); no significant association with years of employment.		(251)
2 037 bus drivers, 100 cases, Denmark	Job title and years of work	No increase in lung cancer risk after adjustment for smoking, city of employment and bus route (urban/rural) (reference group: workers with ≤ 15 of employment; 10-year lag); borderline significant increase observed prior to adjustment.	Adjusted for smoking.	(328)
695 bus drivers/maintenance workers, 17 cases, Sweden	Job title	Non-significant increase in lung cancer risk (SMR 1.2, 95% CI 0.71–2.0).	Few cases.	(134)
Bus drivers, 30 cases, UK	Job title	Non-significant increase in lung cancer risk (RR 1.4, 95% CI 0.94–2.0).	Few cases.	(339)
96 438 professional drivers, 604 cases, Sweden	Job title	Increased lung cancer risk for taxi and short distance truck drivers (borderline significance); more pronounced in urban environments: highest risk for short distance truck drivers in the Stockholm county (RR 1.7, 95% CI 1.3–2.3, 50 cases).	Adjusted for smoking.	(177)
1 726 professional drivers, 77 cases, Switzerland	Job title	Increased lung cancer mortality (SMR 1.5, 95% CI 1.2–1.8).		(128)
52 812 railroad workers, 4 194 cases, US	Semi-quantitative estimates of DEP (based on locomotive emission data)	Increased lung cancer mortality (SMR 1.8, 95% CI 1.5–2.1) for employees hired after introduction of diesel engines; indication of increased risk with years of employment (0–15-year lag); no relation to estimated exposure intensity.		(206)
54 973 railroad workers, 4 351 cases, US	Job title and years of work	Increased lung cancer mortality (SMR 1.4, 95% CI 1.3–1.5) (5-year lag); no relation to years of employment.		(114)
55 407 railroad workers, 1 694 cases, US	Exposed vs non-exposed (based on job titles)	Increasing lung cancer risk with years of exposure (RR 1.7, 95% CI 1.3–2.3) for ≥ 15 years of exposure.		(116)

Table 19. Cohort studies on lung cancer risk related to diesel exhaust exposure [adapted mainly from DFG (82)].

Study population	Exposure characterisation	Results	Comments	Reference
43 826 railroad workers, 933 cases, Canada	Probably, possibly and non-exposed (based on job titles)	Increasing lung cancer risk with increasing exposure ($p < 0.001$).		(163)
8 391 railroad workers, 236 cases, Finland	Job title and years of work	No increase in lung cancer risk (RR 0.86, 95% CI 0.75–0.97); no relation to years of exposure.		(294)
34 156 heavy equipment operators, 309 cases, US	Semi-quantitative (based on proximity of diesel exhaust source)	No increase in lung cancer risk of the whole cohort (SMR 1.0, 95% CI 0.88–1.1); increased risk among retired subjects (SMR 1.6, 95% CI 1.4–1.9, 155 cases); no relation to estimated exposure.		(462)
8 490 transport maintenance workers, 102 cases, UK	Job title	No increase in lung cancer risk (SMR 1.0, 95% CI 0.82–1.2); borderline-significant increase for hand workers (SMR 1.3, 95% CI 1.0–1.7).		(358)
6 071 dock workers, 86 cases, Sweden	Job title	Increased lung cancer risk (RR 1.7, 95% CI 1.4–2.1).		(132)
4 849 road workers, 54 cases, US	Job title	No increase in trachea, bronchus and lung cancer mortality (SMR 0.69, 95% CI 0.52–0.90).		(34)
9 738 truck drivers, 613 heavy equipment operators and 1 828 railroad workers; 18, 5 and 14 cases, US	Job title	Significant increase in lung cancer risk for heavy equipment operators (RR 2.6, 95% CI 1.1–6.1); non-significant increase for truck drivers and railroad workers (RR 1.2, 95% CI 0.77–2.0 and RR 1.6, 95% CI 0.94–2.7), respectively.	Few cases. Adjusted for smoking.	(48)
1 190 231 economically active Finns in population census 1970, 30 137 cases, Finland	Retrospective estimates of diesel derived NO_2 (FINJEM)	No increase in lung cancer risk (men: RR 0.99, 95% CI 0.96–1.0; women: RR 1.2, 95% CI 0.85–1.7); no indication of dose-response (10-year lag).	Adjusted for smoking quartz and asbestos exposure, and socio-economic status.	(130)
Workers in population censuses 1960 and 1970, 5 944 cases, Sweden	Semiquantitative (JEM)	Increasing lung cancer risk with increasing intensity of exposure in men (low: RR 0.95, 95% CI 0.90–1.0; medium: RR 1.1, 95% CI 1.1–1.2; high: RR 1.3, 95% CI 1.3–1.4).		(45)

[a] CI calculated by IARC (167).
CI: confidence interval, DEP: diesel exhaust particles, EC: elemental carbon, JEM: job exposure matrix, RR: relative risk, SMR: standard mortality ratio, TC: total carbon, UK: United Kingdom, US: United States.

Table 20. Case-control studies on lung cancer risk related to diesel exhaust exposure [adapted mainly from DFG (82)].

Study population (cases/controls)	Exposure characterisation	Results	Comments	Reference
Non-metal miners (198/562), US	Retrospective estimates of respirable EC	Increasing lung cancer risk with increasing exposure (OR 2.8, 95% CI 1.3–6.3 for the group with highest exposure in comparison with the group with lowest exposure; 15-year lag), and with years of employment.	Adjusted for smoking.	(382)
Potash miners (68/349), Germany	Retrospective estimates of respirable EC	No association between cumulative exposure and lung cancer risk.	Adjusted for smoking. High exposure levels among controls.	(259)
Trucking industry workers (994/1 085), US	Retrospective estimates of EC	Increasing lung cancer risk with increasing cumulative exposure ($p < 0.05$); significant increase for the highest quartile (5-year lag) (OR 1.6, 95% CI 1.1–2.5).	Adjusted for smoking.	(389)
Trucking industry workers (994/1 085), US	Title of job held longest	Non-significant increase in lung cancer risk for long-haul truck drivers (OR 1.3, 95% CI 0.83–1.9); increasing with years of work ($p < 0.05$) (OR 1.6, 95% CI 1.0–2.5 for ≥ 18 years of work, 213 cases).	Adjusted for smoking and asbestos exposure.	(390)
Professional drivers (2 251/2 251), Denmark	Job title and years of work	Increased lung cancer risk for taxi drivers (OR 1.6, 95% CI 1.2– 2.2, 277 cases), unspecified drivers (OR 1.4, 95% CI 1.3–1.5, 1 002 cases) and truck/bus drivers (OR 1.3, 95% CI 1.2–1.5) increasing risk with increasing years of employment ($p < 0.001$) (10-years lag).	Adjusted for socioeconomic status (surrogate for smoking).	(139)
Bus drivers and maintenance workers (17/102), Sweden	Semi-quantitative (based on tasks and duration of work)	Non-significant increase in lung cancer risk, increasing with increasing exposure.	Few cases. Adjusted for asbestos exposure.	(134)
Dock workers (50/154), Sweden	Semi-quantitative (based on diesel fuel consumption)	Increased lung cancer risk in the highest exposure group (OR 6.8, 90% CI 1.3–35, 19 cases).	Few cases per group. Adjusted for smoking.	(100)
Railroad workers (1 256/2 385), US	Job title and years of work	Increased lung cancer risk for ≥ 20 years of work in diesel-exposed occupation (OR 1.4, 95% CI 1.1–1.9).	Adjusted for smoking and asbestos exposure.	(115)

Table 20. Case-control studies on lung cancer risk related to diesel exhaust exposure [adapted mainly from DFG (82)].

Study population (cases/controls)	Exposure characterisation	Results	Comments	Reference
General population (≥ 40 y, male) (1 681/2 053), Canada	Semi-quantitative (JEM)	Non-significant increase in lung cancer risk, increasing with cumulative exposure ($p < 0.05$)	Adjusted for smoking, exposure to second hand smoke, silica and asbestos (significant increase observed prior to adjustment).	(456)
General population (male) (1 593/1 427), Canada	Semi-quantitative (JEM)	Increased lung cancer risk for ever exposed (OR 1.3, 95% CI 1.1–1.7) (5-year lag); increasing with exposure intensity and cumulative exposure (OR 1.8, 95% CI 1.3–2.6 for group with high cumulative exposure, 142 cases); no relation to years of exposure.	Adjusted for smoking, exposure to asbestos, crystalline silica, cadmium, chromium(VI) and nickel, and socioeconomic status. Study population included that of Parent *et al*. (310).	(330)
General population (male) (857/533 + 1 349 controls with other cancers), Canada	Semi-quantitative (JEM)	Non-significant increase in lung cancer risk (OR 1.2, 95% CI 0.8–1.8 for any exposure; OR 1.6, 95% CI 0.8–2.8 for substantial exposure); no excess risk in comparison with controls with other cancers.	Adjusted for smoking, asbestos and crystalline silica exposure and socioeconomic status.	(310)
General population (≤ 79 y) (595/845), Italy	Semi-quantitative (JEM)	No increase in lung cancer risk (OR 1.0, 95% CI 0.79–1.4); no indication of dose-response relationship.	Adjusted for smoking and occupational exposure to confirmed lung carcinogens.	(346)
General population (40–79 y) (1 042/2 364), Sweden	Retrospective estimates of diesel derived NO_2	Increasing lung cancer risk with increasing cumulative exposure, significant for the highest quartile (RR 1.6, 95% CI 1.1–2.3).	Adjusted for smoking and exposure to other combustion products and asbestos.	(133)
General population (1 004/1 004), Germany	Job description and years of work	Increased lung cancer risk for truck drivers (OR 1.5, 95% CI 1.2–1.9, 396 cases); increasing with years of work (OR 1.7, 95% CI 1.3–2.3 for > 10 years of work, 203 cases).	Adjusted for smoking and asbestos exposure.	(182)

Table 20. Case-control studies on lung cancer risk related to diesel exhaust exposure [adapted mainly from DFG (82)].

Study population (cases/controls)	Exposure characterisation	Results	Comments	Reference
General population (male) (3 498/3 541), Germany	Exposed vs non-exposed (based on job description)	Increased lung cancer risk (OR 1.4, 95% CI 1.2–1.7); most pronounced for heavy equipment operators (OR 2.3, 95% CI 1.4–3.7, 81 cases).	Adjusted for smoking and asbestos exposure.	(55)
General population (male) (1 260/2 084), France	Job title	Increased lung cancer risk for heavy equipment operators (RR 1.4, 95% CI 1.1–1.8, 157 cases) including professional drivers (RR 1.2, 95% CI 1.1–1.9, 128 cases).	Adjusted for smoking and alcohol consumption.	(35)
General population (2 584/5 099), US	Probable, possible and low probability (based on job titles)	No increase in lung cancer risk (OR 0.95, 95% CI 0.78–1.2, 210 cases) for probably occupationally exposed).	Adjusted for smoking and asbestos exposure.	(46)
General population (589/1 035), Sweden	Job title	No increase in lung cancer risk for professional drivers (OR ~ 1.0, 109 cases).	Adjusted for smoking.	(79)
General population (502/502), US	Exposed vs non-exposed (based on job title)	Non-significant increase in lung cancer risk of professional drivers, railroad workers and heavy equipment mechanics (OR 1.4, 95% CI 0.8–2.4, 45 cases).	Adjusted for smoking.	(135)
General population (2 291/2 570), US	Job title and years of work	Increased lung cancer risk for truck drivers with ≥ 10 years of work (OR 1.5, 95% CI 1.1, 2.0, 112 cases).	Adjusted for smoking.	(145)
General population (3 792/1 966), US	Job title	Increased lung cancer risk for truck drivers (OR 2.5, 95% CI 1.1–4.4, 121 cases).	Adjusted for smoking. Controls with colon cancer.	(401)
General population (172/281), UK	Semi-quantitative (based on job titles in death-certificates)	Non-significant increase in lung cancer risk (OR 1.3, 95% CI 1.0–1.6 for all exposed; OR 1.1, 95% CI 0.7–1.8 for the high-exposure group, 32 cases).		(71)

CI: confidence interval, EC: elemental carbon, OR: odds ratio, RR: relative risk, UK: United Kingdom, US: United States.

11. Dose-effect and dose-response relationships

Based on the data reviewed in Chapters 9 and 10, the critical effects of inhalation exposure to diesel exhaust are considered to be pulmonary inflammation and lung cancer. These effects have been demonstrated in both humans and animals. In addition, cardiovascular effects have been reported at slightly higher exposure levels. Dose-response data related to these effects are reviewed in Sections 11.1–11.3. Other effects indicated in the studies are discussed in Section 11.4.

11.1 Pulmonary effects

Animal data

Mild epithelial hyperplasia in terminal bronchioles, alveolar ducts and adjacent alveoli, mild periacinar fibrotic lesions, occasional accumulation of alveolar macrophages and mild progressive decrease in pulmonary function were observed in rats exposed by inhalation to diesel exhaust from a US 2007 compliant heavy-duty diesel engine at 4.2 ppm NO_2 (12 µg DEP/m^3) for 130 weeks (16 hours/day, 5 days/week) (247). Corresponding but slightly milder effects were reported in the same study for rats exposed at 3.6 ppm NO_2 (13 µg DEP/m^3) for 13 weeks (246). No histopathological changes were detected at 130 weeks of exposure at ≤ 0.9 ppm NO_2 (≤ 5 µg DEP/m^3) or at 13 weeks of exposure at ≤ 1.0 ppm NO_2 (≤ 4 µg DEP/m^3) (246, 247).

For comparison, an inhalation study with pure NO_2 indicated no exposure-related changes in rats (25/group) exposed at ≤ 2.2 ppm NO_2 for 13 weeks (6 hours/day, 5 days/week) (25) [as reviewed in (83)]. In a 5-day preliminary study, bronchoalveolar hyperplasia, mononuclear cell infiltration, alveolar histiocytes, and hyperplasia of the tracheal epithelium occurred at ≥ 5 ppm NO_2 (26) [as reviewed in (83)].

In an inhalation study applying a diesel engine without exhaust after-treatment system, no histopathological changes or changes in inflammatory markers in BAL were observed in rats exposed at ≤ 300 µg DEP/m^3 (≤ 0.8 ppm NO_2) for 26 weeks (6 hours/day, 7 days weeks). Accumulation of alveolar macrophages and an increased level of lactate dehydrogenase in BAL were detected at 1 000 µg DEP/m^3 (4.0 ppm NO_2) (343, 374). In another study, mild alveolar type II cell hyperplasia was observed in rats exposed to untreated diesel exhaust at 210 µg DEP/m^3 (0.2 ppm NO_2) for 104 weeks (16 hours/day, 6 days/week). The histopathological changes were more pronounced at ≥ 1 100 µg DEP/m^3 (≥ 1.0 ppm NO_2), including type II cell hyperplasia, Clara-cell hyperplasia and shortening of tracheal and bronchial cilia. Increased neutrophil and decreased macrophage counts in BAL were also detected at ≥ 1 100 µg DEP/m^3 (≥ 1.0 ppm NO_2). Animals exposed to a corresponding filtered exhaust at 1.1 ppm NO_2 (10 µg DEP/m^3) showed mild to moderate Clara-cell hyperplasia and shortening of cilia, and a small increase in neutrophil count in BAL without histopathological changes in the alveoli (171, 186).

In earlier studies, originating mainly from the 1980s, inflammatory and histopathological lung effects were consistently detected in rats exposed to diesel exhaust at ≥ 750–1 100 µg DEP/m^3 (≥ 0.3–0.7 ppm NO_2) for 65–130 weeks (Table 11). In these studies, no lung lesions were reported at exposures below 460 µg DEP/m^3 (0.3 ppm NO_2).

Human data

Dose-response data from single exposure studies in healthy human volunteers are summarised in Table 21. Increased numbers of neutrophils and inflammatory cytokines in bronchial wash (28, 29, 391) and slightly increased airway resistance (265, 391) were observed in healthy volunteers exposed to exhaust from diesel engines without exhaust after-treatment at ~ 100 µg DEP/m^3 (0.2–0.4 ppm NO_2) for 2 hours, indicating that exposure to diesel exhaust causes an acute bronchial inflammatory response in healthy subjects. An increase in lymphocyte count in BAL was observed in one study at 108 µg DEP/m^3 (0.2 ppm NO_2) (391). In three other studies applying similar exposure levels (28, 29, 265), no indications of alveolar inflammation were detected.

In asthmatic volunteers, increased bronchial hyperresponsiveness and a decline in FEV_1 were observed after 1 hour of exposure at 300 µg DEP/m^3 (0.2–1.2 ppm NO_2) (164, 296). These effects were absent at ~ 100 µg DEP/m^3 (≤ 0.4 ppm NO_2) (28, 348, 391).

Table 21. Pulmonary findings in controlled chamber studies in healthy human volunteers exposed to whole diesel exhaust for 1–2 hours.

Effect	Outcome [a]	Exposure level (µg DEP/m^3)	Reference
Lower airway inflammation	–	100	(28, 29)
(increase of inflammatory cells in	–	108	(265)
BAL)	+	108	(391)
	+	270	(375)
	+	300	(365)
Upper airway inflammation (increase	+	100	(28, 29)
of inflammatory cells in bronchial	–	108	(265)
wash or sputum)	+	108	(391)
	–	270	(375)
	+	300	(365)
Increased airway resistance	+	108	(265, 391)
(measured by plethysmography)			
Decreased dynamic lung function	–	108	(265, 391)
(measured by spirometry)	–	270	(375)
	–	276	(466)
	–	300	(365)

[a] Statistically significant difference (+) or no significant difference (–) in comparison with exposure to filtered air.

BAL: bronchoalveolar lavage, DEP: diesel exhaust particles.

11.2 Carcinogenicity

Diesel exhaust have caused lung tumours in several animal studies in which rats have been exposed to whole diesel exhaust at concentrations ≥ 2 200 µg DEP/m^3 for 104–130 weeks (51, 147, 173, 241, 273, 394). Filtered (particle-free) diesel exhaust or whole diesel exhaust at levels ≤ 800 µg DEP/m^3 has not resulted in cancer formation in animals. This suggests an association between the particulate fraction and cancer formation.

Epidemiological studies have shown an association between diesel exhaust exposure and lung cancer in humans. Risk ratios have generally been 1.3–1.6, but higher risks have been reported in some studies in the highest exposure groups (Section 10.5.1, Tables 19–20).

The mechanisms of diesel exhaust related lung cancer are likely to be multi-factorial. Lung overload may play a significant role in cancer development seen at high doses in rats. Since the relevance of lung overload at occupationally relevant exposure levels in humans is unclear, the use of these high-dose rat studies for human risk assessment is questionable.

DEP have been shown to induce genotoxicity (DNA strand breaks, DNA adducts, oxidative DNA damage and mutations) *in vivo* and *in vitro*. In addition to the genotoxicity caused by mutagens bound to DEP (e.g. PAHs and PAH derivatives) or present in the gas phase, DEP-related chronic inflammation, oxidative stress and induction of ROS may contribute to the cancer risk observed in humans. Although it can be hypothesised that the dose-response curve of diesel exhaust related cancer may include a non-linear component, it is not possible to identify a threshold level for the carcinogenicity of diesel exhaust.

Based on a log-linear meta-regression model, Vermeulen *et al*. estimated that 45 years of occupational exposure to diesel exhaust at 1, 10 and 25 µg EC/m^3 result in 17, 200 and 689 excess lung cancer deaths per 10 000, respectively, by the age of 80 years (439).

11.3 Cardiovascular effects

Transient changes in heart rate and heart rate variability have been observed in healthy rats and mice after 1–4 hours exposure to diesel exhaust at ~ 500 µg DEP/m^3 (< 0.5–1.1 ppm NO_2, 4.3–19 ppm CO) (4, 52, 208). The effect was absent with particle-free exhaust (208).

In atherosclerotic mice, an increase in aortic lipid peroxides and macrophage accumulation in atherosclerotic plaques was observed after 7 weeks (6 hours/days, 7 days/week) of exposure to diesel exhaust at ≥ 300 µg DEP/m^3 (≥ 10 ppm CO). No significant effects were observed at 109 µg DEP/m^3 (3.6 ppm CO). Increased lipid peroxidation without macrophage accumulation was detected after exposure to filtered exhaust (31 ppm CO) (60). Bai *et al*. reported changes in atherosclerotic plaque composition characteristic of unstable plaques in atherosclerotic mice exposed to diesel exhaust at 200 µg DEP/m^3 for 7 weeks (6 hours/days, 7 days/week) (16).

Studies on spontaneously hypertensive, heart-failure prone rats and atherosclerotic mice suggest that both untreated and particle-free diesel exhaust may affect cardiac electrophysiology, although no clear-cut dose-response data are available for these effects (59, 61, 62, 208).

In studies on human volunteers, a reduced response to vasodilators was observed in healthy subjects after 1–2 hours exposure to diesel exhaust at 250–350 µg DEP/m^3 (0.2–1.6 ppm NO_2, 3.5–7.5 ppm CO) (23, 230, 255, 257, 418). The effect was absent when DEP was removed from the exhaust (230, 255). Peretz *et al*. reported decreased brachial artery diameter in healthy subjects and subjects with metabolic syndrome after 2 hours of exposure to diesel exhaust at 100 or 200 µg DEP/m^3 (~ 0.02 ppm NO_2, < 1 ppm CO). The effect was statistically significant at 200 µg DEP/m^3 (327). Tong *et al*. observed a borderline significant decrease in brachial artery diameter in healthy volunteers after a 2-hour exposure at 300 µg DEP/m^3 (2.2 ppm NO_2, 6.9 ppm CO) but not at ≤ 214 µg DEP/m^3 (≤ 1.7 ppm NO_2, ≤ 5.4 ppm CO) (409). A transient increase in arterial stiffness was detected in healthy volunteers after 1 hour of exposure at 330 µg DEP/m^3 (0.6 ppm NO_2, 3.1 ppm CO) (232).

Increased diastolic or systolic blood pressure was observed in healthy volunteers exposed for 2 hours to unfiltered diesel exhaust at 300–350 µg DEP/m^3 (0.2–2.2 ppm NO_2, 3.5–6.9 ppm CO) (255, 409) or to a corresponding filtered exhaust (6 µg DEP/m^3, 0.2 ppm NO_2, 3.2 ppm CO) (255). No effect on blood pressure was observed in volunteers exposed to 200 µg DEP/m^3 for 2 hours or to 250–330 µg DEP/m^3 for 1 hour (23, 232, 272, 327, 409, 418).

In subjects with stable coronary artery disease, an increase in ST-segment depression and ischaemic burden was observed during a 1-hour exposure to diesel exhaust at 300 µg DEP/m^3 (1.0 ppm NO_2, 2.9 ppm CO) (256). In subjects exposed to pure CO, an increase in ischaemic burden has been detected at COHb levels corresponding to 14–17 ppm CO and above [reviewed in (395)].

11.4 Other effects

11.4.1 Irritation

No animal studies on irritative effects of diesel engine exhaust were located. Healthy human volunteers exposed to diesel exhaust at 108 µg DEP/m^3 (0.2 ppm NO_2, 0.04 mg/m^3 formaldehyde) for 2 hours reported unpleasant smell (21/25), low-level nasal (14/25) and throat irritation (11/25) and mild eye irritation (6/25) (265). In another study, perceived exposure, rather than true exposure, was associated with self-reported nose and eye symptoms at 100 or 200 µg DEP/m^3 (≤ 0.05 ppm NO_2, ≤ 0.04 mg/m^3 formaldehyde) (65). Redness, secretion and swelling in the nose and in the eyes were reported at ~ 300 µg DEP/m^3 (1.3 ppm NO_2, 0.4 mg/m^3 formaldehyde) (452).

11.4.2 Neurological effects

Female mice exposed to diesel exhaust at 122 µg DEP/m^3 (0.5 ppm NO_2, 2.8 ppm CO) for 13 weeks exhibited a reduced learning performance in the Morris water

maze test. A non-significant reduction of performance was observed at 35 µg DEP/m^3 (0.2 ppm NO_2, 1.1 ppm CO). Mice exposed to particle-free exhaust showed no difference as compared to the control group (459). No effect on learning performance was observed in male mice exposed to diesel exhaust at 149 µg DEP/m^3 (0.5 ppm NO_2, 3.3 ppm CO) for 4 weeks (458).

An increase in the levels of inflammatory cytokines in different regions of the brain, without a constant pattern, was indicated in three inhalation studies on rats (119, 210, 211). Levesque *et al*. detected elevated levels of TNF-α in the midbrain of rats exposed to untreated diesel exhaust at ≥ 100 µg DEP/m^3 (≥ 0.7 ppm NO_2, ≥ 3.6 ppm CO) for 26 weeks. Elevated levels of IL-1β in the midbrain and TNF-α in the frontal and temporal lobe and the olfactory bulb were detected at 992 µg DEP/m^3 (6.9 ppm NO_2, 31 ppm CO). No impact on inflammatory cytokines in any regions of the brain was indicated at 35 µg DEP/m^3 (0.3 ppm NO_2, 1.5 ppm CO) (210).

In the only available human study, increased activity of the left frontal cortex was observed in healthy volunteers during and after a 1-hour exposure to untreated diesel exhaust at ~ 300 µg DEP/m^3 (1.6 ppm NO_2, 7.5 ppm CO) (77).

11.4.3 Immunological effects

In animal inhalation studies, concurrent exposure to diesel exhaust is associated with enhancement of the respiratory response to allergens (Table 10). An exacerbation in ovalbumin-induced lung inflammation was observed in mice concurrently exposed for 8 weeks to diesel exhaust at 169 µg DEP/m^3 (0.5 ppm NO_2) or a corresponding concentration of particle-free exhaust. No significant increase in the response was detected at 36 µg DEP/m^3 (0.2 ppm NO_2). An increase in the level of ovalbumin-specific IgE in serum was indicated for particle-free exhaust only (405). No impact on ovalbumin-induced lung inflammation was detected in mice exposed at 103 µg DEP/m^3 (2.2 ppm NO_2) for 12 weeks (237).

In studies on human volunteers, nasal challenge with 300 µg of DEP prior to or concurrent with an allergen was reported to enhance sensitisation and the allergic response (27, 85, 88). No significant impact on immunological markers in sputum or impact on the response to an accompanying challenge with cat allergen was, however, observed in mildly asthmatic subjects exposed to diesel exhaust by inhalation at 100 µg DEP/m^3 (0.4 ppm NO_2) for 2 hours (348).

11.4.4 Reproductive and developmental effects

A few animal inhalation studies indicate an impact of diesel exhaust exposure on sperm production and testicular morphology (Table 14). A dose-dependent decrease in daily sperm production and an increase in the percentage of degenerative seminiferous tubules were reported in mice exposed to untreated diesel exhaust at ≥ 300 µg DEP/m^3 (≥ 3.8 ppm CO) for 26 weeks (472). No changes in sperm count or testicular morphology were detected in rats exposed to concentrations up to 3 000 µg DEP/m^3 (5.2 ppm NO_2, 14 ppm CO) for 35 weeks (414). Decreased daily sperm production was, however, reported in newborn rats exposed for 13

weeks to diesel exhaust at 5 630 µg DEP/m^3 (4.1 ppm NO_2) or to a corresponding particle-free exhaust (433). An impact of maternal exposure to diesel exhaust during gestation on the reproductive function of male pups is also suggested in a few animal studies (Table 15).

Decreased foetal weight and post-natal weight gain were reported in the offspring of mice exposed prior to mating or during gestation to untreated diesel exhaust at 3 000 µg DEP/m^3 (4.2–11 ppm NO_2, ~ 14 ppm CO) (107, 414). No impact on weight gain was observed at ≤ 1 000 µg DEP/m^3 (1.7–4.6 ppm NO_2, ~ 6.1 ppm CO) (107, 415). Impaired motor coordination and activity and enhanced allergic response have been reported in some studies (Table 15).

There are some epidemiological studies indicating that traffic-related air pollution is associated with preterm births, reduced birth weight and increased post-neonatal mortality [reviewed in (335, 377, 387, 393)]. Although diesel exhaust contributes to ambient air pollution in particular at traffic-intensive urban sites, other contributing emissions may play a role as well. Thus, it is not possible to conclude on the potential risk of diesel exhaust during pregnancy on the basis of these studies.

12. Previous evaluations by national and international bodies

12.1 Diesel engine exhaust

US National Toxicology Program (NTP) 2014
According to NTP, exposure to DEP is reasonably anticipated to be carcinogenic to humans, based on limited evidence of carcinogenicity from studies in humans and supporting evidence from mechanistic studies and studies in experimental animals (297).

International Agency for Research on Cancer (IARC) 2012
In 2012, IARC classified diesel exhaust as carcinogenic to humans (Group 1). The re-evaluation was mainly based on recent epidemiological studies supporting a causal association between diesel exhaust exposure and lung cancer. A positive association was also suggested between diesel exhaust exposure and bladder cancer. IARC concluded that there is strong evidence for the ability of whole diesel exhaust to induce cancer in humans through genotoxicity. The evidence for the carcinogenicity of gas-phase diesel exhaust was considered inadequate (33, 167).

Deutsche Forschungsgemeinschaft (DFG) 2008
The MAK (Maximale Arbeitsplatzkonzentration) Commission placed diesel exhaust into carcinogen category 2. Category 2 contains substances that are considered to be carcinogenic to humans but for which adequate epidemiological evidence of a correlation between exposure and occurrence of cancer is not available. The MAK Commission also stated that epidemiological studies indicate

an association between diesel exhaust exposure and asthma, and that animal and human studies indicate an adjuvant allergen effect. The evaluation was stated to relate to pre-2000 diesel motor emissions. Reproductive and developmental toxicity was not included in the evaluation (82).

Swedish Criteria Group for Occupational Standards 2003
The committee concluded that the critical effects of exposure to diesel exhaust are irritation and inflammation of the respiratory passages. This was based on slight inflammatory reactions observed in healthy subjects exposed for 2 hours to diesel exhaust at about 0.1 mg/m^3 DEP and 0.4 mg/m^3 NO_2, and irritation in healthy subjects and increased bronchial reactivity in asthmatics exposed 1 hour at about 0.3 mg/m^3 DEP and 2 mg/m^3 NO_2. Moreover, based primarily on epidemiological studies, it was stated that occupational exposure to diesel exhaust can increase the risk of lung cancer (261).

US Environmental Protection Agency (US EPA) 2002
According to US EPA (423), diesel exhaust is likely to be carcinogenic to humans by inhalation. The conclusion was based on the association between diesel exhaust exposure and increased lung cancer risk observed in epidemiological studies and supporting evidence such as data on mutagenic and chromosomal effects of diesel exhaust and its constituents. Furthermore, diesel exhaust was judged to pose a chronic non-cancer respiratory hazard to humans. Based on limited human and animal data, short-term exposure to diesel exhaust was concluded to cause sensory irritation and respiratory and neurophysiological symptoms. Also, some evidence for an immunologic effect of diesel exhaust was reported. A reference concentration (RfC) of 5 µg DEP/m^3 for life-time inhalation exposure was based on the no observed adverse effect level (NOAEL) of 460 µg DEP/m^3 for inflammatory lung effects in rats after chronic exposure to exhaust from a heavy-duty diesel engine (173), estimated to correspond to a human equivalent concentration of 114 µg DEP/m^3.

World Health Organization/International Programme on Chemical Safety (IPCS/WHO) 1996
WHO/IPCS concluded (448) that diesel exhaust is probably carcinogenic to humans. Chronic alveolar inflammation, impaired lung clearance and hyperplastic lung lesions were identified as critical non-cancer endpoints of long-term animal studies. For the non-cancer health effects, a guidance value of 5.6 µg DEP/m^3 (corresponding to 2.3 µg DEP/m^3 when applying default uncertainty factors instead of a dosimetric conversion) was given for the life-time exposure of the general population. The value was based on the NOAEL of 410 µg DEP/m^3 for inflammatory lung effects in rats after chronic exposure to exhaust from a light-duty diesel engine (172, 173). For lung cancer, risk estimates ranging from 1.6×10^{-5} to 7.1×10^{-5} per 1 µg DEP/m^3 (geometric mean 3.4×10^{-5}) were derived from four carcinogenicity studies with rats (51, 147, 173, 241).

12.2 Nitrogen dioxide

EU Scientific Committee on Occupational Exposure Limits (SCOEL) 2014
SCOEL recommended a limit value for NO_2 of 0.5 ppm (time-weighted average over 8 hours) (373) based mainly on epidemiological data on the lung function of hard-coal miners exposed to diesel exhaust (263). For short-term exposure, a limit value of 1 ppm (15 min) was suggested (373).

Deutsche Forschungsgemeinschaft (DFG) 2008
The MAK Commission set a limit value of 0.5 ppm for short-term exposure to NO_2 (83). The value was primarily based on data from (sub-)chronic animal inhalation studies showing a NOAEL of approximately 2 ppm (25) and on short-term inhalation studies on volunteers, showing inflammatory changes in BAL at 1.5 and 2 ppm, which were minimal and/or inconsistent at 0.6 ppm [e.g. (106)].

13. Evaluation of human health risks

13.1 Assessment of health risks

In the past years, there has been a significant evolution of the diesel engine and exhaust after-treatment technologies, including introduction of diesel oxidation catalysts and particulate filters. These changes in diesel technology have resulted in changes in the emissions and composition of the exhaust, in particular a significant reduction of the emitted mass of DEP. However, it will take a long time before the older technology diesel engines present at workplaces have been replaced by new technology engines. Also, for small non-road engines the emission regulations and thereby technological requirements are less tight.

Most of the data available on the health effects of diesel exhaust are related to older technology diesel engine models from the 1950s to the early 2000s. Table 22 summarises the key experimental data on health effects and dose-response relationships of diesel exhaust from new technology and older technology diesel engines.

13.1.1 Older technology diesel engine exhaust
In humans, single exposures to diesel exhaust at 100–300 µg DEP/m^3 resulted in an increase in markers of pulmonary inflammation (28, 29, 365, 375, 391) and slightly increased airway resistance (265, 391). Single exposures to diesel exhaust at 250–300 µg DEP/m^3 have been shown to impair vascular function in healthy subjects and to increase the ischaemic burden in subjects with coronary heart disease (23, 230, 255, 257, 418). No impact on vascular function was detected with filtered (particle-free) exhaust (230, 255). There are also human studies associating single exposure to older technology diesel engine exhaust with sensory irritation (65, 265, 356, 452), adjuvant allergenic effects (27, 85, 88) and increased bronchial hyperresponsiveness in asthmatics (164, 296).

Epidemiological studies associate exposure to exhaust from older technology diesel engines with an increased lung cancer risk (Tables 19–20). Based on a meta-analysis of three epidemiological studies by a log-linear meta-regression model, 45 years of occupational exposure to diesel exhaust at 1, 10 and 25 µg EC/m^3 was estimated to result in 17, 200 and 689 extra lung cancer deaths per 10 000 individuals, respectively, by the age of 80 years (439).

Long-term inhalation studies in rats have shown increased occurrence and severity of lung inflammatory and histopathological changes with increasing exposure, ranging from mild alveolar septal cell hyperplasia at 210 µg DEP/m^3 (171, 186) to fibrotic lesions at ≥ 750 µg DEP/m^3 (147, 172, 173, 183) and lung tumours at ≥ 2 200 µg DEP/m^3 (51, 147, 173, 241, 273, 394). In studies applying susceptible animal models, diesel exhaust exposure has been associated with exacerbation of atherosclerosis (16, 60) and changes in cardiac function (59, 61, 62, 208). There are also animal inhalation studies associating inhalation exposure to older technology diesel engine exhaust with adjuvant allergenic effects (Table 10), neuroinflammatory effects (Table 9) and effects on the male reproductive function (Tables 14–15). Developmental effects, such as decreased foetal weight gain, impaired motor coordination and enhanced response to allergens, have been indicated in some animal studies, mainly at high exposure levels (Table 15).

13.1.2 New technology diesel engine exhaust

No human studies related to the health effects of new technology diesel engine exhaust were identified.

In the only animal long-term (130 weeks) inhalation study, mild bronchoalveolar epithelial hyperplasia and mild fibrotic lesions, without indications of tumour development, and a mild progressive decrease in pulmonary function were detected in rats exposed to exhaust with 4.2 ppm NO_2 (12 µg DEP/m^3) from a diesel engine compliant with the US 2007 emission standards. The functional impairment was greater in the smallest airways which is consistent with the morphological changes likewise observed in the smallest airways (247). Corresponding but slightly milder effects were reported in the same study for rats exposed at 3.6 ppm NO_2 (13 µg DEP/m^3) for 13 weeks. The findings were largely associated with NO_2 (246).

Since the emissions of DEP and DEP-associated genotoxic compounds of new technology diesel engines are significantly lower than those of the older technology diesel engines, the cancer risk (per kWh) is expected to be reduced with the new diesel technology. The long-term inhalation study in rats with new technology diesel engine exhaust gave no indication of tumour development (247). Short- and long-term *in vivo* genotoxicity studies with new technology diesel engine exhaust have also shown negative results (31, 32, 136, 137).

There are only limited data available on cardiovascular effects of exhaust from new technology diesel engines. However, on grounds of the decrease in the DEP emissions and no evident increase in the emissions of cardiotoxic gas phase

Table 22. Key experimental data on the health effects and dose-response relationships of diesel exhaust.

Endpoint and type of study	New technology diesel engines with exhaust after-treatment [a]	Older technology diesel engines	
		with particle filter/trap	without exhaust after-treatment
Human inhalation (1–2 h)			
Inflammatory changes in BAL/ BW, increased airway resistance	No data identified	No data identified [b]	LOAEL: 100 µg DEP/m^3 (0.2–0.4 ppm NO_2)
Sensory irritation	No data identified	No data identified [b]	LOAEL: 100–300 µg DEP/m^3 (~1.3 ppm NO_2)
Reduced response to vaso-dilators	No data identified	NOAEL: 3.4 ppm NO_2 (7 µg DEP/m^3)	LOAEL: 250–350 µg DEP/m^3 (0.2–1.6 ppm NO_2)
Increased ischaemic burden	No data identified	No data identified	LOAEL: 300 µg DEP/m^3 (1.0 ppm NO_2) [c]
Animal inhalation			
Histopathological changes in the lungs (104–130 wk, rat)	NOAEL: 0.9 ppm NO_2 (5 µg DEP/m^3) LOAEL: 4.2 ppm NO_2 (12 µg DEP/m^3) [d]	LOAEL: 1.1 ppm NO_2 (10 µg DEP/m^3)	LOAEL: 210 µg DEP/m^3 (0.2 ppm NO_2)
Mild decrease in pulmonary function (104–130 wk, rat)	NOAEL: 0.9 ppm NO_2 (5 µg DEP/m^3) LOAEL: 4.2 ppm NO_2 (12 µg DEP/m^3) [d]	No data identified	NOAEL: 2 000 µg DEP/m^3 (1.5 ppm NO_2) LOAEL: 3 500 µg DEP/m^3 (0.3 ppm NO_2)
Lung tumours (104–130 wk, rat)	NOAEL: 4.2 ppm NO_2 (12 µg DEP/m^3)	No lung tumours (original exposure level 6 600 µg DEP/m^3, no data on final exposure levels)	NOAEL: 800–1 000 µg DEP/m^3 (0.3 ppm NO_2) LOAEL: 2 200 µg DEP/m^3 (~1 ppm NO_2)
DNA damage in the lungs	Negative (comet)	No data identified	Positive (induction of 8-OHdG, *gpt* and *lacI* point mutations, DNA strand breaks and adducts)
Systemic genotoxicity	Negative (8-OHdG, micronuclei)	No data identified	Mostly negative
In vitro			
Genotoxicity	No data identified	Mutagenic to bacteria (limited data)	Mutagenic to bacteria and mammalian cells (DEP extracts)

[a] US 2007 compliant heavy-duty engine, [b] Rudell *et al*. (355, 356) were not included since a considerable amount of particles was present in the filtered exhaust, [c] Stable coronary heart artery diseased, [d] Corresponding but slightly milder effects seen in the same study after 13 weeks exposure at 3.6 ppm NO_2 (13 µg DEP/m^3).
BAL: bronchoalveolar lavage, BW: bronchial wash, DEP: diesel exhaust particles, LOAEL: lowest observed adverse effect level, NOAEL: no observed adverse effect level, NO_2: nitrogen dioxide, 8-OHdG: 8-hydroxydeoxyguanosine.

compounds, e.g. CO, the risk of cardiovascular effects (per kWh) is expected to be lower with new technology diesel engines.

There are, however, still open questions related e.g. to the potential role of the nucleation mode (nanosized) particles, which contribute very little to the DEP mass, on the carcinogenicity and other health effects of diesel exhaust.

13.2 Groups at extra risk

Subjects with chronic respiratory or cardiovascular diseases are likely to be specifically sensitive to the health impacts of diesel exhaust exposure. Data from animal and human studies indicate that exposure to diesel exhaust may exacerbate pre-existing cardiovascular disease, especially coronary artery disease (Sections 9.2.3, 9.4.2 and 10.2.2). Exposure to diesel exhaust is also suggested to exacerbate respiratory disorders, including asthma (Sections 10.2.1 and 10.3).

13.3 Scientific basis for an occupational exposure limit

Diesel engine exhaust is a complex mixture of gaseous and particulate constituents. The composition of the exhaust varies, depending, e.g. on the type, age and operational condition of the engine and on the exhaust after-treatment systems applied. In comparison with pre-2005 diesel engines and the less tightly regulated small non-road engines, exhaust from new technology diesel engines is characterised by a significantly reduced particulate mass, reduction of particle associated EC, reduction of organic compounds in the particulate and gas phase, and an increased proportion of NO_2 of the total NO_X. An occupational exposure limit value for diesel exhaust should be intended to cover the varying exhaust composition.

The critical health effects of diesel exhaust are pulmonary inflammation and lung cancer. For older technology diesel engines, these effects are mainly associated with the particulate fraction of the exhaust, making DEP a good candidate for an exposure indicator. As it is challenging to distinguish between DEP and other respirable dust at a workplace, respirable EC may be applied as a marker for DEP. EC constitutes typically ~ 75% of the DEP mass of the older technology heavy-duty diesel engines (Section 2.2), which is the fraction used below to estimate the EC exposure levels in the critical studies. For new technology diesel engine exhaust with significantly reduced DEP and EC mass concentrations, EC may not be an equally useful exposure indicator. NO_2 is likely to be a more relevant exposure indicator for new technology diesel engine exhaust. Since the age and type of engines and exhaust after-treatment systems applied vary within and between workplaces, it may be appropriate to set an occupational exposure limit value for diesel exhaust both as respirable EC and as NO_2. Both of these values should be fulfilled at a workplace where diesel engines are applied.

In inhalation studies on human volunteers, applying exhaust from older technology diesel engines, slight increases in airway resistance and pulmonary inflammatory markers were observed after single exposures at 100 µg DEP/m^3

(~ 75 µg EC/m^3, 0.2–0.4 ppm NO_2). This was the lowest exposure level applied in these studies and represents the overall lowest observed adverse effect level (LOAEL) for pulmonary inflammatory effects of older technology diesel engine exhaust. Also mild sensory irritation was reported at 100–300 µg DEP/m^3 (~ 1.3 ppm NO_2). In long-term animal inhalation studies, inflammatory and histopathological changes in the lungs have been detected in rats at 210 µg DEP/m^3 or above (~ 160 µg EC/m^3, 0.2 ppm NO_2). Rats exposed to filtered exhaust at 1.1 ppm NO_2 (10 µg DEP/m^3) showed mild bronchial hyperplasia and shortening of cilia. No NOAELs were identified.

In a long-term (130 weeks) inhalation study in rats applying exhaust from a new technology diesel engine, mild alveolar and bronchial epithelial hyperplasia, mild fibrotic lesions, and a mild progressive decrease in pulmonary function mainly in the smallest airways consistent with the morphological changes were observed at 4.2 ppm NO_2 (12 µg DEP/m^3, ~ 3 µg EC/m^3), determined to be the LOAEL of this study. Corresponding but slightly milder effects were reported in the same study for rats exposed at 3.6 ppm NO_2 (13 µg DEP/m^3) for 13 weeks. The findings were largely associated with NO_2. No histopathological changes were detected after a 130-week exposure at ≤ 0.9 ppm NO_2 (5 µg DEP/m^3, ~ 1 µg EC/m^3) or a 13-week exposure at ≤ 1.0 ppm NO_2 (≤ 4 µg DEP/m^3) leading to a NOAEL of 0.9 ppm NO_2.

Epidemiological studies associate exposure to older technology diesel engine exhaust with increased lung cancer risk. In long-term animal inhalation studies applying exhaust from older technology diesel engines, development of lung tumours has been observed in rats at 2 200 µg DEP/m^3 (~1 650 µg EC/m^3) and above. In the only identified long-term animal inhalation study applying new technology diesel engine exhaust, no indication of tumour development was detected in rats at the highest exposure level tested (4.2 ppm NO_2, 12 µg DEP/m^3, ~ 3 µg EC/m^3). However, since it is not possible to exclude a genotoxic mode of action, it is currently not possible to identify a threshold level for the carcinogenicity of diesel exhaust. Based on a meta-analysis of three epidemiological studies by a log-linear meta-regression model, 45 years of occupational exposure to diesel exhaust at 1, 10 and 25 µg EC/m^3 was estimated to result in 17, 200 and 689 extra lung cancer deaths per 10 000, respectively, by the age of 80 years.

Although data allowing a direct comparison of the carcinogenic potential of the DEP emitted by new technology and older technology diesel engines are not available, the significant reduction of the DEP mass concentration in exhaust from new technology diesel engines is expected to reduce the lung cancer risk (per kWh). This is supported by the findings from a single set of animal studies showing reduced or negligible *in vivo* lung genotoxicity and oxidative DNA damage after inhalation exposure to diesel exhaust from new technology diesel engines.

14. Research needs

Numerous studies have been published on the health effects of diesel exhaust. However, except for one set of animal inhalation studies (the ACES programme), all of the identified studies applied diesel engines from the 1950s to the early 2000s. As the tightened emission regulations have caused a significant change in the diesel technology and the composition of diesel exhaust in the past years, corresponding studies on the health effects of the exhaust from new technology diesel engines are needed. Studies allowing for comparison of older and new technology diesel engine exhaust with respect to different endpoints, including genotoxicity and inflammatory effects, would be of specific interest. In addition, updating the epidemiological studies in the forthcoming decades would be necessary for assessing the potential changes in the cancer risk. Determination of relevant exposure indicators for new technology diesel engine exhaust, including consideration of the particle size distribution and different particle exposure metrics (e.g. number vs mass concentration) would be valuable. In addition, it is important to compare the hazard per mass unit of DEP from new and older technology diesel engines. Further information would also be needed on exposure levels at workplaces where new diesel engines are in use.

15. Summary

Taxell P, Santonen T. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals* and *the Dutch Expert Committee on Occupational Safety.* 149. Diesel engine exhaust. Arbete och Hälsa 2016;49(6):1–147.

Diesel engine exhaust is a complex mixture of gaseous and particulate compounds produced during the combustion of diesel fuels. The gas phase includes carbon dioxide, nitrogen oxides (NO_X), carbon monoxide and small amounts of sulphur dioxide and various organic compounds. Diesel exhaust particles (DEP) contain elemental carbon (EC), organic compounds, sulphates, nitrates and trace amounts of metals and other elements. New technology diesel engines are characterised by a significant reduction of the DEP mass emissions. Occupational exposure to diesel exhaust occurs in mining, construction work, professional driving, agriculture and other activities where diesel-powered vehicles and tools are applied.

The critical health effects of diesel exhaust are considered to be pulmonary inflammation and lung cancer. For older technology diesel engines, pulmonary inflammatory responses were observed in human volunteers after single exposure at 100 µg DEP/m^3 (~ 75 µg EC/m^3), and in rats after long-term exposure at 210 µg DEP/m^3 (~ 160 µg EC/m^3). Development of lung tumours was seen in rats at 2 200 µg DEP/m^3 (~ 1 650 µg EC/m^3). For new technology diesel engines, pulmonary inflammatory changes were reported in rats after 13 and 130 weeks of exposure at 3.6 and 4.2 ppm NO_2 (12–13 µg DEP/m^3, ~ 3 µg EC/m^3). The effect was absent at 0.9–1.0 ppm NO_2 (4–5 µg DEP/m^3, ~ 1 µg EC/m^3). No indication of tumour development was detected.

Epidemiological studies associate occupational exposure to exhaust from older technology diesel engines with increased lung cancer risk. Based on a log-linear meta-regression model, 45 years of occupational exposure to diesel exhaust at 1, 10 and 25 µg EC/m^3 was estimated to result in 17, 200 and 689 extra lung cancer deaths per 10 000 individuals, respectively, by the age of 80 years. Although data allowing a direct comparison of the carcinogenic potential of exhaust from new and older technology diesel engines are not available, the significant reduction of the DEP mass concentration in the new technology diesel engine exhaust is expected to reduce the lung cancer risk (per kWh).

In addition to the critical effects, human and animal inhalation studies associate exposure to older technology diesel engine exhaust with sensory irritation, increased airway resistance, cardiovascular effects, genotoxicity and adjuvant allergenic effects. There are also animal studies indicating neuroinflammatory effects, developmental effects and effects on the male reproductive function.

When evaluating the health risk of diesel exhausts it is important to take into account that the transition from “old” to “new” technology diesel engines is expected to take a long time.

Keywords: cancer, cardiovascular, diesel engine, diesel exhaust, elemental carbon, inflammation, nitrogen dioxide, occupational exposure limit, particles, pulmonary, review, risk assessment, toxicity.

16. Summary in Swedish

Taxell P, Santonen T. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals* and *the Dutch Expert Committee on Occupational Safety.* 149. Diesel engine exhaust. Arbete och Hälsa 2016;49(6):1–147.

Dieselmotoravgaser är en komplex blandning av ämnen i ångform och partikelform som bildas vid förbränning av diesel. I gasfasen återfinns koldioxid, kvävedioxider (NO_X), kolmonoxid, små mängder svaveldioxid och organiska ämnen. Partikelfasen (DEP) innehåller elementärt kol (EC), organiska ämnen, sulfater, nitrater och spårmängder av andra ämnen t.ex. metaller. Nya dieselmotorer ger betydligt mindre utsläpp av partiklar (uttryckt som massa). Yrkesmässig exponering för dieselavgaser förekommer i gruvor, vid byggnadsarbete, bland yrkeschaufförer, i jordbruk och andra aktiviteter där dieseldrivna fordon och verktyg används.

De kritiska hälsoeffekterna av dieselavgaser är inflammatoriska förändringar i lungorna samt lungcancer. När det gäller avgaser från äldre dieselmotorer sågs inflammatoriska förändringar i lungorna hos frivilliga försökspersoner efter en enstaka exponering för 100 µg DEP/m^3 (~ 75 µg EC/m^3) och hos råttor efter långtidsexponering för 210 µg DEP/m^3 (~ 160 µg EC/m^3). Utveckling av lungtumörer sågs hos råttor vid 2 200 µg DEP/m^3 (~ 1 650 µg EC/m^3). Avgaser från nya dieselmotorer orsakade inflammatoriska effekter i lungorna hos råttor efter 13 och 130 veckors exponering för 3.6 och 4.2 ppm NO_2 (12–13 µg DEP/m^3, ~ 3 µg EC/m^3). Effekterna sågs inte vid 0.9–1.0 ppm NO_2 (4–5 µg DEP/m^3, ~ 1 µg EC/m^3). Man såg heller inga tecken på tumörutveckling.

I epidemiologiska studier har man sett ett samband mellan yrkesmässig exponering för avgaser från äldre dieselmotorer och en ökad risk för lungcancer. Baserat på en log-linjär meta-regressionsmodell uppskattades att 45 års yrkesexponering för dieselavgaser vid 1, 10 and 25 µg EC/m^3 orsakar 17, 200 respektive 689 extra lungcancerfall per 10 000 individer, fram till 80 års ålder. Det finns i dagsläget inte data för att jämföra den cancerframkallande potentialen hos avgaser från nya och äldre dieselmotorer. Den kraftiga minskningen av halten DEP i avgaser (uttryckt som masskoncentration) från nya dieselmotorer gör att lungcancerrisken (per kWh) förväntas sjunka.

Utöver de kritiska effekterna har exponering för avgaser från äldre dieselmotorer satts i samband med irritation, ökat luftvägsmotstånd, hjärt-kärlsjuklighet, genotoxicitet och allergiska adjuvanteffekter i studier på både djur och människa. Det finns även djurstudier som pekar på neuroinflammatoriska effekter, utvecklingsstörningar och effekter på reproduktionsförmågan hos handjur.

Vid utvärdering av hälsoeffekterna av dieselavgaser är det viktigt att beakta att övergången från äldre till nya dieselmotorer förväntas ta lång tid.

Nyckelord: cancer, dieselmotor, dieselavgaser, elementärt kol, hjärt-kärlsjukdom, hygieniskt gränsvärde, inflammation, kvävedioxid, lungor, partiklar, riskbedömning, toxicitet, översikt.

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18. Data bases used in search of literature

In the search for literature the following data bases were used:
Medline
Toxline
Google Scholar

The last search was performed in June 2015.

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Appendix 1. Occupational exposure limits

Occupational exposure limits (8-hour TWAs) for diesel exhaust in different countries.

Country	Particles, resp. ($\mu g/m^3$)		EC, resp. ($\mu g/m^3$)	TC ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)	Ref.
Austria	100, 300 [a]	400 [b], 1 200 [a, b]	-	-	-	-	(1)
Sweden [c]	-	-	-	-	1	20	(2)
Switzerland	-	-	100	-	-	-	(3)
US (MSHA)	-	-	-	160 [a]	-	-	(4)

[a] Underground mining.
[b] Short-term exposure limit (STEL, 15-min TWA).
[c] General occupational exposure limit for exhaust gas.
EC: elemental carbon. CO: carbon monoxide. MSHA: Mine Safety and Health Administration. NO_2: nitrogen dioxide. Resp.: respirable fraction. TC: total carbon. TWA: time weighted average.

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Appendix 2. The committees

The following experts participated in the elaboration of the document:

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Anne Thoustrup Saber	National Research Centre for the Working Environment, Denmark
Vidar Skaug	National Institute of Occupational Health, Norway
Helene Stockmann-Juvala	Finnish Institute of Occupational Health
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IMCM Rietjens	Wageningen University and Research Centre, Wageningen
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FGM Russel	Radboud University Medical Centre, Nijmegen
GMH Swaen	Maastricht University, Maastricht
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JJAM Hendrix, *advisor*	Social and Economic Council, The Hague
JM Rijnkels, *scientific secretary*	Health Council of the Netherlands, The Hague

Appendix 3. Previous NEG criteria documents

NEG documents published in the scientific serial Arbete och Hälsa (Work and Health).

Substance/agent	Arbete och Hälsa issue
Acetonitrile	1989:22, 1989:37*
Acid aerosols, inorganic	1992:33, 1993:1*
Acrylonitrile	1985:4
Allyl alcohol	1986:8
Aluminium and aluminium compounds	1992:45, 1993:1*, 2011;45(7)*D
Ammonia	1986:31, 2005:13*
Antimony	1998:11*
Arsenic, inorganic	1981:22, 1991:9, 1991:50*
Arsine	1986:41
Asbestos	1982:29
Benomyl	1984:28
Benzene	1981:11
1,2,3-Benzotriazole	2000:24*D
Boric acid, Borax	1980:13
1,3-Butadiene	1994:36*, 1994:42
1-Butanol	1980:20
γ-Butyrolactone	2004:7*D
Cadmium	1981:29, 1992:26, 1993:1*
7/8 Carbon chain aliphatic monoketones	1990:2*D
Carbon monoxide	1980:8, 2012;46(7)*
Carbon nanotubes	2013;47(5)*
Ceramic Fibres, Refractory	1996:30*, 1998:20
Chlorine, Chlorine dioxide	1980:6
Chloromequat chloride	1984:36
4-Chloro-2-methylphenoxy acetic acid	1981:14
Chlorophenols	1984:46
Chlorotrimethylsilane	2002:2
Chromium	1979:33
Cobalt	1982:16, 1994:39*, 1994:42
Copper	1980:21
Creosote	1988:13, 1988:33*
Cyanoacrylates	1995:25*, 1995:27
Cyclic acid anhydrides	2004:15*D
Cyclohexanone, Cyclopentanone	1985:42
n-Decane	1987:25, 1987:40*
Deodorized kerosene	1985:24
Diacetone alcohol	1989:4, 1989:37*
Dichlorobenzenes	1998:4*, 1998:20
Diesel exhaust	1993:34, 1993:35*
Diethylamine	1994:23*, 1994:42
2-Diethylaminoethanol	1994:25*N
Diethylenetriamine	1994:23*, 1994:42
Diisocyanates	1979:34, 1985:19

NEG documents published in the scientific serial Arbete och Hälsa (Work and Health).

Substance/agent	Arbete och Hälsa issue
Dimethylamine	1994:23*, 1994:42
Dimethyldithiocarbamates	1990:26, 1991:2*
Dimethylethylamine	1991:26, 1991:50*
Dimethylformamide	1983:28
Dimethylsulfoxide	1991:37, 1991:50*
Dioxane	1982:6
Endotoxins	2011;45(4)*D
Enzymes, industrial	1994:28*, 1994:42
Epichlorohydrin	1981:10
Ethyl acetate	1990:35*
Ethylbenzene	1986:19
Ethylenediamine	1994:23*, 1994:42
Ethylenebisdithiocarbamates and Ethylenethiourea	1993:24, 1993:35*
Ethylene glycol	1980:14
Ethylene glycol monoalkyl ethers	1985:34
Ethylene oxide	1982:7
Ethyl ether	1992:30* N
2-Ethylhexanoic acid	1994:31*, 1994:42
Flour dust	1996:27*, 1998:20
Formaldehyde	1978:21, 1982:27, 2003:11*D
Fungal spores	2006:21*
Furfuryl alcohol	1984:24
Gasoline	1984:7
Glutaraldehyde	1997:20*D, 1998:20
Glyoxal	1995:2*, 1995:27
Halothane	1984:17
n-Hexane	1980:19, 1986:20
Hydrazine, Hydrazine salts	1985:6
Hydrogen fluoride	1983:7
Hydrogen sulphide	1982:31, 2001:14*D
Hydroquinone	1989:15, 1989:37*
Industrial enzymes	1994:28*
Isoflurane, sevoflurane and desflurane	2009;43(9)*
Isophorone	1991:14, 1991:50*
Isopropanol	1980:18
Lead, inorganic	1979:24, 1992:43, 1993:1*
Limonene	1993:14, 1993:35*
Lithium and lithium compounds	2002:16*
Manganese	1982:10
Mercury, inorganic	1985:20
Methacrylates	1983:21
Methanol	1984:41
Methyl bromide	1987:18, 1987:40*
Methyl chloride	1992:27*D
Methyl chloroform	1981:12

NEG documents published in the scientific serial Arbete och Hälsa (Work and Health).

Substance/agent	Arbete och Hälsa issue
Methylcyclopentadienyl manganese tricarbonyl	1982:10
Methylene chloride	1979:15, 1987:29, 1987:40*
Methyl ethyl ketone	1983:25
Methyl formate	1989:29, 1989:37*
Methyl isobutyl ketone	1988:20, 1988:33*
Methyl methacrylate	1991:36*D
N-Methyl-2-pyrrolidone	1994:40*, 1994:42
Methyl-tert-butyl ether	1994:22*D
Microbial volatile organic compounds (MVOCs)	2006:13*
Microorganisms	1991:44, 1991:50*
Mineral fibers	1981:26
Nickel	1981:28, 1995:26*, 1995:27
Nitrilotriacetic acid	1989:16, 1989:37*
Nitroalkanes	1988:29, 1988:33*
Nitrogen oxides	1983:28
N-Nitroso compounds	1990:33, 1991:2*
Nitrous oxide	1982:20
Occupational exposure to chemicals and hearing impairment	2010;44(4)*
Oil mist	1985:13
Organic acid anhydrides	1990:48, 1991:2*
Ozone	1986:28
Paper dust	1989:30, 1989:37*
Penicillins	2004:6*
Permethrin	1982:22
Petrol	1984:7
Phenol	1984:33
Phosphate triesters with flame retardant properties	2010;44(6)*
Phthalate esters	1982:12
Platinum	1997:14*D, 1998:20
Polychlorinated biphenyls (PCBs)	2012;46(1)*
Polyethylene,	1998:12*
Polypropylene, Thermal degradation products in the processing of plastics	1998:12*
Polystyrene, Thermal degradation products in the processing of plastics	1998:12*
Polyvinylchloride, Thermal degradation products in the processing of plastics	1998:12*
Polytetrafluoroethylene, Thermal degradation products in the processing of plastics	1998:12*
Propene	1995:7*, 1995:27
Propylene glycol	1983:27
Propylene glycol ethers and their acetates	1990:32*N
Propylene oxide	1985:23
Refined petroleum solvents	1982:21
Refractory Ceramic Fibres	1996:30*
Selenium	1992:35, 1993:1*

NEG documents published in the scientific serial Arbete och Hälsa (Work and Health).

Substance/agent	Arbete och Hälsa issue
Silica, crystalline	1993:2, 1993:35*
Styrene	1979:14, 1990:49*, 1991:2
Sulphur dioxide	1984:18
Sulphuric, hydrochloric, nitric and phosphoric acids	2009;43(7)*
Synthetic pyretroids	1982:22
Tetrachloroethane	1996:28*D
Tetrachloroethylene	1979:25, 2003:14*D
Thermal degradation products of plastics	1998:12*
Thiurams	1990:26, 1991:2*
Tin and inorganic tin compounds	2002:10*D
Toluene	1979:5, 1989:3, 1989:37*, 2000:19*
1,1,1-Trichloroethane	1981:12
Trichloroethylene	1979:13, 1991:43, 1991:50*
Triglycidyl isocyanurate	2001:18*
n-Undecane	1987:25, 1987:40*
Vanadium	1982:18
Vinyl acetate	1988:26, 1988:33*
Vinyl chloride	1986:17
Welding gases and fumes	1990:28, 1991:2*
White spirit	1986:1
Wood dust	1987:36
Xylene	1979:35
Zinc	1981:13

* in English, remaining documents are in a Scandinavian language.
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