National Research Centre
for the Working Environment

Diesel exhaust particles:

Scientific basis for setting a health-based occupational exposure limit

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DIESEL EXHAUST PARTICLES: SCIENTIFIC BASIS FOR SETTING A HEALTH-BASED OCCUPATIONAL EXPOSURE LIMIT

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FOREWORD

In 2012, the International Agency for Research on Carcinogenicity (IARC) classified diesel engine exhaust as carcinogenic to humans (Group 1) (IARC 2014). In 2014, the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) and the Dutch Expert Committee on Occupational Safety (DECOS) co-produced a criteria document on diesel engine exhaust (Taxell and Santonen 2016). NEG criteria documents are used by the regulatory authorities of the Nordic countries as the scientific basis for setting occupational exposure limits (OELs) for chemical substances. However, NEG does not suggest OELs.

On this background and at request of the Danish Working Environment Authority, a working group at the National Research Centre for the Working Environment (NFA) has evaluated the possibility to establish a health-based OEL for diesel engine exhaust particles.

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The working group wishes to thank Chief Toxicologist Poul Bo Larsen, DHI, Denmark, for reviewing the report.

Copenhagen, December 2018

EXECUTIVE SUMMARY

In this report, a working group at the National Research Centre for the Working Environment reviewed data relevant to assessing the hazard of diesel exhaust particles (DEPs), i.e. human studies (Chapter 2), toxicokinetics (Chapter 3), animal studies (Chapter 4), mechanisms of toxicity (Chapter 5), previous risk assessments of DEPs (Chapter 6), scientific basis for setting an occupational exposure limit (OEL) (Chapter 7) and finally we summarize and suggest a health based OEL for DEPs (Chapter 8). The focus of this report is only occupational exposure by inhalation.

Diesel is used as fuel for engines in vehicles for transport and power supply. Occupational exposure to diesel exhaust occurs in many different sites, including transportation, construction, railroad and mining industries (IARC 2014). Diesel exhaust consists of a particulate phase (carbon particles with adsorbed organic matter) and a gas/vapor phase which include volatile organic compounds, nitrogen oxides and carbon oxides.

In 2012, the International Agency for Research on Carcinogenicity (IARC) classified diesel engine exhaust as carcinogenic to humans (Group 1). IARC concluded that there is sufficient evidence that diesel engine exhaust is causally related to lung cancer. Additionally, a positive association has been observed between exposure to diesel engine exhaust and increased risk of bladder cancer. Furthermore, IARC found sufficient evidence for carcinogenicity of whole diesel engine exhaust (DEE), DEE particulate matter and extracts of diesel exhaust particles (DEPs) in experimental animals. IARC found inadequate evidence for the carcinogenicity of gas-phase DEE (i.e. particle free DEE) (IARC 2014). This shows that the carcinogenic effect of DEE is driven by (DEPs). Therefore, the present report focusses on the particulate phase of the DEE, the DEPs.

The present working group evaluated the relevant literature on DEPs from both epidemiological and animal inhalation studies. However, since data regarding dose-dependent effects (especially on cancer) following inhalation of DEE in humans are available, data from animal studies are primarily included to strengthen the conclusions drawn upon the human data and to allow comparison of health effects caused by other types of particles.

Endpoints were evaluated based on reported adverse effects of diesel exhaust exposure in reports and in the scientific literature. Especially the recent monography by IARC (IARC 2014) and the criteria document by NEG/DECOS (Taxell and Santonen 2016) are used as basis for the present report. The assessment by IARC evaluates cancer evidence (IARC 2014). NEG/DECOS evaluates all health effects. Based on the evaluation, NEG/DECOS regards lung cancer and pulmonary inflammation as the critical effects and the present working group will therefore include both these endpoints.
A meta-regression of lung cancer mortality and cumulative exposure to elemental carbon (EC)(as a proxy measure of DEPs) estimated the numbers of excess lung cancer deaths for 45 years of occupational exposures of 1, 10, and 25 μg/m^3 EC to be 17, 200, and 689 per 10,000, respectively, by 80 years of age.

Pulmonary inflammation and carcinogenicity was observed in sub-chronic and chronic inhalation studies in rats. Dose-response relationships were observed for inflammation following inhalation of DEE and DEPs and instillation of DEPs in rodents. The working group considers inflammation as a threshold effect.

The present working group found that there is evidence that both the extractable organic fraction and the particulate fraction of DEP contribute to the carcinogenicity of DEP. The molecular mechanisms include formation of bulky DNA adducts as well as oxidative DNA damage likely induced by surface-dependent reactive oxygen species (ROS) generation. In addition, inhalation of DEE and DEPs induced dose-dependent pulmonary inflammation which could cause secondary genotoxicity. Thus, the available data indicated induction of cancer through both direct and indirect genotoxic mechanisms. Based on the observed mechanisms of genotoxicity, the present working group concludes that DEP-induced mutagenicity and carcinogenicity occur by non-threshold mechanisms.

The present working group identified a recent meta-analysis as suitable for risk assessment (Vermeulen et al. 2014b). Five high quality chronic inhalation studies in rats were identified, and the present working group decided also to select two of these for calculation of excess cancer risk: A 2-year chronic cancer inhalation study in rats with relatively low tumor incidence (0, 2.5 and 7 mg/m^3) (Heinrich et al. 1995) and another 2-year chronic inhalation study in rats with a relatively high tumor incidence (0.7, 2.2 and 6.6 mg/m^3) (Brightwell et al. 1989) . In the table below excess lung cancer risk at 1 in 1 000, 1 in 10 000, and 1 in 100 000 using different approaches is presented.

	Suggestion of an OEL for DEP calculated as elemental carbon			
Excess lung cancer incidence	EC levels Meta-analysis of Human studies (Vermeulen)	Rat inhalation study of DEE* Method I, Lung burden (Heinrich)	Rat inhalation study of DEE* Method II ECHA** (Heinrich)	Rat inhalation study of DEE* Method II, ECHA** (Brightwell)
1:1 000	0.45 μg/m^3	5.6 μg/m^3	56 μg/m^3	15 μg/m^3
1: 10 000	0.045 μg/m^3	0.56 μg/m^3	5.6 μg/m^3	1.5 μg/m^3
1: 100 000	0.0045 μg/m^3	0.056 μg/m^3	0.56 μg/m^3	0.15 μg/m^3

***) For traditional diesel engine particles, it is assumed that 75% of the mass is elemental carbon (Taxell and Santonen 2016)**
****) European Chemicals Agency**

Three different approaches were used for calculating excess lung cancer risk. First, lung cancer risk was estimated based on the meta-analysis of epidemiological studies of the association between exposure to DEE and lung cancer. Secondly, lung cancer risk was estimated using two different approaches based on the same chronic inhalation study (Heinrich et al. 1995). In the first approach, lung burden in rats after two years of exposure was used to estimate the exposure limits for occupational exposure. In the

second approach, air concentrations were used directly. Thirdly, lung cancer incidence was estimated based on a second chronic inhalation study in rats (Brightwell et al. 1989). Independently of the applied method for risk assessment, the resulting exposure limits were all very low.

The DEE exposure in the epidemiological studies was traditional DEE. Both of the chronic inhalation studies were performed on traditional DEE. Typically, the proportion of elemental carbon from a traditional heavy-duty diesel engine is 75% of the total particle emission while this proportion is reduced to 13% when using "new technology" diesel engines. Correspondingly, the proportions of sulfates are increased from 1% to 53% when exhaust after-treatment systems are applied (Taxell and Santonen 2016). The present working group notes that there is limited available data on the biological effects of DEP from "new technology" diesel engines and that the DEP concentrations in the performed chronic inhalations studies with new technology engines in rats and mice were likely too low to induce detectable levels of cancer.

The present working group notes that in chronic inhalation studies in rats, carbon black nanoparticles and DEP have very similar carcinogenic potential (Heinrich et al. 1995). Furthermore, the present working group notes that there is limited available data regarding carcinogenicity of "new technology" DEE and there is no available evidence suggesting that new technology DEP are less carcinogenic than DEP and carbon black (CB).

The present working group notes that the risk estimates allowing 1: 10 000 excess lung cancer cases or less are all close to the current ambient air concentrations of EC (ca. 0.4 $\mu g/m^3$ EC for rural measurements in Denmark (Massling et al. 2011) and 2.7 $\mu g/m^3$ EC levels on a major street in Copenhagen, Denmark (Palmgren et al. 2003).

The present working group recommends the approach using the epidemiological data to derive OELs, since this approach relies on data from humans. Thus, the expected excess lung cancer risk based on epidemiological data is 1: 1 000 at 0.45 $\mu g/m^3$, 1: 10 000 at 0.05 $\mu g/m^3$ and 1: 100 000 at 0.005 $\mu g/m^3$ DEPs.

DANSK SAMMENFATNING

I denne rapport vurderer en arbejdsgruppe ved Det Nationale Forskningscenter for Arbejdsmiljø data, der er relevante for at vurdere faren ved dieseludstødningspartikler, dvs. humane studier (kapitel 2), toksikokinetik (kapitel 3), dyreforsøg (kapitel 4)), toksicitetsmekanismer (kapitel 5), tidligere risikovurderinger af dieseludstødningspartikler (kapitel 6), det videnskabelige grundlag for fastsættelse af en grænseværdi for dieseludstødningspartikler i arbejdsmiljøet (kapitel 7) og endelig opsummeres og foreslås en helbredsbaseret grænseværdi for dieseludstødningspartikler i arbejdsmiljøet (kapitel 8). Fokus i denne rapport er alene på erhvervsmæssig eksponering ved indånding.

Diesel bruges som brændstof til motorer i køretøjer til transport og til strømforsyning. Erhvervsmæssig eksponering for dieseludstødning forekommer i mange forskellige brancher og erhverv, herunder ved transport, byggeri, jernbane og minedrift (IARC 2014). Dieseludstødning består af en partikelfase (carbonpartikler med adsorberet organisk stof) og en gas/dampfase, der omfatter flygtige organiske forbindelser, nitrogenoxider og carbonoxider.

I 2012 klassificerede WHO's kræftagentur (IARC) udstødning fra dieselmotorer som kræftfremkaldende for mennesker (Gruppe 1). IARC konkluderede, at der er tilstrækkelig evidens for, at udstødning fra dieselmotorer forårsager lungekræft. Derudover er der en positiv sammenhæng mellem eksponering for udstødning fra dieselmotorer og øget risiko for blærekræft. Desuden fandt IARC tilstrækkeligt bevis for kræftfremkaldende egenskaber for komplet udstødning fra dieselmotorer, udstødningspartikler fra dieselmotorer og ekstrakter af udstødningspartikler fra dieselmotorer i forsøgsdyr. IARC fandt utilstrækkelig evidens for kræftfremkaldende egenskaber af gasfasen af udstødningen (dvs. partikelfri dieselmotorudstødning) (IARC 2014). Dette viser, at den kræftfremkaldende effekt af dieselmotorers udstødning er drevet af dieselpartikler. Derfor er den nærværende rapport fokuseret på partikelfasen af dieselmotorers udstødning, dieseludstødningspartiklerne.

Den nærværende arbejdsgruppe vurderede den relevante litteratur om dieseludstødningspartikler fra både epidemiologiske studier og inhalationsstudier i dyr. Da humane data om dosisafhængige virkninger (især på kræft) efter indånding er tilgængelige, er data fra dyreforsøg primært medtaget for at styrke konklusionerne på humane data og for at muliggøre sammenligning af helbredseffekter forårsaget af andre typer af partikler.

Helbredseffekter forårsaget af udsættelse for dieseludstødning blev vurderet ud fra rapporter og videnskabelig litteratur. Især anvendes den nylige monografi fra IARC (IARC 2014) og et kriteriedokument om dieseludstødning fra Den Nordiske Ekspertgruppe for dokumentation af helbredsrisiko ved kemikalier (NEG) og Den hollandske komité for arbejdsmiljøsikkerhed (The Dutch Expert Committee on Occupational Safety (DECOS)) (Taxell og Santonen 2016) som grundlag for denne rapport. Bedømmelsen fra IARC vurderer evidens for kræft (IARC 2014). NEG/DECOS

rapporten vurderer alle sundhedseffekter. På baggrund af deres evaluering vurderer NEG/DECOS lungekræft og lungeinflammation som værende de kritiske effekter, og forfatterne til den nærværende rapport vil derfor inkludere begge disse endepunkter. En meta-regressionsanalyse af sammenhængen mellem lungekræftmortalitet og kumulativ eksponering for elementært kulstof (anvendt som en proxy for dieseludstødningspartikler) viste, at antallet af overskydende lungekræftdødsfald efter 45 års erhvervsmæssig eksponering for 1, 10 og 25 μg/m^3 elementært carbon til at være henholdsvis 17, 200 og 689 pr. 10.000, ved en alder på 80 år.

Der blev observeret lungeinflammation og kræft i subkroniske og kroniske inhalationsstudier af rotter. Dosis-respons-effekter blev observeret for inflammation efter indånding af dieseludstødning og dieseludstødningspartikler samt instillation af dieseludstødningspartikler i gnavere. Arbejdsgruppen betragter inflammation som en tærskeleffekt.

Den nærværende arbejdsgruppe konstaterede, at både den ekstraherbare organiske fraktion og partikelfraktionen af dieseludstødning bidrager til dieseludstødnings kræftfremkaldende effekt. De molekylære mekanismer inkluderer dannelse af DNA-addukter såvel som oxidative DNA-skader, der sandsynligvis induceres af overfladeafhængig ROS-generering. Desuden inducerede indånding af dieselmotor udstødning og dieseludstødningspartikler dosisafhængig lungeinflammation, som kan forårsage sekundær genotoksicitet. De foreliggende data indikerede således induktion af kræft gennem både direkte og indirekte genotoksiske mekanismer. Baseret på de observerede genotoksicitetsmekanismer konkluderer den nærværende arbejdsgruppe, at dieselpartikel-induceret mutagenicitet og kræft sker via ikke-tærskelmekanismer.

Den nærværende arbejdsgruppe identificerede en ny meta-analyse som egnet til risikovurdering (Vermeulen et al. 2014b). Desuden blev der identificeret 5 kroniske inhalationsundersøgelser hos rotter af høj kvalitet, og den nærværende arbejdsgruppe besluttede at beregne overskydende kræftrisiko ud fra data fra to af disse studier: Et 2-årigt kronisk kræftinhalationsstudie hos rotter med relativt lav tumorincidens (0, 2,5 og 7 mg/m^3) (Heinrich et al. 1995) og et andet 2-års kronisk inhalationsstudie af rotter med en relativt høj tumorincidens (0,7, 2,2 og 6,6 mg/m^3) (Brightwell et al. 1989). I tabellen præsenteres overskydende lungekræftrisiko ved 1 ud af 1 000, 1 ud af 10 000 og 1 ud af 100 000 ved anvendelse af forskellige fremgangsmåder.

	Forslag til grænseværdi for dieseludstødningspartikler beregnet som elementært carbon			
Overskydende lungekræft-incidens	Elementære carbon niveauer Meta-analyse af humane studier (Vermeulen)	Rotte-inhalationsstudie af dieseludstødning* Metode I, Lunge burden (Heinrich)	Rotte-inhalationsstudie af dieseludstødning* Metode II ECHA** (Heinrich)	Rotte-inhalationsstudie af dieseludstødning* Metode II, ECHA** (Brightwell)
1:1 000	0.45 μg/m^3	5.6 μg/m^3	56 μg/m^3	15 μg/m^3
1: 10 000	0.045 μg/m^3	0.56 μg/m^3	5.6 μg/m^3	1.5 μg/m^3
1: 100 000	0.0045 μg/m^3	0.056 μg/m^3	0.56 μg/m^3	0.15 μg/m^3

***) For partikler udledt fra traditionelle dieselmotorer antages det, at 75 % af massen er elementært carbon (Taxell and Santonen 2016)**
****) European Chemicals Agency**

Der blev anvendt tre forskellige metoder til beregning af overskydende lungekræftrisiko. Først blev risikoen for lungekræft vurderet ud fra meta-analysen af epidemiologiske undersøgelser af sammenhængen mellem eksponering for udstødning fra dieselmotorer og lungekræft. For det andet blev risikoen for lungekræft vurderet ved anvendelse af to forskellige metoder baseret på den samme kroniske inhalationsundersøgelse (Heinrich et al. 1995). Ved den første tilgang blev lungebyrden hos rotter efter to års eksponering brugt til at estimere eksponeringsgrænserne for erhvervsmæssig eksponering. Ved den anden tilgang blev luftkoncentrationerne anvendt direkte. For det tredje blev lungekræftincidensen estimeret baseret på et andet kronisk inhalationsstudie i rotter (Brightwell et al. 1989). Uafhængigt af den anvendte metode til risikovurdering, var de resulterende eksponeringsgrænser alle meget lave.

I de epidemiologiske undersøgelser var eksponeringen fra dieselmotorer baseret på ”traditionel teknologi”. Begge de kroniske inhalationsundersøgelser blev udført på udstødning fra dieselmaskiner baseret på ”traditionel teknologi”. Typisk er andelen af elementært kulstof fra en traditionel dieselmotor 75 % af den totale partikelemission, mens denne andel typisk reduceres til 13% ved anvendelse af dieselmotorer baseret på "ny teknologi". Tilsvarende øges andelen af sulfater typisk fra 1% til 53%, når udstødningsefterbehandlingssystemer anvendes (Taxell og Santonen 2016). Den nærværende arbejdsgruppe bemærker, at der er begrænsede tilgængelige data om de biologiske effekter af partikler fra dieselmotorer baseret på "ny teknologi", og at partikelkoncentrationerne i de udførte kroniske inhalationsundersøgelser i mus og rotter med ”nye teknologi”-motorer sandsynligvis var for lave til at fremkalde detekterbare kræftniveauer.

Den nærværende arbejdsgruppe bemærker, at data fra kroniske inhalationsundersøgelser i rotter viser, at carbon black nanopartikler og dieseludstødningspartiklers kræftfremkaldende potentiale er sammenlignelige (Heinrich

et al. 1995). Den nærværende arbejdsgruppe bemærker endvidere, at data for kræft og "ny teknologi" dieseludstødning er begrænset, og at der ikke foreligger nogen evidens for, at "ny teknologi" dieseludstødningspartikler er mindre kræftfremkaldende end dieseludstødningspartikler fra "traditionel teknologi" dieselmaskiner og carbon black.

Den nuværende arbejdsgruppe bemærker, at risikovurderingen, der tillader 1: 10 000 overskydende lungekræftsager eller mindre, ligger tæt på de nuværende omgivende luftkoncentrationer af EC (ca. 0,4 μg / m^3 EC til landmålinger i Danmark (Massling et al. 2011) og 2,7 μg / m^3 EC niveauer på en hovedgade i København, Danmark (Palmgren et al. 2003).

Den nærværende arbejdsgruppe anbefaler, at der til fastlæggelse af grænseværdi anvendes tilgangen, som baserer sig på de epidemiologiske data, da denne tilgang er baseret på data fra mennesker. Således er den forventede overskydende lungekræftrisiko i forbindelse med erhvervsmæssig udsættelse for dieseludstødningspartikler 1: 1 000 ved 0,45 μg/m^3, 1: 10 000 ved 0,05 μg/m^3 og 1: 100 000 ved 0,005 μg/m^3.

CONTENTS

ABBREVIATIONS

ACES	Advanced collaborative emissions study
AF	Assessment factor
BAL	Broncho alveolar lavage
CB	Carbon black
CI	Confidence interval
DECOS	The Dutch Expert Committee on Occupational Safety
DEE	Diesel engine exhaust
DEP	Diesel exhaust particles
EC	Elemental carbon
ECHA	European Chemicals Agency
HO-1	Heme oxygenase 1
HR	Hazard ratio
IARC	The International Agency for Research on Cancer
ICRP	International Commission on Radiological Protection
IL	Interleukin
IP	Intraperitoneal
LOAEC	Lowest observed adverse effect concentration
LOAEL	Lowest observed adverse effect level
NEG	The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals
NIST	National Institute of Standards and Technology
NO	Nitrogen monooxide
NO_x	Nitrogen oxide
NO_2	Nitrogen dioxide
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NFA	National Research Centre for the Working Environment
OEL	Occupational exposure limit
OR	Odd ratio
PAH	Polyaromatic hydrocarbons
ROS	Reactive oxygen species
RR	Relative risk
SE	Standard error
SRM	Standard reference materials
TiO_2	Titanium dioxide
TNF	Tumor necrosis factor
VOC	Volatile organic compounds

INTRODUCTION

Diesel is used as fuel for engines in vehicles for transport and power supply. Occupational exposure to diesel exhaust occurs in many different sites, including transportation, construction, railroad and mining industries (IARC 2014).

Diesel exhaust consists of a particulate phase (carbon particles with adsorbed organic matter) and a gas/vapor phase which include volatile organic compounds, nitrogen oxides and carbon oxides.

In 2012, the International Agency for Research on Carcinogenicity (IARC) classified diesel engine exhaust (DEE) as carcinogenic to humans (Group 1). IARC concluded that there is sufficient evidence in humans that DEE is causally related to lung cancer. Additionally, a positive association has been observed between exposure to DEE and increased risk of bladder cancer in humans. Furthermore, IARC found sufficient evidence for carcinogenicity of whole DEE, DEE particulate matter and extracts of DEPs in experimental animals. IARC found inadequate evidence for the carcinogenicity of gas-phase DEE (i.e. particle free DEE) (IARC 2014). This shows that the carcinogenic effect of DEE is driven by diesel exhaust particles (DEPs).

The present report focuses on the particulate phase of DEE and DEPs.

The first diesel-powered heavy duty diesel vehicles were introduced in the mid-19th century. These "traditional" diesel engines did not control the emission of particulate matter. Increased concern for adverse health effects related to emissions from these "traditional" diesel engines has led to the development of "new-technology" engines. These "new-technology" engines are equipped with particulate filters that reduce the emission of particulate matter considerably (more than 90% by mass)(IARC 2014;Taxell and Santonen 2016). The composition of DEPs emitted from "traditional" and "new technology" diesel engines is also different. Typically, the proportion of elemental carbon from a traditional heavy-duty diesel engine is 75% of the particle emission while this proportion is reduced to 13% when using new technology diesel engines. The proportions of sulfates and organic carbon are increased from 1% to 53% and 19% to 30%, respectively, when exhaust after-treatment systems are applied (Taxell and Santonen 2016).

Currently, "traditional" diesel engines are gradually being replaced by "new-technology" diesel engines. However, it is expected to take a long time before this transition has been completed (Taxell and Santonen 2016).

The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) and the Dutch expert Committee on Occupational Safety (DECOS) co-produced a criteria document on DEE and have compiled occupational exposure limits (OELs) for DEE levels in different countries (Taxell and Santonen 2016). These are presented in Table 1.

Table 1. Occupational exposure limits (8-h TWAs) for diesel engine exhaust in different countries (Table is adapted from (Taxell and Santonen 2016))

Country	Particles (respirable fraction) ($\mu g/m^3$)	Elemental carbon (respirable fraction) ($\mu g/m^3$)	Total carbon, Underground mining ($\mu g/m^3$)	NO_2 (ppm)	CO (ppm)
Austria	100 (15 min STEL is: 400)	-	-	-	-
Austria (Underground mining)	300 (15 min STEL is: 1200)	-	-	-	-
Sweden (general occupational exposure limit for exhaust gas)	-		-	1	20
Switzerland	-	100	-	-	-
US[a] (underground mining)	-	-	160	-	-

[a]: Mine Safety and Health Administration, STEL: Short-term exposure limit,

Health-based occupational cancer risk values for DEE calculated by the DECOS has been in public consultation.

The aim of the present report is to review the data and investigate if the present knowledge allows for a suggestion of a health-based OEL for DEPs. In this document, we review the relevant literature on the adverse effects of DEPs with the recent IARC evaluation (IARC 2014) and the recent NEG/DECOS report (Taxell and Santonen 2016) as central sources of information.

The risk assessment methodology of this report will follow the guidelines suggested by REACH (ECHA 2012b). First, threshold or non-threshold effects are determined. For an OEL based on threshold effects, the following traditional approach is made: 1) identification of critical effect, 2) identification of the no observed adverse effect concentration (NOAEC), 3) calculation of OEL using assessment factors (AFs) adjusting for inter and intra species differences. For non-threshold effects, the current working group will use two different approaches for calculating excess lung cancer risk. In the first approach, lung burden will be used to estimate the exposure levels. In the second approach, air concentrations will be used directly. Conclusively, the calculated OELs will be compared, and lastly, a recommended OEL for DEP exposure will be proposed.

HUMAN STUDIES

Human exposure

IARC recently reviewed occupational DEE exposure data:
"Exposure to diesel exhaust occurs in many different occupational settings, including the mining, railroad, construction and transport industries. The main determinants of exposure are the size, number and use of diesel engines indoors and outdoors, and the degree of ventilation. Several different markers of exposure have been used, such as elemental carbon, nitrogen oxides and polyaromatic hydrocarbons (PAHs). A generally accepted proxy for levels of exposure to diesel engine exhaust is elemental carbon, although this is not specific to diesel engine alone. Miners (in settings where diesel engines are used) and tunnel construction workers are the most highly exposed occupational groups, with average levels of exposure to elemental carbon above 100 μg/m³. Dock workers, diesel mechanics and maintenance personnel are exposed on average to levels between 20 and 40μg/m³; train crews, construction workers and workers involved in loading and unloading ships are exposed to levels of elemental carbon of around 10 μg/m³; and professional drivers are exposed on average to lower levels of around 2 μg/m³. Levels of exposure to elemental carbon vary largely within job titles, and these relative rankings can therefore vary in specific situations. Furthermore, the composition of diesel engine exhaust differs between occupational settings due to variations in use scenarios, operating conditions and engine technology"(IARC 2014).

The exposure to DEP (measured as elemental carbon) varies from 2 to more than 100 μg/m³. The present working group notes that exposure to DEP (measured as elemental carbon) varies at least 50-fold between occupational settings.

Epidemiological studies

A recent evaluation by NEG on DEE (Taxell and Santonen 2016) concluded that: *'The critical health effects of DEE are pulmonary inflammation and lung cancer.'* The present working group has therefore decided to focus on these end points as the critical effects. Furthermore, the present report is focused on the particulate phase of DEE, because IARC found sufficient evidence for carcinogenicity of whole DEE, DEE particulate matter and extracts of DEPs in experimental animals while IARC found inadequate evidence for the carcinogenicity of gas-phase DEE (IARC 2014).

Inflammation

NEG/DECOS has recently reviewed the human inhalation studies on the inflammatory effects of DEE (Taxell and Santonen 2016):

"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/m³ (~ 75 μg EC/m³, 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."

No human studies on the pulmonary effects of exhaust from "new technology" diesel engines were identified.

Controlled exposure of humans to NO_2 has been shown to result in pulmonary inflammation (HEI, 2012).

The present working group notes that pulmonary inflammation was observed in humans exposed to a single exposure of diesel exhaust containing 100 μg DEP/m^3 (~ 75 μg EC/m^3). Since this was the lowest exposure level applied, it was not possible to establish a no observed adverse effect concentration (NOAEC) for DEP-induced inflammation.

Since exposure to NO_2 may cause pulmonary inflammation in humans, it is likely that NO_2 contributes to DEE-induced pulmonary inflammation. Therefore, the present working group does not regard inflammation as a suitable DEP- induced critical effect.

Cancer

IARC evaluated the epidemiological studies on exposure to diesel exhaust and risk of cancer (IARC 2014). IARC concluded *'There is sufficient evidence in humans for the carcinogenicity of diesel engine exhaust. Diesel engine exhaust causes cancer of the lung. A positive association has been observed between exposure to diesel engine exhaust and cancer of the urinary bladder'*.

IARC found inadequate evidence for the carcinogenicity of gas-phase DEE (i.e. particle free DEE) (IARC 2014). This shows that the carcinogenic effect of diesel exhaust is driven by DEPs. The present working group therefore considers lung cancer as the relevant critical effect for diesel exhaust.

A meta-analysis of epidemiological studies was performed including studies with information regarding dose-response relationship between exposure to diesel exhaust quantified as elemental carbon and risk of lung cancer identified at the time of the IARC evaluation (Vermeulen et al. 2014b).

The meta-analysis included all the epidemiological studies on diesel exhaust exposure and lung cancer that fulfilled the following criteria: a) diesel engine exhaust exposure was expressed as cumulative elemental carbon in the exposure response analysis, b) an appropriate unexposed/low exposure reference group was used, c) no major methodological shortcomings were identified. The identified studies which were included in the IARC evaluation were one American study of non-metal miners (Attfield et al. 2012;Silverman et al. 2012) and two American studies of trucking industry workers (Garshick et al. 2012;Steenland et al. 1998). In addition, a fourth study of potash miners (Möhner et al. 2013) that was published after the IARC evaluation was identified. However, this study was not included in the meta-analysis. The reasons for this were: a) the reference group had a relatively high exposure, b) the study contributed with only 68 lung cancer cases, and c) the derivation of the elemental carbon metric was not described in detail.

The three studies were used as described in (Vermeulen et al. 2014b):
'From the nested case–control study by Steenland et al. (1998) of trucking industry workers, we used odds ratios (ORs) for cumulative elemental carbon (EC) exposure categories with a 5-year lag. Steenland et al. (1998) included 994 lung cancer deaths and 1,085 controls. All cases and controls had died in 1982–1983 and were long-term Teamsters enrolled in the pension system. Cases and controls were divided by job categories based on the longest held job. In 1988–1989, submicrometer elemental carbon (EC) was measured in 242 samples covering the major job categories in the trucking industry (Steenland et al. 1998). Estimates of past exposure to elemental carbon (EC), for participants in the epidemiologic study were made by assuming that a) average 1990 levels for a job category could be assigned to all subjects in that job category, and b) levels prior to 1990 were directly proportional to vehicle miles traveled by heavy duty trucks and the estimated emission levels of diesel engines.'

'From the cohort study of trucking industry workers by Garshick et al. (2012), we used hazard rations (HRs) for cumulative elemental carbon (EC) exposure categories with a 5-year lag based on analyses that excluded mechanics. In that study, work records were available for 31,135 male workers employed in the unionized U.S. trucking industry in 1985. Mortality was ascertained through the year 2000 and included 779 lung cancer deaths. From 2001 through 2006 a detailed exposure assessment was conducted (> 4,000 measurements) that included personal and work-area submicrometer elemental carbon (EC) measurements covering the major job categories in the trucking industry. Exposure models based on terminal location in the United States were developed. Historical trends in ambient terminal elemental carbon (EC) were modeled based on historical trends in the coefficient of haze, a measurement of visibility interference in the atmosphere. In addition to changes in ambient exposure, the historical model accounted for changes in job-related exposures based on a comparison of elemental carbon (EC) measurement data obtained in 1988 through 1989 with the newly collected elemental carbon (EC) measurements.'

'From the nested case–control miner study by Silverman et al. (2012), we used odd ratios (ORs) for cumulative elemental carbon (EC) with a 15-year lag; we chose to use risk estimates from the nested case–control study instead of estimates from the cohort analysis (Attfield et al. 2012) because of their control for confounding, particularly from smoking, in the nested case–control study. The case–control study was nested within a cohort of 12,315 workers in eight non-metal mining facilities and included 198 lung cancer deaths and 562 incidence density–sampled controls. Respirable elemental carbon (EC) was estimated for each surface and underground job from the year of introduction of diesel-powered equipment in the facilities to 31 December 1997. Between 1998 and 2001, a detailed exposure assessment was conducted measuring personal respirable elemental carbon (EC) levels (> 700 measurements) covering the majority of job titles in the facilities (Stewart et al. 2010). These estimates were back-extrapolated for underground jobs per mine based on historical carbon monoxide measurement data and diesel engine exhaust (DEE)-related determinants (e.g., diesel engine horsepower and ventilation rates).'
Study-specific categorical relative risk (RR) estimates for lung cancer mortality associated with cumulative diesel exhaust particle levels (measured as elemental carbon) relative to the lowest category of exposure for each study."

The obtained exposure-response relationships for the three individual studies and for all the studies combined are shown in table 2 (Vermeulen et al. 2014b):

Table 2. Exposure-response estimates (lnRR for a 1-μg/m³-increase in elemental carbon from individual studies and the primary combined estimate based on a log-linear model (reproduced from (Vermeulen et al. 2014b)).

Model[a]	Intercept	β (95%CI)
All studies combined	0.088	0.00098 (0.00055, 0.00141)
Silverman et al. (2012) only	–0.18	0.0012 (0.00053, 0.00187)
Steenland et al. (1998) only	–0.032	0.00096 (0.00033, 0.00159)
Garshick et al. (2012) only	0.24	0.00061 (–0.00088, 0.00210)

[a]Log-linear risk model (lnRR = intercept + β x exposure. Exposure defined as elemental carbon in μg/m³.

Thus, according to the meta-analysis, the risk of lung cancer from exposure to DEPs measured as elemental carbon can be determined by:

lnRR (lung cancer)=0.088 +0.000982 μg/m³-years EC

The slopes for the three studies included in the meta-analysis (i.e., the lnRR estimated for a 1-μg/m³-year increase in EC) were within a factor of two, and 95% confidence intervals (CIs) overlapped (Table 1). The combined slope estimate was 0.000982 (95% CI: 0.00055, 0.00141). The exposure-response curve from the meta-analysis is shown in figure 1 (Vermeulen et al. 2014b).

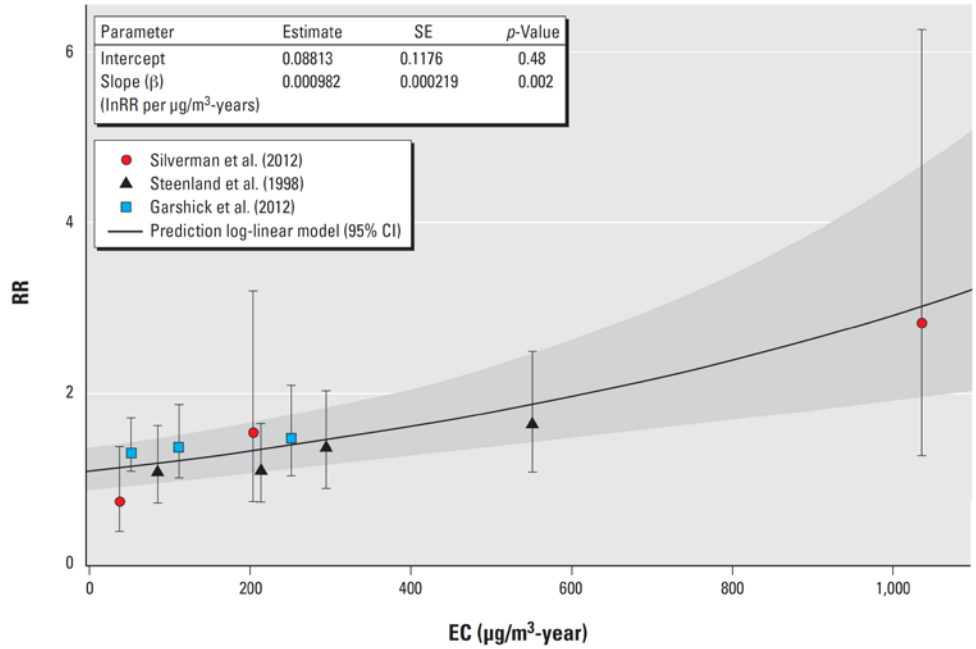

Figure 1. Predicted exposure–response curve based on a log-linear regression model using relative risk (RR) estimates from three cohort studies of DEE and lung cancer mortality. Individual RR estimates [based on hazard ratios (HRs) reported by Garshick et al. (2012) or odds ratios (ORs) reported by Silverman et al. (2012) and Steenland et al. (1998)] are plotted with their 95% CI bounds indicated by the whiskers. The shaded area indicates the 95% CI estimated based on the log-linear model. The insert presents the

estimates of the intercept and beta slope factor, the standard error (SE) of these estimates, and the associated p-values. Reproduced from (Vermeulen et al. 2014b).

The meta-analysis by Vermeulen et al. has been extensively discussed in the literature (Vermeulen and Portengen 2016;Vermeulen and Portengen 2017;Möhner and Wendt 2017;Möhner 2017a;Möhner 2017b;Vermeulen et al. 2014a) especially regarding the choice of different time lags in the three included studies. In a sensitivity analysis, different lags (0, 5, 10, 15 years) were used, the study by Möhner et al. (Möhner et al. 2013) was included or the highest dose from the study by Silverman (Silverman et al. 2012) was excluded. All these changes had limited effect on the slopes (β) of the risk estimates, which varied from 0.000608 to 0.001021. The risk estimates were further examined in another publication (Vermeulen and Portengen 2016), where the meta-analyses from the above-mentioned sensitivity analysis were further elaborated and the corresponding acceptable air concentrations of elemental carbon leading to acceptable risk (4×10^{-5} or one in 25 000 individuals) or maximum tolerated risk (4×10^{-3} or one in 250 individuals) were estimated. The exposure levels for acceptable and maximum tolerated risks were estimated to be 0.011 $\mu g/m^3$ and 1.03 $\mu g/m^3$, respectively, for the published meta-analysis, and the different estimates varied less than two-fold (Vermeulen and Portengen 2016).

The present working group considers this study by Vermeulen et al. as an important and relevant study that builds on the critical literature review performed by members of the IARC working group. Moreover, it provides information on dose-response relationship between exposure to diesel exhaust based on exposure measurements and risk of lung cancer.

The present working group furthermore notes that the studies use cohorts of workers and nested case-control designs thus minimizing the risk of potential confounding caused by population-based comparison groups. Moreover, all three studies include exposure assessment in terms of EC exposure measurements.

The present working group is of the opinion that the meta-analysis can be used for quantitative risk assessment of DEPs even though the exposure was DEE and not only DEPs. Animal exposure studies have clearly shown that DEE and DEPs are carcinogenic, whereas filtered DEE is not (IARC 2014; HEI, 2012), thus showing that it is the particulate fraction of diesel exhaust that causes lung cancer.

TOXICOKINETICS

Exposures to DEPs occur in many occupational settings; primarily via inhalation but to a lesser extent also dermal exposure and secondary ingestion occur. Focus in this section will be on inhalation, the most critical exposure pathway.

Toxicokinetics has recently been reviewed and summarized by NEG as follows: *"Upon inhalation of diesel exhaust, DEP deposition will occur throughout the respiratory tract, with a majority of the particles reaching the alveolar region (Oravisjärvi et al. 2014;EPA 2002). 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 (Rissler et al. 2012). Applying measurement data on DEP number-size distributions and the International Commission on Radiological Protection (ICRP) 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 (Oravisjärvi et al. 2014). From the tracheobronchial region, DEP is cleared by mucociliary clearance and removed into the gastrointestinal system within 24 hours (WHO 1996). 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 (EPA 2002). 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 (WHO 1996). 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 (EPA, 2002). 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."*(Taxell and Santonen 2016).

In the human experimental exposure study described in the NEG/DECOS report (Rissler et al. 2012), the deposited fraction of DEPs was 0.27-0.28. Sixty percent of the deposited DEPs are retained in the alveolar region. This equals an alveolar deposition of 16.8% (0.6*28%=16.8%). The present working group also notes that exercise may increase the alveolar deposition by 5–6-fold. Furthermore, the present working group notes that the retention half-time for insoluble particles in humans is several hundred days and longer than the clearance rate observed in rats (60-100 days).

ANIMAL STUDIES

Selection of studies and endpoints

Since data on effects (especially on cancer) following inhalation of DEPs in humans are available, data from animal studies are primarily included to strengthen the conclusions drawn upon the human data and to allow comparison of health effects caused by other types of particles. Rats are the preferred animal model in particle toxicology and are more sensitive than mice to particle-induced lung cancer and fibrosis.

IARC found sufficient evidence for the carcinogenicity of whole DEE, DEE particulate matter and extracts of DEPs in experimental animals while there was inadequate evidence for the carcinogenicity of gas-phase DEE (i.e. particle free DEE) (IARC 2014). This shows that DEPs rather than the gas-phase of the diesel exhaust are causative agents for the carcinogenic effects observed. Therefore, in the present report, the focus is on the particulate phase of the DEE.

In the present report, inhalation studies will be prioritised. For the description of toxicological endpoints and mechanism of toxicity, studies using pulmonary deposition such as intratracheal instillation will be included because intratracheal instillation studies make it possible to evaluate the effects of the particulate phase of the diesel exhaust alone. Dose-response assessments, however, are in the current report solely conducted based on sub-chronic and chronic inhalation studies.

Endpoints were evaluated based on reported adverse effects of diesel exhaust exposure in reports and in the scientific literature. Especially the recent monography by IARC (IARC 2014) and the criteria document by NEG/DECOS (Taxell and Santonen 2016) are used as basis for the present report. The assessment by IARC evaluates cancer evidence (IARC 2014). NEG/DECOS evaluates all health effects. Based on their evaluation NEG/DECOS considers lung cancer and pulmonary inflammation as the critical effects and the authors of the present report will therefore include both these endpoints.

Pulmonary inflammation

NEG/DECOS has recently reviewed the studies on inflammatory effects in rats following pulmonary deposition of DEPs (Taxell and Santonen 2016):

"In long-term animal inhalation studies, inflammatory and histopathological changes in the lungs have been detected in rats at 210 μg DEP/m³ or above (~ 160 μg EC/m³, 0.2 ppm NO_2). Rats exposed to filtered exhaust at 1.1 ppm NO_2 (10 μg DEP/m³) showed mild bronchial hyperplasia and shortening of cilia". No NOAELs were identified, as effects were observed with lowest tested dose.

"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/m³, ~ 3 μg EC/m³), 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."

There are a number of relevant diesel engine exhaust inhalation studies and intratracheal instillation studies of DEP addressing pulmonary inflammation. The studies considered most relevant are described below.

In a chronic inhalation study by Mauderly and co-workers, male and female rats were exposed to exhaust from "traditional diesel" engine by inhalation (Mauderly et al. 1994). The diesel exhaust was generated by light-duty engines burning certification fuel and operating on an urban-duty cycle .The dosage regimen was a mass concentration of 2.5 or of 6.5 mg/m^3 for 16 h/day, 5 days/week for 3, 6, 12, 18 or 24 months. The low and high concentration diesel exhaust contained 0.7 and 3.8 ppm NO_2, respectively, and 8.8 and 23 NO_x, respectively. Neutrophils were measured in bronchoalveolar lavage fluid (BAL) in lungs after 12 months of exposure. In terms of increased neutrophil numbers in BAL, the mass concentrations of 2.5 mg/m^3 and 6.5 mg/m^3 were determined to be the no observed adverse effect concentration (NOAEC) and the lowest observed adverse effect concentration (LOAEC), respectively. Other endpoints investigated in the study included additional BAL fluid endpoints, organ weight, as well as neoplastic lesions. The incidence of animals with neoplastic lesions is further described in the paragraph on cancer. Concerning other BAL fluid endpoints, lactate dehydrogenase and beta glucoronidase were increased at both dose levels. Concerning lung weight, the weight was increased for both DEP dosed groups at 18 months and at later time points. The authors of the report suggested that this reflected the inflammatory, proliferative and fibrotic lesions resulting from the exposure. Notably the relative lung weights (lung weight/body weight) were not increased.

Sub-chronic (16 h/day, 5 days/week, 13 weeks) exposure of rats to exhaust from "new technology" diesel engines (12-13 μg/m^3 DEP ~ 3μg EC/m^3, ~4 ppm NO_2) resulted in pulmonary inflammation (increase in inflammatory markers in broncheoalveolar lavage). In mice, a similar exposure resulted in increased number of neutrophils in bronchoalveolar lavage). No inflammatory effects were detected in rats exposed to a lower concentration (4-5 μg DEP /m^3 ~ 1 EC μg/m^3, 0.9-1.0 ppm NO_2) (McDonald et al. 2012).

There are also a number of relevant short-term inhalation studies with pulmonary inflammation as endpoint. Of these we consider the following to be the most important.

Campen et al. investigated the effects of diesel exhaust in mice. Mice were exposed to diesel exhaust (0.5 and 3.6 mg/m^3) for 3 days (6 h/day) in whole-body inhalation chambers with or without particulates filtered. Increased pulmonary inflammation (measured as PMNs) was detected in mice exposed at the highest concentration of whole exhaust. No effect on the number of PMNs was detected in mice exposed to filtered exhaust (Campen et al. 2005).

McDonald et al. compared lung inflammation in mice exposed by inhalation 6 h/day for 7 days to exhaust generated from a diesel engine in two different cases. In the first case, the diesel engine was operated with socalled "2003" fuel and no exhaust after-treatment was applied (234 μg DEP/m^3, 0.04 ppm NO_2). In the other case, the engine was operated with low-sulfur-fuel and a particle trap (7 μg DEP/m^3, 0.04 ppm NO_2). Otherwise, the engine was operated identically in both cases. Mice exposed to full diesel exhaust operated with "2003" fuel had increased levels of lung inflammatory markers (including tumor necrosis factor (TNF), IL-6 and HO-1). No significant effects of inflammatory markers were observed in mice exposed to filtered exhaust from engine operated with low-sulfur-fuel (McDonald et al. 2004) .

The inflammatory effect of DEP alone has also been evaluated in studies where rodents have been exposed to standard reference materials (SRM) from the US National Institute of Standards and Technology (NIST). Of these, we consider the following to be among the most important:

Short-term effects of SRM1650 DEP originating from heavy-duty diesel engines on markers of inflammation were evaluated in mice. Mice were exposed by inhalation to either a single 90 min exposure (20 or 80 mg/m^3 DEP) or as 4 repeated 90 min exposures (5 or 20 mg/m^3). Inhalation of DEP induced a dose-dependent inflammatory response with infiltration of neutrophils and elevated gene expression of IL-6 in the lungs of mice (Dybdahl et al. 2004).

The inflammatory response to the SRM1650b DEP was evaluated in mice 1, 3 and 28 days after a single intratracheal instillation of 18, 54 or 162 μg/mouse. A time- and dose-dependent inflammatory response was observed. The response had returned to baseline 28 days post-exposure (Kyjovska et al. 2015).

Exposure of rats to NO_2 has been shown to induce pulmonary inflammation (HEI, 2012).

The present working group notes that pulmonary inflammation has been detected in rats following chronic exposure to diesel exhaust at 210 μg DEP/m^3 or above (~ 160 μg EC/m^3, 0.2 ppm NO_2). Low grade inflammation was observed in mice and rats exposed to a new technology diesel engine, but the effects were ascribed to the relatively high levels of NO_2 rather than to the DEPs. The present working group further notes that the studies by Campen et al. and McDonald et al. demonstrate that the inflammatory effects are reduced when DEPs are filtered away. Furthermore, based on the studies using DEP standard reference materials, it is noted that DEP alone induces a dose-dependent inflammatory response in the lungs of mice. Since exposure to NO_2 may cause pulmonary inflammation in rats, it is likely that NO_2 contributes to DEE-induced pulmonary inflammation.

Genotoxicity and cancer

Genotoxicity and cancer are well studied, possible adverse effects of exposure to DEPs.

Cancer

IARC recently reviewed chronic diesel exhaust inhalation studies in animals. IARC found sufficient evidence for carcinogenicity of whole DEE, DEE particulate matter and extracts of DEPs in experimental animals. In contrast, IARC found inadequate evidence for the carcinogenicity of gas-phase DEE in experimental animals (IARC 2014). The studies on whole DEE were generated from fuels and engines produced before the year 2000 (IARC 2014).

Inhalation studies

According to the IARC evaluation, whole diesel exhaust has been tested for carcinogenicity by inhalation exposure in 19 studies in rats, 4 studies in mice, 3 studies in hamsters and 1 study in monkeys. The study in monkeys was a 2-year inhalation study and therefore too short for an evaluation of cancer. NEG/DECOS concluded that there was no clear evidence of carcinogenicity of diesel exhaust in mice or hamsters even at high particle loads (Taxell and Santonen 2016). Therefore, with regard to the endpoint cancer the present working group has only focused on studies in rats, the most sensitive species. In total, 11of the 19 rat studies identified by IARC showed an increased cancer incidence in rats exposed to diesel exhaust. Of these 11 studies, 10 were on exhaust from light-duty engines and 1 study was on exhaust from a heavy-duty engine. Based on the IARC review (IARC 2014), 5 chronic dose-response studies in rats exposed to whole diesel exhaust by inhalation were identified as well-designed and well-powered with group size of more than 70 animals per sex (Mauderly et al. 1987;Brightwell et al. 1989;Mauderly et al. 1994;Heinrich et al. 1995;Stinn et al. 2005). These 5 studies are described below and an overview of the studies is presented in Table 3.

Table 3. Diesel engine exhaust inhalation studies in rats with observed dose carcinogenicity response

Reference	Strain (sex) Group size	Exposure	DEP mg/m³	NO_2	NO ppm	NOx	Lung tumor incidence M	F
Mauderly et al., 1986	F344 (M/F)	Clean air and DEE (1980 5.7-L V8)	0				1.4%	
	N = 221-230	7 h/d, 5 d/w for up to 30 months	0.35	0.01			0.7%	
			3.5	0.3			4.6%*	
			7.0	0.7			16.1%*	
Brightwell, 1989	F344 (M/F)	Cond. air and DEE (VW Rabbit 1.5-L)	0				1.5%	0.8%
	N = 72 or 144	16 h/d, 5d/w for 24 + 6 months	0.7				1,.4%	0.0%
			2.2	0.9-2.8			4.2%	15.3%*
			6.6				22.5%*	54.2%*
Mauderly et al., 1994	F344 (M/F)	Cond. air and DEE (Two ´88 LH6 GM 6.2L V8)	0				3.0%	0.0%
	N = 100	16 h/d, 5d/w for 24 + 6 months	2.5	0.7		8.8	5.0%	8.0%
			6.5	3.8		24	9.0%	29.0%*
Heinrich et al., 1995	Wistar (F)	Clean air and DEE (Two VW 40-kW 1.6-L)	0					0.5%
	N = 100-220	18 h/d, 5d/w for 24 + 6 months	0.84	0.3		4.7		0.0%
			2.5	1.2		14		5.5%*
			7.0	3.8		33		22.0%*
Stinn et al., 2005	Wistar (M/F)	Clean air and DEE (VW 1.6-L)	0				4.0%	0.0%
	N = 99	6 h/d, 7 d/w for 24 + 6 months	3		7	9	18.0%*	28.0%*
			10		23	28	34.7%*	56.9%*

The table is adapted from IARC Table 3.2 (IARC 2014). DEE, DEPs (measured particulate matter in mg/m³). Cond.: Conditioned. Brightwell also included a filtered exhaust exposure with 99.7% of the mass removed. No increased tumor incidence was observed.

Mauderly et al. 1986:
Groups of 221-230 male and female Fischer 344 rats were exposed by inhalation to 0.35, 3.5 and 7 mg/m³ DEE for 7 h/day, 5 days/week for up to 30 months. Control rats were exposed to filtered air. The diesel exhaust was generated by a 1980 model 5.7-L V8 diesel engines (Volkswagen). The exhaust was diluted with clean air to reach the average diesel particle concentrations of 0.35, 3.5 and 7 mg/m³. The diesel exhaust contained 0.1±0.1, 0.3±0.2 and 0.7±0.5 ppm NO_2, respectively. Compared with controls, the incidence of rats with lung tumors was significantly increased in rats at the high-dose exposure (16.1%). These were combined data from both sexes. The article did not report the incidence stratified by sex (Mauderly et al. 1986).

Brightwell et al. 1989:
Groups of 72 male and 72 female F344 rats were exposed by inhalation to 0.7, 2.2 and 6.6 mg/m³ DEE or particle-filtered exhaust for 16 hrs/day, 5 days/week for 24 months followed by 6 months in clean air. Control rats were exposed to conditioned air (n=144 per sex). The diesel exhaust was generated by a Volkswagen Rabbit 1.5-L diesel engine and diluted with clean air to reach the mean diesel particle concentrations of 0.7, 2.2 and

6.6 mg/m^3. The diesel exhaust contained 0.9±0.05, 2.7±0.08 and 8±1 ppm NO_x, respectively, and 0.7±0.5, 2.1±0.8 and 6±2 ppm NO, respectively. The medium dose particle-filtered exhaust contained 2.8±0.5 ppm NO_x and 2.2±0.7 ppm NO. The high dose particle-filtered exhaust contained 8±2 ppm NO_x and 7±2 ppm NO. Compared with controls, the incidence of rats with lung tumors was significantly increased in both female groups of both mid-dose exposure (15.3%) and high-dose exposure (54.2%); and male group of high-dose exposure (22.5%). No increase in the number of rats with lung tumors were observed in rats exposed to filtered diesel exhaust (Brightwell et al. 1989).

Mauderly et al.1994:
Groups of approximately 100 male and 100 female F344 rats were exposed by inhalation to 2.5 and 6.5 mg/m^3 DEE for 16 h/day, 5 days/week for 24 months followed by 6 months in clean air. Control rats were exposed to conditioned air (n=144). The diesel exhaust was generated by two 1988 model LH6 General Motors 6.2-L V8 diesel engines and diluted with clean air to reach the mean diesel particle concentrations of 2.5 and 6.5 mg/m^3. Compared with controls, the incidence of rats with lung tumors was significantly increased in groups of female low-dose exposure (8%); and female and male high-dose exposure (9% and 29%, respectively)(Mauderly et al. 1994).

Heinrich et al. 1995 :
Groups of 100-220 female Wistar rats were exposed by inhalation to 0.8, 2.5 and 7 mg/m^3 DEE for 18 hours/day, 5 days/week for 24 months followed by 6 months in clean air (Heinrich et al. 1995). The diesel exhaust was generated by two 40-kW 1.6-L diesel engines (Volkswagen). The exhaust was diluted with clean air (1:80, 1:27, 1:15 and 1:9) to reach the average diesel particle concentrations of 0.8, 2.5 and 7 mg/m^3. The diesel exhaust contained 0.3-3.8 ppm NO_2, 4.7-33.1 ppm NO_x, 2.6-21.1 ppm CO and 0.2-0.7 % CO_2. In addition, small amounts of SO_2, CH_4 and C_nH_m (0.3-3.4 ppm) were measured in the diesel exhaust. The number of rats with tumors after 30 months is given in Table 3. Compared to rats exposed to clean air, exposure to diesel exhaust induced a significant increase of cancer in the groups exposed to the two highest concentrations (2.5 and 7 mg/m^3), while no rats developed cancer in the group exposed to the lowest concentration (0.8 mg/m^3).

Stinn et al. 2005:
Groups of 99 male and 99 female Wistar rats were exposed by nose-only inhalation to 3 and 10 mg/m^3 diesel engine exhaust for 6 h/day, 7 days/week for 24 months followed by 6 months in clean air (Stinn et al. 2005). The diesel exhaust was generated by a 40 kW 1.6-L diesel engine (Volkswagen). The exhaust was diluted with air to reach the average diesel particle concentrations of 3 and 10 mg/m^3. The diesel exhaust contained 7 ppm NO and 9 ppm NO_x (low dose), and 23 ppm NO and 28 ppm NO_x (high dose). Control animals were exposed to clean air. Compared with controls, the incidence of rats with lung tumors was significantly increased in both male and female groups of both low (9 out of 50 males (18%)); 14 out of 50 females (28%)) and high exposure (17 out of 49 males (35%); 29 out of 51 males (57%)).

The study by Brightwell et al. showed no increase in lung tumor incidence when rats exposed to filtered diesel exhaust (Brightwell et al. 1989). According to IARC, filtered

diesel exhaust without particles has been tested for carcinogenicity by inhalation exposure in 7 studies in rats, 3 studies in mice, 3 studies in hamsters (IARC 2014). None of the studies in rats and hamsters showed increased lung cancer incidences. One of the studies in mice showed increased lung cancer incidence in mice exposed to filtered exhaust. However, this study was considered inadequate by IARC because the incidence of tumors in the control group was significant lower than historical controls in the same laboratory. Furthermore, when the study was repeated performed in the same way, no increase in the incidence of lung tumors was detected.

After the publication of the IARC monography, a chronic cancer study on "new technology" DEE was published that met the above criteria for the selection of cancer studies on exhaust from diesel engines (well designed and well powered with group size of more than 70 animals per sex):

New technology diesel exhaust, ACES study:
Groups of 100 male and 100 female rats were exposed by inhalation to 3, 5 and 12 μg/m^3 DEE for 16 hours/day, 5 days/week for more than 24 months (McDonald et al. 2015). The diesel exhaust was generated by US 2007 compliant heavy-duty diesel engine. The diesel exhaust contained 0.1, 0.9 and 4.2 ppm NO_2, respectively. Compared with controls, no increase in the incidence of rats with lung tumors was observed.

Intratracheal instillation

In the review by IARC, 2 intratracheal instillation studies in rats were considered adequate for an assessment of DEP- induced carcinogenicity. Both studies showed an increased incidence of lung tumors (IARC 2014).

Summary

In summary, IARC found sufficient evidence for carcinogenicity of whole DEE, DEE particulate matter and extracts of DEPs in experimental animals. IARC found inadequate evidence for the carcinogenicity of the gas-phase of DEE (IARC 2014). The studies on whole DEE were generated from fuels and engines produced before the year 2000 (IARC 2014).

When considering well-performed studies with multiple dose levels, 5 chronic 2-year inhalation cancer studies on "traditional technology" whole diesel exhaust in rats were identified based on the review performed by IARC (IARC 2014). In 4 of the studies, increased lung cancer incidence occurred at exposure concentrations between 2.2 and 3.5 mg/m^3 DEP's (Brightwell et al. 1989;Mauderly et al. 1987;Heinrich et al. 1995;Stinn et al. 2005). In the fifth study, increased cancer incidence was observed at 6.5 mg/m^3 DEPs (Mauderly et al. 1994). No increase in the incidence of lung tumors was found in 7 studies of rats exposed to filtered exhaust (particles removed) (IARC 2014) or in rats exposed to whole diesel exhaust at ≤ 800 μg/m^3 for ≥ 2 years (Taxell and Santonen 2016;Taxell and Santonen 2016). Intratracheal instillation of DEP showed an increased incidence of lung tumors. This shows that the carcinogenic effect is caused by the particulate phase of the diesel exhaust.

One chronic rat inhalation study of exhaust from "new-technology" diesel engines was identified (ACES) (McDonald et al. 2015): No increase in cancer incidence was detected in rats exposed in mass concentrations of DEP up to 12 μg/m^3 (4.2 ppm NO_2) for 130 weeks.

The present working group notes that the particle concentration in this "new-technology" diesel exhaust study is very low (≤ 12 μ/m^3) compared to the particle concentrations in the cancer studies on "traditional diesel" exhaust (effects at ≥ 2200 μg/m^3). This may explain the lack of effect in the study. This is also in agreement with the considerations by McDonald et al.: "*It is reasonable to speculate that the markedly lower, and possibly nearly negligible, levels of exposure to diesel exhaust particles in the present study may preclude effects attributable to the particles*" (McDonald et al. 2015).

NEG/DECOS (Taxell and Santonen 2016) concluded:
"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 (Ishinishi et al. 1988;Brightwell et al. 1986;Mauderly et al. 1987;Stinn et al. 2005;Nikula et al. 1995;Heinrich et al. 1995). 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 (Mohr et al. 1986;Brightwell et al. 1986;Heinrich et al. 1986;Mauderly et al. 1987;Heinrich et al. 1995). 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) (McDonald et al. 2015). No clear evidence of carcinogenicity of diesel exhaust in mice or hamsters has been observed even at high particle loads (Brightwell et al. 1986;Heinrich et al. 1986;Heinrich et al. 1995)."

Conclusion

In conclusion, the present working group notes that chronic inhalation studies in rats have shown elevated incidence of lung tumors in rats exposed to diesel exhaust compared to control rats exposed to air. The importance of the particulate phase of DEE for the carcinogenic effect is supported by studies showing 1) no increase in the incidence of lung tumors in rats when the particulate phase is removed before exposure, and 2) increase in incidence of lung tumors in rats intratracheally instilled with DEPs.

Genotoxicity

IARC recently summarized DEE-induced genotoxicity (IARC 2014):

> "*Diesel engine exhausts and the mechanisms by which they induce cancer in humans are complex in nature, and no single mechanism appears to predominate. Organic solvent and physiological fluid extracts of diesel engine exhaust particles and several of the individual components of these exhausts are genotoxic, and some are carcinogenic, generally through a mechanism that involves DNA mutation. These modifications include the formation of bulky DNA adducts and oxidized DNA bases. Both the organic and particulate components of diesel engine exhaust emissions can generate ROS, leading to oxidative stress. ROS can be generated from washed particles, fresh particles, arene quinones formed by photochemical or*

enzymatic processes, metals and the phagocytosis process, and as a result of the inflammatory process. ROS can lead directly to the formation of oxidatively modified DNA and DNA adducts from by-products of lipid peroxidation (Voulgaridou et al. 2011), can cause lipid peroxidation, which generates cytotoxic aldehydes (Barrera et al. 2008), and can also initiate a signalling cascade that leads to inflammation, resulting in further induction of oxidative stress, which in turn leads to cell prolif- eration and cancer (Milara and Cortijo 2012). In response to the inflammatory insult, cyclooxygenase-2 is upregulated and is a potent mediator of cell proliferation (Speed and Blair 2011)."

NEG/DECOS recently summarised DEE-induced genotoxicity (Taxell and Santonen 2016):

"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."(Taxell and Santonen 2016).

There are a number of studies evaluating the genotoxic effects of DEP. Some of the studies considered most relevant are described below.

Short-term effects of SRM 1650 DEP originating from heavy-duty diesel engines on markers of genotoxicity were evaluated in mice. Mice exposed to DEP by inhalation to either a single 90 min exposure (20 or 80 mg/m^3) or as 4 repeated 90 min exposures (5 or 20 mg/m^3) resulted in DNA strand breaks in BAL cells, oxidative DNA damage and DNA adducts in lungs of mice (Dybdahl et al. 2004).

Genotoxicity was evaluated in mice and rats exposed to exhaust from "new technology" diesel engines at three exposure-doses: Low: 3 μg DEP/m^3/0.1 ppm NO_2, Medium: 5 μg DEP/m^3/0.9 ppm NO_2, High: 12 μg DEP/m^3/4.2 ppm NO_2). Mice and rats were exposed 16 h/day, 5 days/week for up to 3 months and 24 months, respectively. Compared to controls, no increase in genotoxicity (DNA damage (comet assay) in the lungs, 8-OHdG

in serum and micronuclei in peripheral blood) was detected (Hallberg et al. 2015;Bemis et al. 2015;Hallberg et al. 2012;Bemis et al. 2012).

The present working group concludes that diesel exhaust is genotoxic. This is based on the fact that inhalation studies with DEE and intratracheal instillation studies of DEPs in rodents have shown increased levels of genotoxicity. Therefore, the present working group agrees with the evaluation by NEG/DECOS that the genotoxic potency of diesel exhaust most likely is attributed to the particulate phase of the exhaust. A single study of genotoxicity of exhaust from "new technology" diesel engines was identified. In that study, no genotoxicity was observed when tested in rodents. The highest particle concentration in that study was very low (12 μg DEP/m^3) compared to the particle concentrations in studies of exhaust from "traditional engines" resulting in effects. This may explain the lack of effect in the study.

MECHANISMS OF TOXICITY

Pulmonary inflammation, genotoxicity and cancer

IARC concluded recently:

> *"there is strong mechanistic evidence that diesel engine exhaust, as well as many of its components, can induce lung cancer in humans through genotoxic mechanisms that include DNA damage, gene and chromosomal mutation, changes in relevant gene expression, production of reactive oxygen species and inflammatory responses. In addition, the co-carcinogenic, cell-proliferative, and/or tumour-promoting effects of other known and suspected human carcinogens present in diesel-engine exhaust probably contribute to its carcinogenicity in the human lung." (IARC 2014).*

NEG/DECOS:

"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 hypothesized 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" (Taxell and Santonen 2016).

The mechanisms by which DEPs cause cancer are likely mediated by both primary (particle driven) and secondary (cell driven) genotoxicity.

Chronic inhalation studies of DEE alongside filtered diesel exhaust showed that the particulate fraction of diesel exhaust was required for diesel exhaust-mediated tumor formation, as rats exposed to diesel exhaust developed tumors in contrast to rats exposed to filtered diesel exhaust (Brightwell et al. 1989).

Both inhalation of diesel exhaust and pulmonary instillation of DEP and DEP extracts increased the mutant frequency in lung tissue in *gpt* delta transgenic mice (Hashimoto et al. 2007). Mice were exposed to 3 mg/m^3 for 12 weeks or to single instillations of 0.2 mg DEP extract or 0.5 mg DEP. The mutant frequency potency of DEP extract and DEP was and 2.7 x 10^{-5} and 5.6 x 10^{-5}, respectively. DEP extracts constituted 50% of DEP mass, and the authors concluded that the mutagenic potential of DEP could be explained by the extractable mutagens including PAH and nitrated PAH (Hashimoto et al. 2007). However, chronic inhalation exposure to TiO_2 NPs, carbon black NPs and DEE in rats performed in the same study showed that the cancer incidence from the three types of exposure was very similar (table 4), and thus, the authors concluded that the study supported the hypothesis that the carbon core of the diesel soot is the main causative agent for DEE-related carcinogenicity (Heinrich et al. 1995). The CB and TiO_2 exposure concentrations were changed during the experiment to obtain similar lung particle load as in the diesel exposed animals

Similar conclusions were made based on a study comparing tumor formation in rats following inhalation of DEE and CB nanoparticles (Nikula et al. 1995).

The present working group does not regard the change in exposure concentrations in the Heinrich study as problematic.

Table 4. Lung cancer incidence in rats exposed to diesel exhaust, CB and TiO_2 after 30 months (24 months of exposure followed by 6 months in clean air) (Heinrich et al. 1995)

	Average particle exposure (mg/m^3)					
	Clean air, control	DEP			CB	TiO_2
	0	0.8	2.5	7.0	11.6	10
Number of rats with tumors[a,b)]	1/217	0/198	11/200** (4/200**)	22/100*** (9/100***)	39/100 (28/100)	32/100 (19/100)

a) Count without benign keratinising cystic squamous-cell tumors in parentheses.

b) *,**,***: Statistically significant compared to clean air control at $p \leq 0.05$, 0.01 and 0.001, respectively.

Inhalation and pulmonary instillation of the standard DEP NIST1650 induced increased levels of DNA strand breaks in lung tissue and bronchoalveolar lavage cells and bulky DNA adducts in lung tissue (Kyjovska et al. 2015;Dybdahl et al. 2004) suggesting that DNA damage is caused both by formation of bulky DNA adducts and by oxidative DNA damage.

The SRM DEP NIST 1650 and carbon black Printex 90 also had similar mutagenic potential in vitro in the murine lung fibroblast cell line FE-1 (Jacobsen et al. 2008;Jacobsen et al. 2007). The CB nanoparticles induced much more reactive oxygen species compared to the DEP. On the other hand, the mutagenic potential of the PAH content could not account for the observed mutagenicity. The authors concluded that the observed mutagenicity was likely caused by both the organic fraction of DEP and by particle-mediated ROS production (Jacobsen et al. 2008).

Thus, evidence suggests that both the extractable organic fraction and the particulate fraction of DEP contribute to the carcinogenicity of DEP. The molecular mechanism includes formation of bulky DNA adducts as well as oxidative DNA damage likely induced by surface-dependent ROS generation. In addition, inhalation of DEE and DEP-induced dose-dependent pulmonary inflammation which could cause secondary genotoxicity. Thus, the available data indicated induction of cancer through both direct and indirect genotoxic mechanisms. Based on the observed mechanisms of genotoxicity, the present working group concludes that DEP-induced mutagenicity and carcinogenicity occurs by non-threshold mechanisms.

DOSE-RESPONSE RELATIONSHIPS

Inflammation

Human data
NEG/DECOS has recently reviewed studies on the inflammatory response in the lungs of human volunteers exposed to whole diesel exhaust for 1-2 hours. Exposure levels at ~100 μg DEP/m^3 resulted in increased numbers of neutrophils and inflammatory cytokines in BAL in healthy volunteers (Taxell and Santonen 2016).

Animal data
Dose-response relationships have been observed for inflammation following inhalation of DEE (Mauderly et al. 1994) and DEP (Dybdahl et al. 2004) and instillation of DEP (Kyjovska et al. 2015).

Cancer

Human data
Vermeulen et al. performed a meta-regression of lung cancer mortality and cumulative exposure to EC (as a proxy measure of DEPs), based on RR estimates reported by two studies of workers in the trucking industry and one study of miners (Vermeulen et al. 2014b). Based on a linear meta-regression model, a lnRR of 0.000982 (95% CI: 0.00055, 0.0014) for lung cancer mortality with each 1-μg/m^3-year increase in cumulative EC was estimated (Figure xx in the paragraph on epidemiological studies). The numbers of excess lung cancer deaths for 45 years of occupational exposures of 1, 10, and 25 μg/m^3 EC were estimated to 17, 200, and 689 per 10,000, respectively, by 80 years of age.

Animal data
Strong dose-response relationships have been observed for lung cancer in rats following inhalation of diesel exhaust (Figure 2). The present working group notes that female rats are more sensitive than male rats. In figure 2, the cancer incidences in rats from 4 of the 5 identified chronic inhalation studies are shown (Brightwell et al. 1989;Heinrich et al. 1995;Mauderly et al. 1986;Mauderly et al. 1994;Stinn et al. 2005). The data from the fifth study by Mauderly et al. is omitted on the figure because that study did not report the incidence stratified by sex (Mauderly et al. 1986).

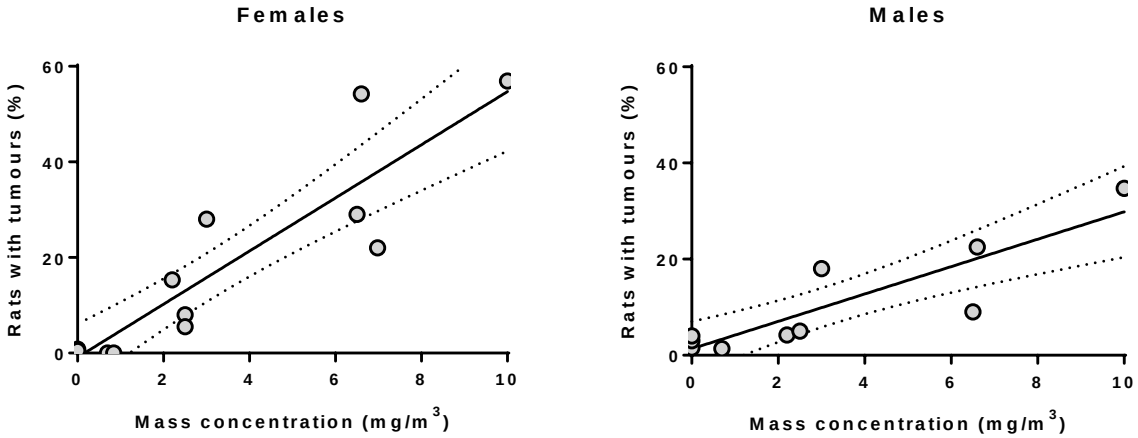

Figure 2. **Frequency of female and male rats with tumors as a function of DEP mass concentrations in the chronic inhalation studies by (Brightwell et al. 1989;Heinrich et al. 1995;Mauderly et al. 1994;Stinn et al. 2005). Dotted lines represent 95% confidence interval for the regression lines. Females: y = 5.6x – 0.088; Males: y = 2.8x +1.4**

PARTICLE CHARACTERISTICS

Diesel exhaust consists of a particulate phase (carbon particles with adsorbed organic matter) and a gas/vapor phase which includes volatile organic compounds (VOCs), nitrogen oxides and carbon monoxide. The focus of the present report is on the particulate phase of diesel exhaust.

"Traditional" diesel engines did not control the emission of particulate matter. In contrast, "new-technology" engines are equipped with particulate filters that reduce the emission of particulate matter considerable (more than 90% by mass)(IARC 2014;Taxell and Santonen 2016). The composition of DEPs emitted from "traditional" and "new technology" diesel engines is also different. Typically, the proportion of elemental carbon from a traditional heavy-duty diesel engine is 75% while this proportion is reduced to 13% when using new technology diesel engines. The proportions of sulfates and organic carbon are increased from 1% to 53% and 19% to 30%, respectively, when exhaust after-treatment systems are applied (Taxell and Santonen 2016).

Since the composition of DEPs is complex, different metrics to measure the exposure to DEPs has been applied (Taxell and Santonen 2016): Measurement of EC is considered the most specific and sensitive marker of DEP because in most occupational settings only DEE contributes significantly to EC. In contrast, measurement of the total mass of particles in the occupational setting does not allow a separation of DEP from other particles in the environment. Another metric for DEP exposure is to quantify PAH or other organic compounds adhered to the DEPs chemically.

These years, "traditional" diesel engines are gradually being replaced by "new-technology" diesel engines. This will most likely result in lower concentrations of DEPs in occupational settings. However, it is expected to take a long time before this transition has been completed (Taxell and Santonen 2016). Furthermore, it should be emphasised that the nanosized fraction of DEPs contributes very little to the DEP mass.

No studies were identified comparing the toxicity on a mass base of DEPs from "traditional technology" and "new technology".

The present working group notes that EC is considered the best marker of DEP. Further, the present working group notes that there is a lack of knowledge regarding the toxicity of DEPs from "new technology" compared to DEPs from "traditional technology" engines.

PREVIOUS EVALUATIONS OF DIESEL EXHAUST

The most recent evaluations of DEE are presented below.

IARC (2012)

In 2012, the IARC classified DEE as carcinogenic to humans (group 1). This classification was based on sufficient evidence that DEE is causally related to lung cancer. In addition, IARC found some evidence for a positive association between exposure to DEE and an increased risk of bladder cancer. Furthermore, IARC found sufficient evidence for carcinogenicity of whole DEE, DEE particulate matter and extracts of DEPs in experimental animals. IARC found inadequate evidence for the carcinogenicity of gas-phase DEE (i.e. particle free DEE) (IARC 2014). IARC does not differentiate between exhaust from traditional and new technology diesel engines in their classification (IARC 2014). In Denmark, substances classified as group 1, 2A and 2B by IARC are considered carcinogenic.

NEG/DECOS (2014)

In 2014, NEG and DECOS co-produced a criteria document on diesel exhaust (Taxell and Santonen 2016).

NEG/DECOS summarised as follows:

"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/m3, ~ 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."

SCIENTIFIC BASIS FOR SETTING AN OCCUPATIONAL EXPOSURE LIMIT

Different methods exist for calculating OELs. The choice of method depends on the mode of action of the substance, and can fundamentally be split up in two approaches: Threshold effects or non-threshold effects. The threshold effect approach relies on the assumption that the organism can withstand a certain dose before adverse effects occur, whereas for non-threshold effects it is assumed that any exposure to the substance can result in adverse effects. The present working group considers cancer as the most severe critical effect. Furthermore, the present working group considers that DEP-induced mutagenesis and cancer occurs by non-treshold mechanisms. Therefore, in this report, we will calculate proposed OELs based on non-threshold effects for lung cancer. The calculations will be performed based on data from both human and animal studies.

Health-based exposure limit based on epidemiological data

DEE was recently classified as carcinogenic to humans by IARC (class 1). In a recent meta-analysis of three studies of occupational exposure to diesel engine exhaust (Vermeulen et al. 2014b), the association between lung cancer incidence and DEE measured as EC was modelled.

lnRR for lung cancer= intercept + slope x exposure

Exposure was measured as EC in $\mu g/m^3$-years. The intercept was set at 0, and the slope was determined to be 0.000982 with a standard error of 0.000219:

lnRR for lung cancer = 0.000982 x exposure

In Denmark, the life time risk of developing lung cancer (0-74 years) is 4.9% for men and 4.5% for women. The relative risk caused by occupational exposure to a carcinogen, which causes cancer at the different risk levels (1%, 0.1% and 0.01%) are given in table 5.

Table 5. Relative risk of lung cancer for carcinogens that cause 1%, 0.1% or 0.01% excess lung cancer risk in a population with the current Danish lung cancer incidence

	Men	Women
Life time risk (0-74 years) 2011-2015 in Denmark[1]	4.9%	4.5%
Excess lung cancer risk level	RR	RR
1:100	RR= (4.9+1)/ 4.9= 1.20	RR= (4.5+1)/4.5= 1.22
1:1 000	RR= (49+1)/49= 1.02	RR= (45+1)/45=1.02
1:10 000	RR= (490+1)/490= 1.002	RR= (450+1)/450= 1.002
1:100 000	RR= (4900+1)/4900= 1.000 2	RR= (4500+1)/4500= 1.000 2

1) http://www-dep.iarc.fr/NORDCAN/DK/StatsFact.asp?cancer=180&country=208

Assuming 1:1 000 excess lung cancer incidences among men, the calculation would be:

EC concentration in μg/m^3-years = Ln (1.02)/ 0.000982= 20.16 μg/m^3-years

For a 45-year work life this would correspond to 20.16 μg/m^3-years/45 years = 0.45 μg/m^3

Assuming 1:100 000 excess lung cancer incidences among men, the calculation is:

EC concentration in μg/m^3-years = Ln (1.0002)/ 0.000982= 0.2016 μg/m^3-years

For a 45-year work life this would correspond to 0.0045 μg/m^3

Table 6. Estimated lung cancer risk based epidemiological data from (Vermeulen et al. 2014b)

Excess lung cancer risk	DEP air concentration
1: 1 000	0.45 μg/m^3
1: 10 000	0.045 μg/m^3
1: 100 000	0.0045 μg/m^3

Health-based exposure limit based on two chronic inhalation studies in rats

The present working group notes that the five identified high quality chronic inhalation studies in rats show that female rats are more sensitive to DEP-induced cancer than male rats (Figure 2 in the Dose-response chapter of the present report). Furthermore, the studies have somewhat different cancer incidences. The current working group has therefore calculated health-based exposure limits based on two different studies, representing low or high carcinogenic response, respectively, in female rats.

Low response (Heinrich study)

The chronic inhalation study by Heinrich (Heinrich et al. 1995) was identified as representative of a relatively low response and was selected because the carcinogenicity of CB and TiO_2nanoparticles was assessed alongside DEP, thus allowing comparison of the carcinogenic potency between the three types of particles.

Table 7. Observed cancer incidence following DEP exposure in (Heinrich et al. 1995)

DEP concentration	0	2.5 mg/m^3	7.0 mg/m^3
Cancer Incidence	1/217	11/200	22/100
Lung burden (mg/lung)		23.7	63.9

Method I

A non-threshold effect is assumed. The genotoxic effects induced by DEP are probably caused by a number of mechanisms including surface-associated PAH, carbon-core-

induced ROS and inflammation-induced ROS. DNA damage in terms of increased levels of DNA strand breaks in the comet assay was observed following intratracheal instillation of the standard DEP NIST1650b at dose levels that did not induce statistically significant increases in neutrophil influx 1 and 28 days post-exposure (Kyjovska et al. 2015). This suggests that surface-associated PAH and carbon-core-induced ROS are likely mechanisms of action. These are considered non-threshold effects.

The current working group has chosen to use the approach used by Kasai et al.(Kasai et al. 2016) and Erdely et al. (Erdely et al. 2013), who use the measured lung burden in rats exposed by inhalation and the alveolar surface area of rats and humans to estimate the human equivalent lung burden:

Observed cancer incidence at 2.5 mg/m^3:
(11/200 – 1/217)/(1-1/217) = 0.05 = 5%

Lung deposited dose in rats at 2.5 mg/m^3: 23.7 mg/lung.

The human equivalent dose is:

(Rat deposited dose) x (human alveolar surface area)/(rat alveolar surface area) =
23.7 mg x 102 m^2/0.4 m^2= 6 043.5 mg DEP per human lung.

We assume the following standardised constants:

The standard value of human ventilation is 20 L/min during light work (1.2 m^3/h).
An average work day is 8 h per day.
An average work week is 5 days.
In Denmark, an average employee work 45 weeks per year.
An average working life is 45 years.
Assuming 16.8% deposition as previously reported for humans by (NEG/DECOS)(Taxell and Santonen 2016).

Using the values above, a lung burden of 6 043.5 mg in humans would require that workers are exposed to:

Air concentration =
6 043.5 mg/(8h/day x 5 days/week x 45 weeks/year x 45 years x 1.2 m^3/h x 0.168) =
0.37 mg/m^3

Thus, at an air concentration of 0.37 mg/m^3 during a 45-year work life, an excess lung cancer incidence of 5% is expected. Assuming a linear dose-response relationship, then 1% excess lung cancer is expected at:

(0.37 mg/m^3)/5 = 0.074 mg/m^3

Accordingly, assuming linear dose-response relationship, the excess lung cancer risk is estimated:

Table 8. Excess cancer risk

Excess lung cancer risk	DEP air concentration
1: 1 000	7.4 μg/m^3
1: 10 000	0.74 μg/m^3
1: 100 000	0.074 μg/m^3

Method II

Risk estimates were calculated as recommended by ECHA (ECHA 2012a; SCHER/SCCP/SCENIHR 2009), based on the 2 year DEE inhalation study in rats by (Heinrich et al. 1995) (Table 4):

Excess cancer risk:

Observed excess cancer incidence at 2.5 mg/m^3:

(5/200- 1/217)/(1-1/217)= 0.0506 = 5 %

Correction of dose metric for humans during occupational exposure (8h/d):

2.5 mg/m^3 x (18 h/day)/(8 h/day) x (6.7 m^2/10 m^2) = 3.769 mg/m^3

Calculation of unit risk for cancer:

Risk level = exposure level x unit risk
0.0506 = 3 769 μg/m^3 x unit risk
Unit risk = 1.34 x 10^{-5} per μg/m^3

At a dose of 1 μg/m^3, 1.34 x 10^{-5} excess cancers are expected.

Calculation of dose levels corresponding to risk level of 10^{-5} (and other risk levels)

10^{-5} risk level = exposure level x unit risk (1.34 x 10^{-5} per μg/m^3)
Exposure level (10^{-5}) = 0.74 μg/m^3

Thus, at 0.74 μg/m^3, 1:100 000 excess lung cancer cases can be expected.

Table 9. Calculated excess lung cancer incidence at DEP mass concentrations based on method II

Excess lung cancer incidence	DEP Air concentration (μg/m^3)
1: 1 000	74
1: 10 000	7.4
1: 100 000	0.74

High response (Brightwell et al.)

The study by Brightwell et al. was selected as an example of a high response study.

Here, rats were exposed 16 h/day, 5 days/week for 2 years to diesel engine exhaust at particle concentrations of 0.7, 2.2 or 6.6 mg/m^3 particles (Brightwell et al. 1989). In addition, filtered diesel engine exhaust was included at the middle and high doses. Lung burden was not assessed. Females were identified as the most sensitive sex and the middle dose (2.2 mg/m^3) was selected as the lowest dose inducing lung tumors.

Table 10. Cancer incidence (Brightwell study)

Exposure	0 mg/m^3	0.7 mg/m^3	2.2 mg/m^3	6.6 mg/m^3	Filtered DE (6.6 mg/m^3)
Cancer incidence in female rats	1/126	0/71	11/72	39/72	0/72
Cancer incidence in male rats	2/134	1/72	3/72	16/71	0/71

For the study by Brightwell et al, excess lung cancer incidence at DEP mass concentrations were calculated based on method II for (Brightwell et al. 1989).

Calculations based on Method I were not performed due to lack of information on deposited dose in the study by Brightwell.

Excess cancer risk:

Observed excess cancer incidence at 2.2 mg/m^3:

(11/72- 1/126)/(1-[1/126])= 0.146 =15 %

Correction of dose metric for humans during occupational exposure (8h/d):

2.2 mg/m^3 x (16 h/day)/(8 h/day) x (6.7 m^2/10 m^2) = 2.948 mg/m^3

Calculation of unit risk for cancer:

Risk level = exposure level x unit risk
0.146 = 2 948 μg/m^3 x unit risk
Unit risk = 5.0 x 10^{-5} per μg/m^3

At a dose of 1 μg/m^3, 4.4 x 10^{-5} excess cancers are expected.

Calculation of dose levels corresponding to risk level of 10^{-5} (and other risk levels)

10^{-5} risk level = exposure level x unit risk (5.0 x 10^{-5} per μg/m^3)
Exposure level (10^{-5}) = 0.20 μg/m^3

Thus, at 0.20 μg/m^3, 1:100 000 excess lung cancer cases can be expected.

Table 11. Calculated excess lung cancer incidence at DEP mass concentrations based on method II for (Brightwell et al. 1989)

Excess lung cancer incidence	DEP Air concentration (μg/m^3)
1: 1 000	20
1: 10 000	2.0
1: 100 000	0.20

Summary

In summary, excess lung cancer risks were estimated based on the meta-analysis of epidemiological studies and for two different 2-year inhalation studies in rats:

Table 12. Summary of risk estimates for DEP-induced lung cancer

Excess lung cancer incidence	Human study μg/m^3	Method I, μg/m^3, Heinrich	Method II, μg/m^3	
			Heinrich	Brightwell
1: 1 000	0.45	7.4	74	20
1: 10 000	0.045	0.74	7.4	2.0
1: 100 000	0.0045	0.074	0.74	0.20

In the human study, DEE exposure was measured as elemental carbon (EC), whereas the animal studies used total PM. For comparison, total PM was converted to EC assuming that DEP contains 75% EC as found for traditional DEP (Taxell and Santonen 2016).

Table 13. Overview of exposure levels in terms of EC, resulting in extra cancer risk levels at 1:1000, 1:10 000 and 1: 100 000 based on a non-threshold based mechanism of action using different approaches

	Suggestion of an OEL for DEP calculated as EC			
Excess lung cancer incidence	Human studies	Method I, μg/m^3 Rat inhalation study of DEE*	Method II, μg/m^3 Rat inhalation study of DEE*	
	Vermeulen	Heinrich	Heinrich	Brightwell
1: 1 000	0.45 μg/m^3	5.6 μg/m^3	56	15
1: 10 000	0.045 μg/m^3	0.56 μg/m^3	5.6	1.5
1: 100 000	0.0045 μg/m^3	0.056 μg/m^3	0.56	0.15

Method I is based on lung deposition. Method II is based on air concentrations and following ECHA guidelines.*For traditional DEPs, it is assumed that 75% of the mass is EC (Taxell and Santonen 2016).

The suggested health-based OELs for DEP can be compared to the unit risk values for TiO_2 NPs and carbon black NPs based on chronic inhalation studies performed in parallel (Heinrich et al. 1995).

Table 14. Unit risk values for TiO_2 NPs, CB and DEE in chronic inhalation studies performed in parallel

	Control	10 mg/m^3 TiO_2	2.5 mg/m^3 Carbon black	2.5 mg/m^3 DEE
Total cancer incidence	1/217	32/100	8/107	11/200
Lung burden (mg/g)		39	21	23.7
Unit risk pr ug/m^3		2.1×10^{-5}	1.97×10^{-5}	1.34×10^{-5}

Overall, the OEL suggestions derived from the meta-analysis of epidemiological studies were 20 -100 fold lower than the OEL suggestions that were based on chronic inhalation studies in rats. The present working group notes that one of the explanations may be that the clearance rate of nanosized particles has been estimated to be ca. 60-100 days in rats and mice, but substantially lower, several hundred days, in humans (Taxell and Santonen 2016).

CONCLUSION

The present working group evaluated the relevant literature on diesel exhaust from both epidemiological and animal inhalation studies.

A recent evaluation by NEG/DECOS on DEE (Taxell and Santonen 2016) concluded that: *'The critical health effects of diesel engine exhaust are pulmonary inflammation and lung cancer.'*

IARC evaluated the epidemiological studies on exposure to DEEs and risk of cancer (IARC 2014) and concluded: *'There is sufficient evidence in humans for the carcinogenicity of diesel engine exhaust. Diesel engine exhaust causes cancer of the lung. A positive association has been observed between exposure to diesel engine exhaust and cancer of the urinary bladder'.*

The present working group considers lung cancer as the most severe critical effect.

A meta-analysis of epidemiological studies was performed including only studies with information regarding dose-response relationship between exposure to DEE and risk of lung cancer identified at the time of the IARC evaluation (Vermeulen et al. 2014b).

Dose-dependent tumor formation was observed in lungs of rats in chronic inhalation studies in rats. Chronic exposure to filtered DEE did not induce lung tumor formation in rats. The present working group regards carcinogenicity as the critical adverse effect of DEE exposure. Furthermore, the present working group regards the carcinogenic effect as caused by the particulate fraction of DEE.

The present working group found that the mechanism of action of the carcinogenic effect of DEP has not been fully clarified. Primary genotoxicity caused by PAH and surface-dependent generation of reactive oxygen species has been demonstrated. Secondary genotoxicity due to particle-induced inflammation is an important and well-documented mechanism of action for the development of lung cancer. However, the available data indicated induction of cancer through both direct and indirect genotoxic mechanisms. Therefore, the present working group considers carcinogenicity as a non-threshold effect. Consequently, the present working group decided to perform the risk assessment based on a non-threshold mechanism of action.

The present working group identified a recent meta-analysis as suitable for risk assessment (Vermeulen et al. 2014b). However, 5 highly quality chronic inhalation studies in rats were identified, and the present working group decided also to select two of these for calculation of excess cancer risk: A 2-year chronic cancer inhalation study in rats with relatively low tumor incidence (0, 2.5 and 7 mg/m^3) (Heinrich et al. 1995), and another 2-year chronic inhalation study in rats with a relatively high tumor incidence (0.7, 2.2 and 6.6 mg/m^3) (Brightwell et al. 1989) . In Table 15, excess lung cancer risk at 1 in 1 000, 1 in 10 000, and 1 in 100 000 using different approaches is presented.

Table 15. Overview of exposure levels in terms of elemental carbon (EC), resulting in extra cancer risk levels at 1:1000, 1:10 000 and 1: 100 000 based on a non-threshold based mechanism of action using different approaches

	Suggestion of an OEL for DEP calculated as elemental carbon			
Excess lung cancer incidence	EC levels Meta-analysis of Human studies (Vermeulen)	Rat inhalation study of DEE* Method I, Lung burden (Heinrich)	Rat inhalation study of DEE* Method II ECHA (Heinrich)	Rat inhalation study of DEE* Method II, ECHA (Brightwell)
1:1 000	0.45 μg/m^3	5.6 μg/m^3	56 μg/m^3	15 μg/m^3
1: 10 000	0.045 μg/m^3	0.56 μg/m^3	5.6 μg/m^3	1.5 μg/m^3
1: 100 000	0.0045 μg/m^3	0.056 μg/m^3	0.56 μg/m^3	0.15 μg/m^3

***) For traditional DEPs, it is assumed that 75% of the mass is EC (Taxell and Santonen 2016)**

The DEE exposure in the epidemiological studies was traditional DEE. Both of the chronic inhalation studies were performed on traditional DEE. The present working group notes that there is limited available data on the biological effects of DEP from "new technology" diesel engines. Typically, the proportion of EC from a traditional heavy-duty diesel engine is 75% of the total particle emission while this proportion is reduced to 13% when using "new technology" diesel engines. Correspondingly, the proportions of sulfates are increased from 1% to 53% when exhaust after-treatment systems are applied (Taxell and Santonen 2016).

The current working group notes that in chronic inhalation studies in rats, CB nanoparticles and DEPs have very similar carcinogenic potential (Heinrich et al. 1995). The present working group furthermore notes that there is no available evidence suggesting that "new technology" DEPs are less carcinogenic than "traditional" DEP and carbon black.

Three different approaches were used for calculating excess lung cancer risk. First, lung cancer risk was estimated based on the meta-analysis of epidemiological studies of the association between exposure to DEE and lung cancer. Secondly, lung cancer risk was estimated using two different approaches based on the same chronic inhalation study (Heinrich et al. 1995). In the first approach, lung burden in rats after two years of exposure was used to estimate the exposure limits for occupational exposure. In the second approach, air concentrations were used directly. Thirdly, lung cancer incidence was estimated based on a second chronic inhalation study in rats (Brightwell et al. 1989). Independently of the applied method for risk assessment, the resulting exposure limits were all very low.

The present working group notes that the risk estimates allowing 1: 10 000 excess lung cancer cases or less are all close to the current ambient air concentrations of EC (ca. 0.4 μg/m^3 EC for rural measurements in Denmark (Massling et al. 2011) and 2.7 μg/m^3 EC levels on a major street in Copenhagen, Denmark (Palmgren et al. 2003). The present working group recommends the approach using the epidemiological data, since this

approach relies on data from humans. Thus, the expected excess lung cancer risk based on epidemiological data is 1: 1 000 at 0.45 μg/m^3, 1: 10 000 at 0.05 μg/m^3 and 1: 100 000 at 0.005 μg/m^3 DEPs.

REFERENCES

Attfield MD, Schleiff PL, Lubin JH, Blair A, Stewart PA, Vermeulen R, Coble JB, Silverman DT. The Diesel Exhaust in Miners study: A cohort mortality study with emphasis on lung cancer. J Natl Cancer Inst 2012;104:869-883.

Barrera G, Pizzimenti S, Dianzani MU. Lipid peroxidation: Control of cell proliferation, cell differentiation and cell death. Mol Aspects Med 2008:29:1-8.

Bemis JC, Torous DK, Dertinger SD. Part 2. Assessment of genotoxicity after exposure to diesel exhaust from U.S. 2007 - Compliant diesel engines: Report on 1- and 3-month exposures in the ACES bioassay. Advanced Collaborative Emissions Study (ACES) Subchronic Exposure Results: Biologic Responses in Rats and Mice and Assessment of Genotoxicity. Research Report 166. Boston, MA: Health Effects Institute, 2012.

Bemis JC, Torous DK, Dertinger SD, HEI Health Review Committee. Part 2. Assessment of micronucleus formation in rats after chronic exposure to new-technology diesel exhaust in the ACES bioassay. Res Rep Health Eff Inst 2015;184:69-82.

Brightwell J, Fouillet X, Cassano-Zoppi AL, Gatz R, Duchosal F. Neoplastic and functional changes in rodents after chronic inhalation of engine exhaust emissions. In: Ishinishi N, Koizumi A, McClellan RO, Stober W, (eds.). Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust: Proceedings of the International Satellite Symposium on Toxicological Effects of Emissions from Diesel Engines. Amsterdam: Elsevier Science Publishers BV, 1986. pp. 471-485.

Brightwell J, Fouillet X, Cassanozoppi AL, Bernstein D, Crawley F, Duchosal F, Gatz R, Perczel S, Pfeifer H. Tumors of the respiratory-tract in rats and hamsters following chronic inhalation of engine exhaust emissions. J Appl Toxicol 1989;9:23-31.

Campen MJ, Babu NS, Helms GA, Pett S, Wernly J, Mehran R, McDonald JD. Nonparticulate components of diesel exhaust promote constriction in coronary arteries from ApoE-/- mice. Toxicol Sci 2005;88:95-102.

Dybdahl M, Risom L, Bornholdt J, Autrup H, Loft S, Wallin H. Inflammatory and genotoxic effects of diesel particles in vitro and in vivo. Mutat Res 2004;562:119-131.

ECHA. Guidance on information requirements and chemical safety assessment. Appendix R8-15 Recommendations for nanomaterials applicable to: Chapter R.8 Characterisation of dose [concentration] - response for human health. ECHA-12-G-09-EN. European Chemical Agency, 2012a.

ECHA. Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. ECHA-2010-G-19-EN. European Chemical Agency, 2012b.

EPA. Health assessment document for diesel engine exhaust. EPA/600/8-90/057F. Washington DC: National Center for Environmental Assessment, U.S. Environmental Protection Agency (EPA), 2002.

Erdely A, Dahm M, Chen BT, Zeidler-Erdely PC, Fernback JE, Birch ME, Evans DE, Kashon ML, Deddens JA, Hulderman T, Bilgesu SA, Battelli L, Schwegler-Berry D, Leonard HD, McKinney W, Frazer DG, Antonini JM, Porter DW, Castranova V, Schubauer-Berigan MK. Carbon nanotube dosimetry: From workplace exposure assessment to inhalation toxicology. Part Fibre Toxicol 2013;10:53.

Garshick E, Laden F, Hart JE, Davis ME, Eisen EA, Smith TJ. Lung cancer and elemental carbon exposure in trucking industry workers. Environ Health Perspect 2012;120:1301-1306.

Hallberg LM, Ward JB, Hernandez C, Ameredes BT, Wickliffe JK. Part 3. Assessment of genotoxicity and oxidative stress after exposure to diesel exhaust from U.S. 2007-compliant diesel engines: Report on 1- and 3-month exposures in the ACES bioassay. Advanced Collaborative Emissions Study (ACES) Subchronic Exposure Results: Biologic Responses in Rats and Mice and Assessment of Genotoxicity. Research Report 166. Boston, MA: Health Effects Institute, 2012.

Hallberg LM, Ward JB, Hernandez C, Ameredes BT, Wickliffe JK, HEI Health Review Committee. Part 3. Assessment of genotoxicity and oxidative damage in rats after chronic exposure to new-technology diesel exhaust in the ACES bioassay. Res Rep Health Eff Inst 2015;184:87-105.

Hashimoto AH, Amanuma K, Hiyoshi K, Sugawara Y, Goto S, Yanagisawa R, Takano H, Masumura K, Nohmi T, Aoki Y. Mutations in the lungs of gpt delta transgenic mice following inhalation of diesel exhaust. Environ Mol Mutagen 2007;48:682-693.

HEI. Advanced Collaborative Emissions Study (ACES). Subchronic Exposure Results: Biological Responses in Rats and Mice and Assessment of Genotoxicity. Research Report 166. Boston, MA: Health Effects Institute, 2012.

Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, Levsen K. Chronic inhalation exposure of wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal Toxicol 1995;7:533-556.

Heinrich U, Muhle H, Takenaka S, Ernst H, Fuhst R, Mohr U, Pott F, Stober W. Chronic effects on the respiratory-tract of hamsters, mice and rats after long-term inhalation of high-concentrations of filtered and unfiltered diesel-engine emissions. J Appl Toxicol 1986;6:383-395.

IARC. Diesel and gasoline engine exhaust and some nitroarenes. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Vol. 105. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France: WHO; International Agency for Research to Cancer, 2014.

Ishinishi N, Kuwabara N, Takaki Y. Long-term inhalation experiments on DE. In: Research Committee for HERP Studies. Diesel Exhaust and Health Risks: Results of the HERP studies. Tsukuba, Ibaraki, Japan: Japan Automobile Research Institute, 1988. pp. 11-84.

Jacobsen NR, Møller P, Cohn CA, Loft S, Vogel U, Wallin H. Diesel exhaust particles are mutagenic in FE1-MutaMouse lung epithelial cells. Mutat Res 2008;641:54-57.

Jacobsen NR, Saber AT, White P, Møller P, Pojana G, Vogel U, Loft S, Gingerich J, Soper L, Douglas GR, Wallin H. Increased mutant frequency by carbon black, but not quartz, in the lacZ and cII transgenes of muta mouse lung epithelial cells. Environ Mol Mutagen 2007;48:451-461.

Kasai T, Umeda Y, Ohnishi M, Mine T, Kondo H, Takeuchi T, Matsumoto M, Fukushima S. Lung carcinogenicity of inhaled multiwalled carbon nanotube in rats. Part Fibre Toxicol 2016;13:53.

Kyjovska ZO, Jacobsen NR, Saber AT, Bengtson S, Jackson P, Wallin H, Vogel U. DNA strand breaks, acute phase response and inflammation following pulmonary exposure by instillation to the diesel exhaust particle NIST1650b in mice. Mutagenesis 2015;30:499-507.

Massling A, Nøjgaard JK, Ellermann T, Ketzel M, Nordstøm C. Particle project report 2008 - 2010. Particulate contribution from traffic in Copenhagen. NERI Technical Report, No. 837. Aarhus, DK: National Environmental Research Institute, Aarhus University, 2011.

Mauderly JL, Jones RK, Griffith WC, Henderson RF, McClellan RO. Diesel exhaust as a pulmonary carcinogen in rats exposed chronically by inhalation. Fundam Appl Toxicol 1987;9:208-221.

Mauderly JL, Jones RK, McClellan RO, Henderson RF, Griffith WC. Carcinogenicity of diesel exhaust inhaled chronically by rats. Dev Toxicol Environ Sci 1986;13:397-409.

Mauderly JL, Snipes MB, Barr EB, Belinsky SA, Bond JA, Brooks AL, Chang IY, Cheng YS, Gillett NA, Griffith WC. Pulmonary toxicity of inhaled diesel exhaust and carbon black in chronically exposed rats. Part I: Neoplastic and nonneoplastic lung lesions. Res Rep Health Eff Inst 1994;(68 Pt 1):1-75.

McDonald JD, Doyle-Eisele M, Gigliotti A, Miller RA, Seilkop S, Mauderly JL, Seagrave J, Chow J, Zielinska B. Part 1. Biologic responses in rats and mice to subchronic inhalation of diesel exhaust from U.S. 2007-compliant engines: Report on 1-, 3-, and 12-month exposures in the ACES bioassay. Advanced Collaborative Emissions Study (ACES) Subchronic Exposure Results: Biologic Responses in Rats and Mice and Assessment of Genotoxicity. Research Report 166. Boston, MA: Health Effects Institute, 2012.

McDonald JD, Doyle-Eisele M, Seagrave J, Gigliotti AP, Chow J, Zielinska B, Mauderly JL, Seilkop SK, Miller RA. Part 1. Assessment of carcinogenicity and biologic responses in rats after lifetime inhalation of new-technology diesel exhaust in the ACES bioassay. Res Rep Health Eff Inst 2015;184:9-44.

McDonald JD, Harrod KS, Seagrave J, Seilkop SK, Mauderly JL. Effects of low sulfur fuel and a catalyzed particle trap on the composition and toxicity of diesel emissions. Environ Health Perspect 2004;112:1307-1312.

Milara J, Cortijo J. Tobacco, inflammation, and respiratory tract cancer. Curr Pharm Des 2012;18:3901-3938.

Mohr U, Takenaka S, Dungworth DL. Morphologic effects of inhaled diesel engine exhaust on lungs of rats: Comparison with effects of coal oven flue gas mixed with pyrolyzed pitch. Dev Toxicol Environ Sci 1986;13:459-470.

Möhner M. On the approach for calculating occupational exposure limits for diesel motor exhaust. Occup Environ Med 2017a;74:926.

Möhner M. Re: Occupational diesel exposure, duration of employment, and lung cancer: An application of the parametric G-formula. Epidemiology 2017b;28:e63-e64.

Möhner M, Kersten N, Gellissen J. Diesel motor exhaust and lung cancer mortality: Reanalysis of a cohort study in potash miners. Eur J Epidemiol 2013;28:159-168.

Möhner M, Wendt A. A critical review of the relationship between occupational exposure to diesel emissions and lung cancer risk. Crit Rev Toxicol 2017;47:185-224.

Nikula KJ, Snipes MB, Barr EB, Griffith WC, Henderson RF, Mauderly JL. Comparative pulmonary toxicities and carcinogenicities of chronically inhaled diesel exhaust and carbon black in F344 rats. Fundam Appl Toxicol 1995;25:80-94.

Oravisjärvi K, Pietikäinen M, Ruuskanen J, Niemi S, Laurén M, Voutilainen A, Keiski RL, Rautio A. Diesel particle composition after exhaust after-treatment of an off-road diesel engine and modeling of deposition into human lung. J Aerosol Sci 2014;69:32-47.

Palmgren F, Wåhlin P, Loft S. Luftforurening med partikler i København. En oversigt. Faglig rapport fra DMU nr. 433. København: Danmarks Miljøundersøgelser, Miljøministeriet, 2003.

Rissler J, Swietlicki E, Bengtsson A, Boman C, Pagels J, Sandström T, Blomberg A, Löndahl J. Experimental determination of deposition of diesel exhaust particles in the human respiratory tract. J Aerosol Sci 2012;48:18-33.

SCHER/SCCP/SCENIHR. Risk assessment methodologies and approaches for genotoxic and carcinogenic substances. European Commission Health & Consumer Protection, 2009.

Silverman DT, Samanic CM, Lubin JH, Blair AE, Stewart PA, Vermeulen R, Coble JB, Rothman N, Schleiff PL, Travis WD, Ziegler RG, Wacholder S, Attfield MD. The Diesel Exhaust in Miners study: A nested case-control study of lung cancer and diesel exhaust. J Natl Cancer Inst 2012;104:855-868.

Speed N, Blair IA. Cyclooxygenase- and lipoxygenase-mediated DNA damage. Cancer Metastasis Rev 2011;30:437-447.

Steenland K, Deddens J, Stayner L. Diesel exhaust and lung cancer in the trucking industry: Exposure-response analyses and risk assessment. Am J Ind Med 1998;34:220-228.

Stinn W, Teredesai A, Anskeit E, Rustemeier K, Schepers G, Schnell P, Haussmann HJ, Carchman RA, Coggins CRE, Reininghaus W. Chronic nose-only inhalation study in rats, comparing room-aged sidestream cigarette smoke and diesel engine exhaust. Inhal Toxicol 2005;17:549-576.

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

Vermeulen R, Portengen L. Is diesel equipment in the workplace safe or not? Occup Environ Med 2016;73:846-848.

Vermeulen R, Portegen L. On the approach for calculating occupational exposure limits for diesel motor exhaust: Authors' response. Occup Environ Med 2017;74:927-928.

Vermeulen R, Portengen L, Silverman DT, Garshick E, Steenland K. Meta-analysis of lung cancer risk from exposure to diesel exhaust: Vermeulen et al. respond. Environ Health Perspect 2014a;122:A230-A231.

Vermeulen R, Silverman DT, Garshick E, Vlaanderen J, Portengen L, Steenland K. Exposure-response estimates for diesel engine exhaust and lung cancer mortality based on data from three occupational cohorts. Environ Health Perspect 2014b;122:172-177.

Voulgaridou GP, Anestopoulos I, Franco R, Panayiotidis MI, Pappa A. DNA damage induced by endogenous aldehydes: Current state of knowledge. Mutat Res 2011;711:13-27.

WHO. Diesel fuel and exhaust emissions. Environmental Health Criteria, Vol. 171. Geneva: World Health Organization, 1996.

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