National Research Centre
for the Working Environment

Polycyclic aromatic hydrocarbons containing benzo[a]pyrene

Scientific basis for setting a health-based occupational exposure limit

(Polycycliske aromatiske hydrocarboner indeholdende benzo[a]pyren: Videnskabelig dokumentation for helbredsbaserede risikoestimater)

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Foreword

The Danish Working Environment Authority has asked the National Research Centre for the Working Environment (NFA) to reassess the documentation for the Danish occupational exposure limit (OEL) for benzo[a]pyrene (BaP) and other polycyclic aromatic hydrocarbons (PAH).

The latest report evaluating PAH containing BaP is from the Scientific Committee on Occupational Exposure Limits (SCOEL) from 2016, who summarized previous published reports and findings from the literature (SCOEL, 2016). Their recommendation was mainly based on two previous reports from the Dutch Expert Committee on Occupational Standards (DECOS) from 2006 and Ausschuss für Gefahrstoffe (AGS) from 2011, respectively (AGS, 2011; DECOS, 2006). These reports used BaP as a marker for total PAH content. The same approach will be used in the present report. Both reports established dose-response relationships for BaP-induced excess lung cancer risk for health-based OELs. These three previous risk assessment reports will lay the foundation for the re-evaluation of the current Danish OEL in the present report, in conjunction with a literature search on epidemiological PAH exposure studies.

The OEL derivation and risk assessment methodology of this report will follow the guidelines outlined by REACH guidance documents (ECHA-RAC/SCOEL, 2017b; ECHA, 2012, 2019; ECHA/RAC-SCOEL, 2017a).

Documentarist Elizabeth Bengtsen, NFA, is gratefully acknowledged for assistance with the literature search.

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

Contents

Abbreviations

3-MC	3-methylcholanthrene
AhR	Aryl hydrocarbon-receptor
AKR	Aldo-keto reductase
BaP	Benzo[a]pyrene
BaPeq	BaP toxic equivalent concentration
BPDE	Benzo(a)pyrene-diol-epoxide
BSM	Benzene soluble matter
Carc	Carcinogenicity
CB	Carbon black particle
CIs	Confidence intervals
CYP	Cytochrome
DMBA	Dimethylbenz-(a)-anthracene
DNEL	Derived No-Effect Level
E-HAP	Exporisq-HAP
GSH	Glutathione
HBC-OCRV	Health-based calculated - occupational cancer risk value
LOAEL	Lowest observed adverse effect level
Muta	Mutagenicity
NOAEL	No observed adverse effect level
OEL	Occupational exposure limit
PAH	Polycyclic aromatic hydrocarbons
Repr	Reproductive toxicity
ROS	Reactive oxygen species
RR	Relative risk
Skin Sens	Skin sensitisation
SMRs	Standardized mortality ratios
TEF	Toxic equivalent factor
TWA	Time-weighted average
UNC	Unexposed controls
URRs	Unit relative risks

Executive summary

In this report, the working group at the National Research Centre for the Working Environment reviewed data relevant to assess the hazard of benzo[a]pyrene (BaP) and other polycyclic aromatic hydrocarbons (PAH) and calculate health-based occupational exposure limits (OELs) based on data from both human and animal studies. The current Danish OEL is 0.2 mg/m^3 for PAH (benzene soluble fraction). No specific OEL exists for BaP in Denmark.

PAH consist of two or more fused aromatic rings of carbon and hydrogen atoms. They are formed as bi-products during combustion and pyrolysis processes of organic materials. Man-made sources provide the greatest source of release. More than 100 single PAH have been identified. PAH do not exist isolated, but as components in complex mixtures that contain many different PAH and related compounds. The mixture composition is primarily determined by combustion temperature. The most extensively studied PAH as surrogate for total PAH exposure is BaP, which consists of five aromatic benzene rings.

BaP is classified as carcinogenic to humans by IARC (class 1) and in EU, the substance is classified for carcinogenicity (Carc 1B), mutagenicity (Muta. 1B), reproductive toxicity (Repr 1B), and for skin sensitisation (Skin Sens. 1). BaP is considered to contribute significantly to the carcinogenic potency of PAH-rich mixtures. Other PAH have been classified as possibly or probably carcinogenic to humans (class 2A or 2B) by IARC. National and international authorities have decided to use BaP as an indicator for total PAH exposure. Similarly, the current working group is of the opinion that BaP can be used as a quantitative indicator of airborne PAH exposure in the working environment.

Occupational exposure to PAH mainly occurs in the major PAH-generating industries, including coal liquefaction and gasification, coke production, coal-tar distillation, roofing and paving, iron and steel foundries, wood impregnation with creosote, aluminium production, carbon-electrode manufacture, chimney sweeping, and power plants. Based on the presented data, the present working group notes that BaP exposure levels vary up to a 1000-fold between occupational settings, with the highest exposure levels ranging from 26 to around 100 μg BaP/m^3.

Airborne PAH can be absorbed though inhalation, (secondary) ingestion and skin contact. Thus, there are several occupationally relevant routes of exposure. Metabolism occurs at the site of exposure, and metabolites can subsequently be found in blood and in urine. Furthermore, the current working group notes that PAH and their metabolites are able to pass the placental barrier, leading to potential reproductive and developmental toxicity. PAH metabolites and their conjugates do not persist in the body, but are rapidly excreted in urine and faeces. The current working group notes that 1-hydroxypyrene in urine is the most commonly used biomarker of BaP and PAH exposure, but that urine levels of the BaP metabolite 3-hydroxy-benzo[a]pyrene may prove to be a more suitable biomarker for biomonitoring.

The most well-described toxicological endpoint of PAH, and especially BaP, exposure is cancer. Due to the complexity of PAH exposure, no epidemiological data on BaP exposure alone is available. From the literature search, three key risk assessment studies were identified, which comprise cohort data ranging from the 1970s to 2014, and use different approaches for calculating and describing their data. Based on available epidemiological evidence, the current working group considers lung and bladder cancer as critical effects of BaP and PAH exposure. Animal data support this conclusion. The main mechanism of action for cancer is metabolism of PAH and BaP, which leads to bio-activated DNA-reactive metabolites through the diol epoxide pathway, the radical cation pathway, and the *o*-quinone and reactive oxygen species (ROS) pathways. The reactive metabolites directly damage the DNA by formation of DNA adducts and ROS-mediated damage. The current working group considers metabolite-induced mutagenesis as the primary mode of action and as a non-threshold mechanism leading to genotoxicity, mutagenicity and cancerous changes.

The most well-documented non-cancerous effects of PAH and BaP exposure relate to reproductive and developmental effects. Male reproductive toxicity includes lowered sperm quality, primarily through genotoxicity, altered Leydig cell function, and oxidative stress. Female reproductive toxicity probably occurs through stimulation of apoptosis (resulting in follicle depletion), alterations in the estrous cycle, hormone imbalance and genotoxicity. BaP exposure induces developmental effects primarily through genotoxicity and mutagenicity, altered cell signalling, and oxidative stress. US EPA identified male and female reproductive system effects as well as developmental toxicity (including developmental neurotoxicity), as a human hazard of BaP exposure. Based on the available information in both human and animal reproductive toxicity studies, the current working group considers both male and female reproductive toxicity, and developmental toxicity as critical effects of BaP exposure through a threshold mechanism.

Thus, based on the reviewed literature, the current working group concluded that lung cancer, reproductive toxicity and developmental toxicity are all critical effects of PAH and BaP exposure. An OEL based on non-threshold lung cancer data from epidemiological studies and DNELs based on threshold effects from animal reproductive and developmental toxicity studies were therefore calculated.

Prior to the risk assessment calculations, the most recent and relevant risk assessments of PAH and BaP were reviewed. Both reports calculated their risk levels for lung cancer after 45 work years based on data from the meta-study by Armstrong and colleagues (see table below)(Armstrong et al., 2004; Armstrong, 2003).

Overview of health-based OELs calculated in previous assessments after 45 work years based on a non-threshold mechanism of action.

Lung cancer			
Previous assessments			
DECOS 2006		AGS 2011	
HBC-OCRV	BaP air concentration (µg/m^3)	Excess lung mortality risk	BaP air concentration (µg/m^3)
4 per 1,000	0.454	4: 1,000	0.64
4 per 10,000	0.049	4: 10,000	0.066
4 per 100,000	0.0051	4: 100,000	0.0066
1 per 1,000	0.114	1: 1,000	0.160
1 per 10,000	0.012	1: 10,000	0.017
1 per 100,000	0.0013	1: 100,000	0.0017

OEL based on lung cancer data from human studies

The present working group calculated and proposed health-based OELs for BaP (as a proxy for total PAH content) for the Danish working environment based on the lung cancer data from the meta-analysis by Armstrong and colleagues (Armstrong et al., 2004; Armstrong, 2003), taking the same approach as previous reports. The calculated excess lung cancer incidences after 45 work years are presented in the table below. High comparability with previous risk assessments were observed. In addition to the lung cancer assessment, the current working group also recommends a skin notation for BaP and other PAH.

Calculated excess lung cancer incidence based on epidemiological data.

Lung cancer	
Present report	
Excess lung cancer incidence	BaP air concentration (µg/m^3)
1: 1,000	0.24
1: 10,000	0.024
1: 100,000	0.0024

DNEL based on reproductive toxicity and developmental toxicity in mice

DNELs for male and female reproductive toxicity and developmental toxicity based on animal studies were calculated as recommended by ECHA for toxicological effects having thresholds (ECHA, 2012). A lowest observed adverse effect level (LOAEL) of 75 µg BaP/m^3 for 4 hours a day for 60 days was identified for male reproductive effects, and a no observed adverse effect level (NOAEL) of 75 µg BaP/m^3 for 4 hours a day for 14 days was identified for female reproductive effects. A LOAEL for developmental effects was identified at 25 µg BaP/m^3 for exposure for 4 hours a day for 10 days (gestation days 11–20). Due to the range in assessment factors for LOAEL to NOAEL (3 or 10), $AF_{overall\ min}$ and $AF_{overall\ max}$ were calculated for male reproductive and developmental toxicity. The current working group has decided to present calculations based on $AF_{overall\ min}$, but notes the lower DNELs when using the $AF_{overall\ max}$. The current working group notes that the

severity of the reported reproductive and developmental effects, combined with introduction of sensitive groups, could warrant the use of the more strict assessment factor. DNELs for male and female reproductive toxicity and developmental toxicity are presented in the table below.

Overview of DNEL for BaP air concentration based on a threshold based mechanism of action.

Non-neoplastic toxicity					
Developmental toxicity		**Reproductive toxicity**			
		Male		Female	
DNEL	0.223 µg/m^3	DNEL	0.335 µg/m^3	DNEL	0.335 µg/m^3

The current working group considers both cancer and the identified non-neoplastic toxicities as critical effects. Therefore, the current working group recommends that both outcomes are taken into consideration.

Dansk sammenfatning

Ved fastsættelse af grænseværdier i arbejdsmiljøet indgår en række hensyn. Det drejer sig om helbredsrisikoen, men også tekniske og samfundsmæssige hensyn.

I NFA's arbejde med grænseværdidokumentation anvendes risikoestimater, som er et teoretisk mål for hvor mange, der ved daglig udsættelse for stoffet ved grænseværdien efter et helt arbejdsliv (typisk efter 40-45 år) vil blive syge. I disse beregninger, er der *ikke* taget hensyn til personlige værnemidler eller andre kendte foranstaltninger til beskyttelse mod eksponering.

NFA udarbejder dokumentation for helbredsbaserede grænseværdier. Der tages udgangspunkt i publiceret systematisk litteraturgennemgang af epidemiologiske studier, dyrestudier og cellestudier af sammenhængen mellem udsættelse og risiko for forskellige helbredsudfald og de biologiske virkningsmekanismer. På baggrund af dette videnskabelige arbejde beregnes risikoestimaterne.

Dokumentation for helbredsbaserede grænseværdier vil sammen med de tekniske og samfundsmæssige betragtninger ligge til grund for forhandlinger mellem arbejdsmarkedets parter om endelig fastsættelse af grænseværdierne.

I denne rapport vurderer en arbejdsgruppe ved NFA data, der er relevante for at evaluere faren ved udsættelse for polycykliske aromatiske kulbrinter (PAH) og benzo[a]pyrene (BaP), og beregner helbredsbaserede græseværdier for disse i arbejdsmiljøet. Beregningerne baseres på data fra både humane studier og dyreforsøg. Den nuværende danske grænseværdi for PAH i arbejdsmiljøet er 0,2 mg/m^3. Der er ikke pt en dansk grænseværdi for BaP.

PAH består af en eller flere aromatiske ringe af kulstof og brintatomer. De bliver dannet som biprodukter under forbrænding og pyrolyse af organiske materialer, primært fra menneskeskabte kilder. Der findes mere end 100 typer PAH. PAH findes dog ikke som isolerede stoffer, men indgår som komponenter i komplekse blandinger som indeholder mange forskellige PAH og tilhørende materialer, som fx i forbrændingspartikler. Sammensætningen i disse blandinger bestemmes primært af forbrændingens temperatur. BaP består af fem aromatiske ringe og er det mest undersøgte markørstof for total PAH eksponering.

BaP er klassificeret som kræftfremkaldende for mennesker af IARC (klasse 1) og i EU er BaP klassificeret som kræftfremkaldende (Carc. 1B), mutagent (Muta. 1B), reproduktionsskadeligt (Repr. 1B), og hudsensibiliserende (Skin Sens. 1). BaP anses for at bidrage væsentlig til den kræftfremkaldende effekt af PAH blandinger. Andre PAH typer er blevet klassificeret som sandsynligvis eller muligvis kræftfremkaldende for mennesker (klasse 2A eller 2B) af IARC. Både nationale og internationale autoriteter har besluttet at bruge BaP som indikator for total PAH eksponering. Denne arbejdsgruppe er enig i beslutningen og vil i nærværende rapport bruge BaP som en kvantitativ indikator for luftbåren PAH eksponering i arbejdsmiljøet.

Arbejdsmiljømæssig eksponering for PAH sker hovedsagelig i PAH-genererende processer, så som kulforarbejdning, koksproduktion, kultjæreproduktion, tagdækning og vejbelægning, jern- og stålstøberier, træimprægnering med kreosot, aluminiums-produktion, fremstilling af kulstofelektroder, kraftværker, samt skorstensfejning. Arbejdsgruppen noterer, baseret på de præsenterede data, at BaP eksponeringsniveauer varierer med op til 1000-fold mellem arbejdspladsmålinger, med de højeste eksponeringsniveauer liggende fra 26 til omkring 100 µg BaP/m^3.

Luftbåren PAH kan blive optaget i kroppen via indånding, (sekundær) indtagelse og gennem huden. Der er derfor flere arbejdsmiljømæssige relevante eksponeringsveje. Metabolisme af PAH starter på eksponeringsstedet og PAH metabolitter kan efterfølgende findes i blod og urin. Derudover noterer arbejdsgruppen, at PAH og dets metabolitter kan passere moderkagens barrier, hvilket potentielt kan lede til fosterskader. PAH metabolitter og dets konjugater ophobes ikke i kroppen, men udskilles i urin og fæces. Måling af 1-hydroxypyrene i urinen er den mest almindelige biomarkør for BaP og PAH eksponering, men urinniveauer for BaP metabolitten 3-hydroxy-benzo[a]pyren kunne vise sig at være en bedre biomarkør til biomonitorering.

Den mest velbeskrevne helbredseffekt af udsættelse for PAH, og specielt BaP, er kræft. På grund af kompleksiteten i PAH sammensætningen ved eksponering, findes der ikke epidemiologisk data for BaP eksponering alene. Der blev i litteratursøgningen identificeret tre hovedstudier, som inkluderer kohortedata fra studier publiceret fra 1970erne til 2014, og som bruger forskellige tilgange for at udregne og beskrive deres data. Baseret på de tilgængelige epidemiologiske data, anser arbejdsgruppen lungekræft og blærekræft som kritiske effekter af BaP og PAH eksponering. Data fra dyr understøtter dette. Hovedmekanismen bag kræftudviklingen opstår når PAH og BaP omsættes i kroppen. Dette medfører dannelsen af DNA-reaktive metabolitter via forskellige molekylære pathways kaldet: "The diol epoxide pathway, the radical cation pathway, and the *o*-quinone and ROS pathways". De reaktive metabolitter kan direkte skade DNA'et via dannelsen af DNA addukter og via ROS-medieret DNA skader. Arbejdsgruppen anser derfor metabolit-induceret DNA skade som hovedmekanisme for effekten af PAH og BaP eksponering. På grund af den DNA-skadende virknings-mekanisme for BaP-induceret kræft, er der ingen nedre grænse for effekt, og arbejdsgruppen anser derfor BaP-induceret kræft for at være en ikke-tærskeleffekt, der fører til genotoksicitet, mutationer og kræft.

De mest dokumenterede, ikke-kræftrelaterede effekter af PAH og BaP eksponeringer er skader på hanlig og hunlig fertilitet og fosterudvikling. Skader på fertilitet hos mænd inkluderer nedsat sædkvalitet (hovedsagligt via genotoksicitet), ændret Leydig cellefunktion, og oxidativt stress. Hos kvinder sker de reproduktive effekter sandsynligvis gennem programmeret celledød, hvilket fører til fald i folikelantal, gennem ændringer i menstruations- eller østruscyklussen, gennem ændringer i hormonniveauer eller gennem genotoksicitet. BaP eksponering forsager primært skader på fosterudvikling gennem genotoksicitet og mutationer, ændret cellesignalering, og oxidativt stress. Det amerikanske miljøagentur (US EPA) har konkluderet, at udsættelse for BaP forårsager skader på hanlig og hunlig fertilitet og fosterudvikling. Baseret på både epidemiologiske studier og dyrestudier, anser arbejdsgruppen både skader på

hanlig og hunlig fertilitet og fosterudvikling igennem tærskeleffekt mekanismer som kritiske effekter af BaP og PAH eksponering.

Baseret på den gennemgåede litteratur, konkluderer arbejdsgruppen derfor, at lungekræft, skader på hanlig og hunlig fertilitet og fosterudvikling alle er kritiske effekter af BaP og PAH eksponering. Arbejdsgruppen udregner, på den baggrund, helbredsbaserede grænseværdier baseret på lungekræftdata fra humane studier, og derudover Derived No-Effect Levels (DNELs) for tærskeleffekternes skader på hanlig og hunlig fertilitet og fosterudvikling.

De nyeste og mest relevante risikovurderinger for PAH og BaP blev gennemgået. Disse bygger alle på data fra det samme metastudie (Armstrong et al., 2004; Armstrong, 2003), og estimerer overskydende lungekræftdødelighedsrisiko efter 45 arbejdsår (se tabel nedenfor).

Oversigt over tidligere risikovurderinger og estimeret overskydende lungekræftdødelighedsrisiko efter 45 arbejdsår baseret på en ikke-tærskeleffektmekanisme.

Lungekræft			
Tidligere vurderinger			
DECOS 2006		AGS 2011	
HBC-OCRV	BaP luft-koncentration (µg/m^3)	Overskydende lungedødeligheds-risiko	BaP luft-koncentration (µg/m^3)
4 per 1.000	0,454	4: 1.000	0,64
4 per 10.000	0,049	4: 10.000	0,066
4 per 100.000	0,0051	4: 100.000	0,0066
1 per 1.000	0,114	1: 1.000	0,160
1 per 10.000	0,012	1: 10.000	0,017
1 per 100.000	0,0013	1: 100.000	0,0017

Beregning af grænseværdi baseret på dødelighedsdata for lungekræft fra humane studier

De foreslåede helbredsbaserede grænseværdier for BaP (som surrogat for PAH) i det danske arbejdsmiljø er udregnet med baggrund i det tidligere nævnte metastudie fra Armstrong og kollegaer (Armstrong et al., 2004; Armstrong, 2003). Derved bruger arbejdsgruppen samme tilgang som tidligere risikovurderinger. De udregnede overskydende lungekræftstilfælde efter 45 arbejdsår er vist i tabellen nedenunder. Der ses stor lighed med tidligere risikovurderinger, og den hollandske grænseværdi for BaP, som er 0,55 µg/m^3 ifølge GESTIS databasen. Derudover anbefaler arbejdsgruppen også en hudnotifikation for BaP og PAH.

Udregnet overskydende lungekræftdødelighedsrisiko baseret på epidemiologisk data.

Lungekræft	
Nærværende rapport	
Overskydende lungekræfts-tilfælde	BaP luft-koncentration (µg/m^3)
1: 1.000	0,24
1: 10.000	0,024
1: 100.000	0,0024

Beregning af grænseværdier baseret på skader på hanlig og hunlig fertilitet og fosterudvikling i mus

DNELs blev udregnet for nedsat mandlig og kvindelig fertilitet og for skader på fosterudvikling som anbefalet af ECHA for toksiske effekter igennem tærskeleffekt mekanismer (ECHA, 2012). Et LOAEL på 75 µg BaP/m^3 for eksponering i 4 timer om dagen i 60 dage blev identificeret for nedsat hanlig fertilitet, og et NOEAL på 75 µg BaP/m^3 for eksponering i 4 timer om dagen i 14 dage blev identificeret for nedsat hunlig fertilitet. For påvirkning af fosterudvikling blev et LOAEL på 25 µg BaP/m^3 identificeret for eksponering i 4 timer om dagen i 10 dage (drægtighedsdage 11–20). For påvirkning af mandlig fertilitet og for skader på fosterudvikling blev der udregnet to forskellige DNELs, som varierer med hensyn til størrelsen af sikkerhedsfaktor for brugen af en LOAEL-værdi i stedet for NOAEL (3-10). Arbejdsgruppen har valgt at fremstille DNELs udregnet med den mindste sikkerhedsfaktor. Men arbejdsgruppen noterer også at alvorligheden af den observeret toksicitet, kombineret med mulighed for eksponering af følsomme grupper, indikerer at en mere konservativ tilgang kunne overvejes. DNELs for skader på hanlig og hunlig fertilitet og fosterudvikling er præsenteret i tabellen nedenfor.

Oversigt over DNELs for BaP luftkoncentration baseret på tærskeleffektmekanismer.

Ikke-kræftrelateret toksicitet					
Skader på fosterudvikling		**Skader på fertilitet**			
		Mand		Kvinde	
DNEL	0,223 µg/m^3	DNEL	0,335 µg/m^3	DNEL	0,335 µg/m^3

Arbejdsgruppen anser både kræft, skader på fertilitet og skader på fosterudvikling som kritiske effekter. Derfor anbefales det, at alle endepunkter tages i betragtning.

Introduction

Literature search

Polycyclic aromatic hydrocarbons (PAH) and benzo[a]pyrene (BaP) are well-established substances with extensive literature catalogues. For the present report, the current working group conducted a literature search in the bibliographic database, PubMed, identifying the recent peer reviewed original journal publications on BaP (1978-2020). However, certain criteria were incorporated to exclude studies not relevant for risk assessment. The following search term was used:

Search: ((("50 32 8"[EC/RN Number]) OR ((((("benzo a pyrene") OR ("benzo (a) pyrene")) OR ("benzo(a)pyrene")) OR ("benzo[a]pyrene")) OR (benzo a pyrene[MeSH Terms])) AND (((((exposure*[Title/Abstract]) OR (exposed[Title/Abstract])) OR (occupational exposure[MeSH Terms])) AND (humans[MeSH Terms])) AND (English[Language])) AND ((("Meta-Analysis" [Publication Type] OR "Meta-Analysis as Topic"[Mesh]) OR (((((("Meta-Analysis" [Publication Type] OR "Meta-Analysis as Topic"[Mesh]) OR ("meta analysis"[Title/Abstract])) OR ("meta-analysis"[Title/Abstract])) OR ("meta analyses"[Title/Abstract])) OR ("meta-analyses"[Title/Abstract])) AND (humans[MeSH Terms])) AND (English[Language]))) OR (((((("exposure-response"[Title/Abstract]) OR ("dose-response"[Title/Abstract])) OR ("dose response"[Title/Abstract])) OR ("risk assessment"[Title/Abstract])) OR (risk assessment[MeSH Terms])) AND (humans[MeSH Terms])) AND (English[Language]))))

The search resulted in 332 publications. Of these, 278 publications were excluded based on a manual review of the titles, resulting in 54 publications selected for abstract review. Of these, 17 publications were excluded and 15 publications were selected for full text in-depth review. The remaining 22 publications were set aside and divided into different categories depending on their topic, as they could provide important information on certain aspects of the current report, other than OEL calculation (e.g. mechanistic information). Of the 15 in-depth reviewed publications, three were selected for descriptive review in the report (Armstrong et al., 2004; Petit et al., 2019; Rota et al., 2014).

Polycyclic Aromatic Hydrocarbons

PAH are a large class of organic compounds consisting of two or more fused aromatic rings of carbon and hydrogen atoms. Numerous configurations of conjugated aromatic rings are possible. The physicochemical properties of PAH are largely determined by the number of rings and molecular mass. Examples of PAH and some of their chemical properties are presented in Table 1. Most PAH are highly lipophilic and therefore soluble in many organic solvents, whereas their water solubility is low. Depending on the vapour pressure, airborne PAH with three or lower aromatic rings are sufficiently volatile to be present as gaseous compounds in the working environment. PAH with four rings may be present both in the gas phase and bound to airborne particulates, and PAH with higher molecular weights (>228) are typically bound to airborne particulates (IARC, 2010). Furthermore, considerable amounts of PAH can also be found in tars and pitches and during the processing of such materials, they are released in the gaseous state, adsorbed onto particles or in form of dust into the surrounding air. In the air, they

show chemical and photochemical reactions, despite them being rather chemically inert (DECOS, 2006).

In the present report, the term PAH will comprise of unsubstituted non-heterocyclic PAH (including alkyl-substituted derivates) only, and only these will be considered for evaluation. This is in line with previous reports (DECOS, 2006; IARC, 2010; IPCS, 1998).

Table 1. Chemical properties of typical constituents of PAH mixtures (DFG, 2012).

Name	CAS No.	Molecular formula	Molecular mass (g/mol)	Melting point (°C)	Boiling point (°C)	Vapour pressure (Pa at 25 °C)	log K_{ow}	Genotoxicity (WHO 1998)	Carcinogenicity (WHO 1998)
anthanthrene	191-26-4	$C_{22}H_{12}$	276.3	264	547			(+)	+
benzo[*b*]fluoranthene	205-99-2	$C_{20}H_{12}$	252.3	168.3	481	6.7×10^{-5}	6.12	+	+
benzo[*a*]anthracene	56-55-3	$C_{18}H_{12}$	228.3	160.7	400	2.8×10^{-5}	5.61	+	+
benzo[*j*]fluoranthene	205-82-3	$C_{20}H_{12}$	252.3	165.4	480	2.0×10^{-6}	6.12	+	+
benzo[*k*]fluoranthene	207-08-9	$C_{20}H_{12}$	252.3	215.7	480	1.3×10^{-8}	6.84	+	+
benzo[*b*]naphtho[2,1-*d*]-	239-35-0	$C_{16}H_{10}S$	234.3	185–188	160–180	–	–	+	+
benzo[*a*]pyrene	50-32-8	$C_{20}H_{12}$	252.3	178.1	496	7.3×10^{-7}	6.50	+	+
chrysene	218-01-9	$C_{18}H_{12}$	228.3	253.8	448	8.4×10^{-5}	5.91	+	+
cyclopenta[*cd*]pyrene	27208-37-3	$C_{18}H_{10}$	226.3	170	439	–	–	+	+
dibenzo[*a*,*h*]anthracene	53-70-3	$C_{22}H_{14}$	278.4	266.6	524	1.3×10^{-8}	6.50	+	+
dibenzo[*a*,*l*]pyrene	191-30-0	$C_{24}H_{14}$	302.4	162.4	595	–	–	(+)	+
dibenzo[*a*,*e*]pyrene	192-65-4	$C_{24}H_{14}$	302.4	244.4	592	–	–	+	+
dibenzo[*a*,*h*]pyrene	189-64-0	$C_{24}H_{14}$	302.4	317	596	–	–	(+)	+
dibenzo[*a*,*i*]pyrene	189-55-9	$C_{24}H_{14}$	302.4	282	594	3.2×10^{-10}	7.3	+	+
indeno[1,2,3-*cd*]pyrene	193-39-5	$C_{22}H_{12}$	276.3	163.6	536	1.3×10^{-8}	6.58	+	+
naphthalene	91-20-3	$C_{10}H_8$	128.2	81	217.9	10.4	3.4	–	
phenanthrene	85-01-8	$C_{14}H_{10}$	178.2	100.5	340	1.6×10^{-2}	4.6		
pyrene	129-00-0	$C_{16}H_{10}$	202.3	150.4	393	6.0×10^{-4}	5.18		
1-methylpyrene	2381-21-7	$C_{17}H_{12}$	216.3	70–71	410				

PAH are bi-products, formed during combustion and pyrolysis processes of organic materials. Although naturally occurring, man-made sources provide the greatest source of release. More than 100 single PAH have been identified. However, in practice, PAH do not exist isolated but as components in complex mixtures that contain many different PAH and related compounds. This is due to the way they are produced or processed both naturally and artificially (DECOS, 2006; SCOEL, 2016). Combustion temperature is a main determinant of the PAH composition of the mixture (DFG, 2012).

The current Danish occupational exposure limit (OEL) is 0.2 mg/m^3 for PAH (benzene soluble fraction). ECHA classification of BaP can be seen in Table 2.

Table 2. Summary of classification from ECHA.

Classification		Specific Concentration limits, M-Factors, Acute Toxicity Estimates
Hazard Class and Category Code(s)	Hazard Statement Code(s)	
Skin Sens. 1	H317	Carc. 1B; H350: C ≥ 0,01 %
Muta. 1B	H340	
Carc. 1B	H350	
Aquatic Acute 1	H400	
Aquatic Chronic 1	H410	
Repr. 1B	H360FD	

Measurement

Due to the very complex and variable compositions of PAH, several approaches for categorizing PAH exposure have been used in the literature:

1. Using total PAH or the benzene soluble matter (BSM).
2. Using a selection of PAH.
3. Using BaP as an indicator for PAH exposure.
4. Using the BaP toxic equivalent concentration (BaPeq).

1. As the carcinogenic effects observed in epidemiological studies due to exposure to PAH-rich sources are an overall effect of all substances in the mixture, it is of interest to monitor PAH-mixtures as a whole. A way to asses total PAH is to sample the BSM, which is a method that often has been used in the past (DECOS, 2006). Basically, this method involves extracting the content of the collecting filters with benzene and then gravimetrically determining the benzene-soluble fraction. Several OELs are established based on this method, including the current Danish OEL. BSM is prepared from airborne particles and contains not only all unsubstituted and non-heterocyclic PAH, but also other PAH and non-PAH substances. This makes BSM values source dependent and as a result, they may strongly be influenced by additional non-PAH sources near the sampling site. Taken together, this makes BSM less attractive for risk assessment and regulatory purposes.

2. By default, quantification of a series of single PAH should give a clearer picture of the overall PAH exposure and mixture composition than measuring BaP alone. Ideally, such a PAH profile should contain a selection of carcinogenic PAH, which are representative for the overall physicochemical properties of PAH and are present in most PAH-sources. However, no generally accepted selection of PAH as a marker for total PAH exposure is available. In fact, various authorities have proposed different selections of PAH to assess overall PAH exposure (DECOS, 2006). An example of this is the widely used list of 16 PAH issued by the U.S. Environmental Protection Agency (EPA) in 1976 (Keith, 2015). This list was developed for assessing risks to human health from drinking water, and its utility for assessing airborne occupational exposure is questionable. The general lack of

consensus regarding which PAH to select makes comparisons across studies and reports troublesome.

3. The most extensively studied PAH as surrogate for total PAH exposure is BaP. BaP consists of five aromatic benzene rings (Figure 1) and is released from a great variety of PAH-sources. Identified as the predominant carcinogenic compound in coal tar almost a century ago (Kennaway, 1955; Kennaway, 1930; Phillips, 1983; Yamagiwa K., 1977), BaP has historically been the main focus of exposure assessment and health effect studies among all PAH.

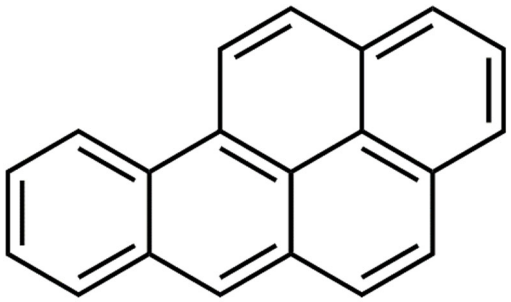

Figure 1. Chemical structure of benzo[a]pyrene

BaP is classified as carcinogenic to humans by IARC (class 1), due to strong and extensive experimental evidence for the carcinogenicity of BaP in many animal species, supported by the consistent and coherent mechanistic evidence from experimental and human studies. BaP is therefore considered to contribute significantly to the carcinogenic potency of PAH-rich mixtures. Various validated analytic techniques are available to measure BaP (IARC, 1983; NRC, 1972, 1983; RIVM, 1989), and most available studies have preferred to use BaP as a marker substance for overall airborne PAH exposure for practical reasons. BaP's behaviour in exposure scenarios has been described as follows:

"At ambient temperatures, BaP is virtually exclusively present in the form of airborne particulate matter. This means, that no significant fluctuation due to condensation, absorption and evaporation has to be expected, when the compound is in the air or during sampling. However, it also means that BaP is a poor predictive marker for the effects of gaseous PAH compounds. This should be kept in mind, because the environmental fate between gaseous and particle–bound PAH may differ. On the other hand, none of the more volatile PAH substances (less than 5 rings) have been shown to be carcinogenic". (DECOS, 2006)

Although BaP may react and decompose during sampling time, BaP's instability does not lead to a complete decomposition, and the influence on its chemical reactivity will most probably be slight (DECOS, 2006). In addition, two studies investigating PAH release in different types of aluminum smelting processes by the Söderberg method have shown a strong relationship between the concentrations of BaP and that of individual and total PAH in the air (the particulate phase PAH) (Sanderson et al., 2005; Farant & Gariepy, 1998). This highlights the applicability of BaP as an indicator for PAH exposure. Indeed, various national and international authorities have decided to use BaP as an indicator for total PAH exposure (AGS, 2011; DECOS, 2006; SCOEL, 2016). Therefore, the current working group considers the use of BaP as a quantitative indicator of the general

airborne PAH exposure to be an acceptable procedure in practice and BaP will be used as a risk indicator in the present report. As of date, Denmark does not have a specific OEL for BaP.

4. Many studies use a benzo[a]pyrene toxic equivalent concentration (BaPeq) as proxy for total PAH in the risk estimation (Bostrom et al., 2002; Cincinelli et al., 2007; Liu et al., 2013; Petit et al., 2019). The aim of this approach is to take the total carcinogenic potency of a mixture of PAH compounds with different carcinogenic potency into account. The concentration of each measured PAH is expressed as BaPeq by using a toxic equivalent factor (TEF), which utilizes BaP as a reference compound to adjust the original concentration depending on the differences in the carcinogenic potency between BaP and the PAH in question (Bostrom et al., 2002). This approach also enables toxicity ranking of PAH. Total BaPeq then corresponds to the sum of BaP and all BaPeqs. The downside to this approach is that studies use different TEFs, and these are not necessarily based on the inhalation route and could therefore misestimate the actual risk when dealing with complex mixtures (Bostrom et al., 2002; Dreij et al., 2017; Petit et al., 2019; Pufulete et al., 2004). In addition, the same PAH are not measured across studies and often only a handful of PAH are taken into account, which could lead to an underestimation of the carcinogenic potency (Samburova et al., 2017).

Ideally, the gold standard for PAH air measurements would be a set of universally approved PAH congeners. However, as of now, no such set have been identified and selected for lung exposure. Many studies therefore use BaP as a surrogate for total PAH exposure. BaP is extensively studied with a vast and consistent amount of data on exposure, and BaP has been identified as one of the more potent PAH carcinogens. Based on this, there is a general acceptance of the use of BaP as surrogate among authorities. This is backed up by studies showing that during Söderberg smelting, BaP air levels correlate with the levels of individual PAH and total PAH (Sanderson et al., 2005; Farant & Gariepy, 1998). An additional study shows that atmospheric BaP and BaPeq levels were very similar across 93 exposure groups belonging to 9 industries (Petit et al., 2019). However, there are negative consequences of simplifying the monitoring process. Many, perhaps important, nuances of PAH exposure are missed. As an example, effects of gaseous PAH compounds can be overlooked, as BaP is a better representative of particle-bound PAH. The current working group acknowledge the issues of using BaP as a proxy for total PAH levels, however ultimately, the current working group is of the opinion that BaP can be used as a quantitative indicator of the general airborne PAH exposure in the working environment.

Toxicokinetics

Although inhalation is considered the primary route of occupational PAH exposure, dermal exposure, and to a lesser extent secondary ingestion, also occur. This section will focus on pulmonary and dermal exposure. However, it should be noted that uptake of PAH in the gastrointestinal tract has been shown to be more rapid than in the lungs and the skin. Hence, a larger portion may reach the internal system by oral exposure as compared to the other exposure routes (Pelkonen & Nebert, 1982).

Uptake and distribution

General uptake and distribution data for PAH was summarized by DECOS:

"PAH are lipophilic compounds and, therefore, they are easily absorbed through the epithelia of the respiratory, gastrointestinal tract and the skin. When absorbed, PAH are distributed via the bloodstream throughout all internal organs, and particularly in those with high fat contents. However, in the literature it is suggested that only a minor portion will reach the circulation, because PAH are metabolised. Furthermore, PAH are able to pass the placental barrier." (DECOS, 2006)

For pulmonary exposure, PM-bound PAH are partly cleared from the lungs by desorption and uptake in the blood, and partly by particle clearance mechanisms. The clearance rate of PM-bound PAH is slow and depends on particle size and lung burden (Gerde et al., 2001).

For dermal exposure, the main question is if absorbed PAH leads to a significant additional cancer risk at other sites than the skin. For this, it is important to know if dermal PAH exposure significantly contributes to the total PAH body burden. This aspect was investigated and summarized in the report from DECOS:

"Both human and animal studies clearly showed that PAH penetrate the skin and reach the circulation. The absorption rates were affected by the viscosity of the vehicle of administration, the anatomical site of application, and the molecular weight of the single PAH. Concerning the distribution of PAH in the body following dermal exposure, no data were found in humans. From the few animal data that are available, it is reasonable to conclude that PAH after dermal exposure are distributed through various internal organs, including the lungs." (DECOS2006)

A more recent study from 2018 in firefighters reported a correlation between dermal PAH exposure and DNA damage in peripheral blood mononuclear cells, as well as a correlation between dermal PAH exposure and urinary 1-hydroxypyrene levels (Andersen et al. 2018). This indicates that PAH absorbed dermally reaches systemic circulation, thereby supporting the conclusions made by DECOS.

The current working group notes that PAH can be absorbed through inhalation, (secondary) ingestion and skin contact. Thus, there are several occupationally relevant routes of exposure. Furthermore, the current working group notes that PAH are able to pass the placental barrier, leading to potential reproductive toxicity.

Metabolism

The metabolism of PAH, and especially BaP, is well studied and has been comprehensively described in previous reports (DECOS, 2006; IARC, 2010, 2012). In general, the PAH are highly lipophilic compounds of low chemical reactivity that need to undergo metabolic activation to become carcinogenic. PAH metabolism starts when they are absorbed through the epithelia of the lungs and the skin, and the longer the retention time, the more PAH will be metabolised (SCOEL, 2016).

BaP metabolism was summarized by IARC as follows:
"Benzo[a]pyrene is metabolized by both phase-I and phase-II enzymes to form a series of arene oxides, dihydrodiols, phenols, and quinones and their polar conjugates with glutathione, sulfate, and glucuronide. Benzo[a]pyrene-7,8-diol is a key metabolite that is formed by the action of epoxide hydrolase on benzo[a]pyrene-7,8-epoxide. This dihydrodiol can be further metabolized by cytochrome P450s (CYPs) to a series of benzo[a]pyrene-7,8-diol-9,10-epoxides, which form one class of ultimate carcinogenic metabolites of benzo[a]pyrene." (IARC, 2012)

This metabolism pathway is called the diol epoxide mechanism and is depicted in Figure 2.

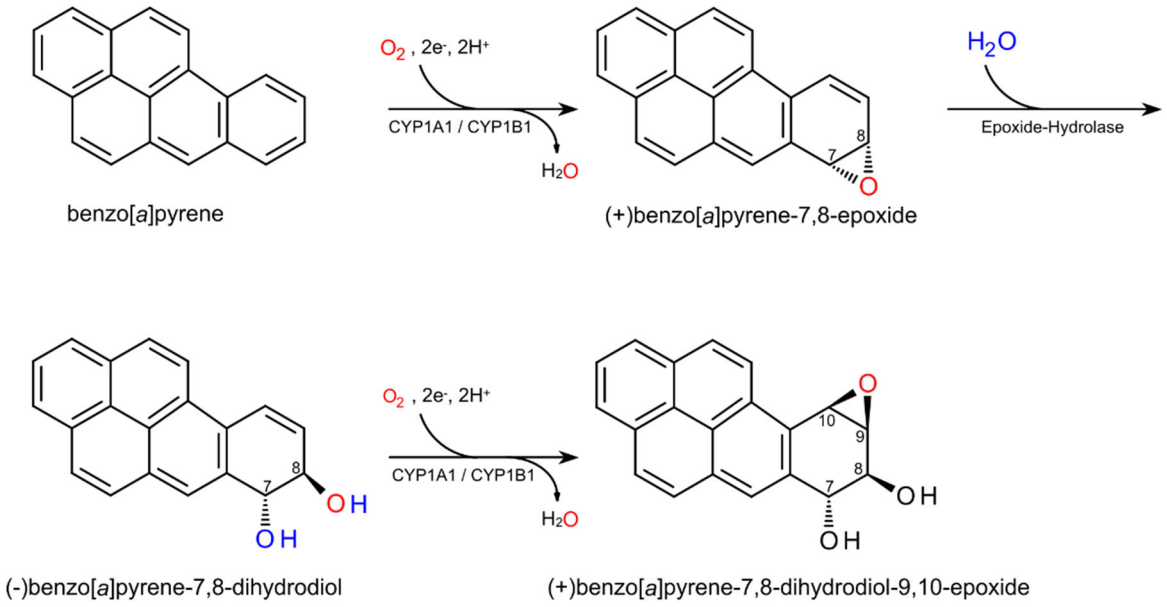

Figure 2. Metabolism of BaP through the diol epoxide mechanism.

IARC continues:

"Both CYPs and peroxidases (e.g. prostaglandin-H synthase) can oxidize benzo[a]pyrene. The major cytochrome P450s involved in the formation of diols and diolepoxides are CYP1A1, CYP1A2 and CYP1B1. Cytochrome P450s are inducible by benzo[a]pyrene and other PAHs through binding to the aryl hydrocarbon-receptor (AhR) nuclear complex, leading to changes in gene transcription of CYPs and phase-II enzymes. Mice lacking the AhR receptor are refractory to benzo[a]pyrene-induced tumorigenesis. Both CYPs and peroxidases can form radical cations by one-electron oxidation. These cations comprise another class of ultimate carcinogenic metabolites" (IARC, 2012)

The carcinogenic properties of BaP and PAH metabolites will be covered in the mechanisms of toxicity section. PAH metabolites and their conjugates do not persist in the body, but are rapidly excreted in the urine and faeces.

The present working group notes that metabolism occurs at the site of exposure and that metabolites can subsequently be found in circulation and in the urine.

Human exposure
Monitoring
Monitoring airborne PAH and BaP

Occupational exposure levels are assessed by monitoring concentrations of airborne PAH or BaP. The common method is to collect particle-bound PAH or BaP by pumping air through a filter or an impactor followed by a backup filter. The gaseous PAH are then absorbed from the air stream when passing this filter by an adsorbent. Various filters and adsorbents are available. Sampling sites may vary depending on the study design, but often involve personal air sampling, static environmental sampling, and outdoor and indoor sampling (DECOS, 2006). The choice of sampling site greatly affects the obtained results, as PAH composition may change rapidly after emission from the source. For example by evaporation, condensation, deposition, adsorption, or by chemical conversion.

Preferred methods for extraction, purification and concentration determination have been summarized (DECOS, 2006):

"In general, after sampling, the filters and the sorbents are extracted with organic solvents (e.g., benzene, toluene, cyclohexane, dichloromethane, acetone and methanol). Standard extraction techniques, such as soxhlet and ultrasonic techniques, and solid-phase extraction may be used. After extraction, samples are cleaned-up or purified. Several techniques may be used for this, including liquid-liquid partition and (semi) preparative normal-phase chromatography. Three analytical-chemical methods are routinely used to determine the concentrations of PAH in environmental samples. These include: separation of single PAH compounds with thin-layer chromatography (TLC) combined with visual fluorescence detection and identification by reference spots; separation by gas chromatography (GC) combined with flame ionisation detection (FID) and/or mass spectrometry (MS) for detection or/and identification; and, separation by reversed-phase high-performance chromatography (HPLC), combined with ultraviolet and/or fluorescence detection and/or MS for detection and identification." (DECOS, 2006)

Biomonitoring

PAH metabolites are excreted in the urine, and can thus be used as biological indicators of exposure to PAH. However, similar to airborne monitoring, biological monitoring of PAH exposure is complicated by the fact that PAH always consist of complex mixtures. In addition, biotransformation is both specific and complex for individual PAH, which may result in metabolites with potentially different carcinogenic potential.

Several metabolites have been proposed as biomarkers of PAH exposure. These include 1-hydroxypyrene and the hydroxyphenanthrenes (metabolites of pyrene and phenathrenes), 3-hydroxybenzo[a]pyrene (a metabolite of benzo[a]pyrene), as well as metabolites of benzo[a]anthracene, benzo[a]phenanthrene, chrysene, fluorene, fluoroanthene and naphthalene (SCOEL, 2016). However, the most frequently used is currently 1-hydroxypyrene (Andersen et al., 2018; IARC, 2010; Jongeneelen, 2014; SCOEL, 2016), as it is easy to measure in human urine and currently the most reliable and practical marker for monitoring individual exposures (Dor et al., 1999; IARC, 2010).

A method for measuring 1-hydroxypyrene was introduced in 1985 (Jongeneelen et al., 1987; Jongeneelen et al., 1985). Since then, several methods have been developed for 1-hydroxypyrene biomonitoring (Hansen et al., 1993; Hatjian et al., 1995; Roos et al., 1997; Wu et al., 1998), and the presence of 1-hydroxypyrene in the urine of workers exposed to PAH in several occupational environments has been demonstrated (DECOS, 2006). As a consequence, the method is currently applied in several PAH-emitting industries (especially coke ovens, primary aluminium industry and coal tar distillation). However, studies using personal air sampling showed that urinary 1-hydroxypyrene levels and PAH-concentration in the ambient air do not always correlate, despite significant correlation between 1-hydroxypyrene and other parameters such as DNA adducts in peripheral lymphocytes (DECOS, 2006; Jongeneelen, 2014). This may be because urinary 1-hydroxypyrene levels not only reflect inhalation exposure, but also dermal and oral exposure. Moreover, the metabolic activity at the site of entry into the body may also heavily affect the concentration of urinary 1-hydroxypyrene and the distribution of PAH over the body, resulting in large inter-individual variation (DECOS, 2006).

As 1-hydroxypyrene is a metabolite of pyrene, which is not considered to be carcinogenic, methods for determination of markers for cancerous PAH have been developed. Specific and sensitive determination of relatively small amounts of urinary 3-hydroxybenzo[a]pyrene as exposure markers of BaP have been developed (SCOAL2016). In 2013, Deutsche Forschungsgemeinschaf (DFG) issued the following correlation between airborne BaP exposure (µg/m^3, 8h time-weighted average (TWA)) and urinary 3-hydroxybenzo[a]pyrene levels (ng/g creatinine) determined by hydrolysis (DFG, 2013)(Figure 3). The sampling was conducted 16 hours after last exposure, before the following shift (Gendre et al., 2004; Gendre et al., 2002).

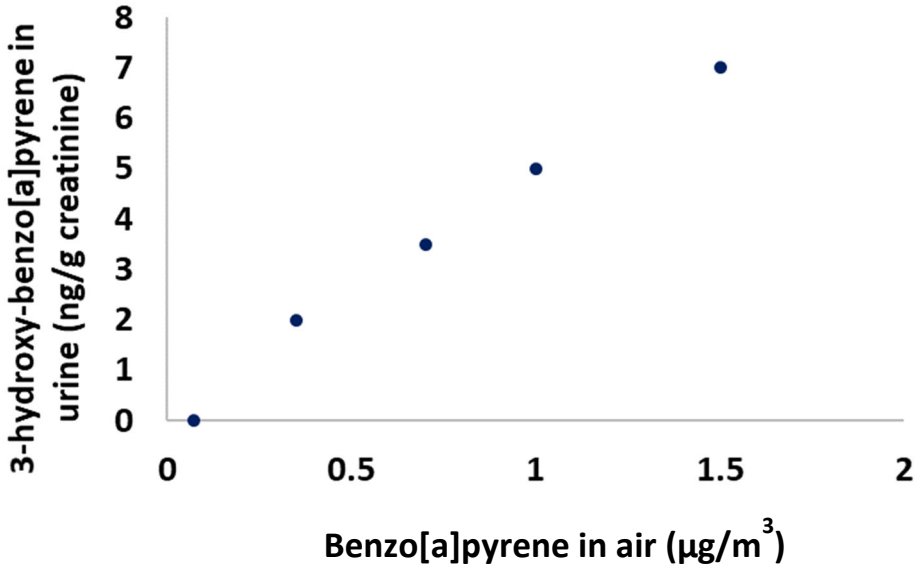

Figure 3. Correlation between airborne BaP exposure and urinary 3-hydroxybenzo[a]pyrene levels. Constructed based on data from (DFG, 2013).

The current working group notes that 1-hydroxypyrene in the urine is the most commonly measured biomarker of BaP and PAH exposure, but that 3-hydroxy-benzo[a]pyrene levels in urine may prove to be a more suitable biomarker for biomonitoring.

Exposure to PAH in the general population

The general population is continuously exposed to PAH, through tobacco smoke, ambient air, water, soils, food and pharmaceutical products. In fact, a study investigating the 1-hydroxypyrene content in the urine of occupationally non-exposed individuals or in representative samples of the general population in the US showed detectable levels in nearly all individuals. The levels in smokers were generally 3 times higher than in non-smoking individuals (Huang et al., 2004). Mainstream and sidestream smoking are major sources of PAH in indoor air (IARC, 2010). However, the largest single source comes from the household burning of wood (ATSDR, 1995), whereas other major indoor sources are unvented radiant and convective kerosene space heaters, gas cooking, and heating appliances (ATSDR, 1995).

Another major source of non-occupational exposure to PAH is ambient air pollution, as summarized by (IARC, 2010):

PAHs are widely detected as ambient air pollutants, primarily bound to particulate matter but also in the gas phase (especially the lower-molecular-weight PAHs). Average concentrations of individual PAHs in the ambient air of urban areas typically range from 1 to 30 ng/m^3 (excluding naphthalenes), and the more volatile PAHs are generally more abundant; however, concentrations up to several tens of nanograms per cubic metre have been reported in road tunnels or in large cities that use coal or other biomasses as residential heating fuels extensively. Estimates of annual emissions of PAHs from anthropogenic sources in the 1990s were 8600 tonnes/year in Europe and 2000 tonnes/year in Canada.

Outdoor PAH-producing sources are primarily combustion processes (i.e. from automobiles and trucks emissions). Other major sources of PAHs in ambient air are industrial emissions and forest fires (IARC, 2010).

A summary of PAH levels in water and food were provided in (IARC, 2010):

Most PAHs in water originate from surface run-off, particularly in urban areas; smaller particles derive from atmospheric fall-out and larger particles from the abrasion of asphalt pavement. Industrial effluents can also contribute to PAH loads in surface waters, and sediment levels may range up to several thousand micrograms per kilogram.
Although concentrations of PAHs in water are usually very low because of the low solubility of these compounds, surface water concentrations are typically 1–50 ng/L, with higher concentrations in some contaminated areas (IPCS, 1998). Comparison of PAH levels in rainwater with those in surface waters showed higher levels in rainwater (10–200 ng/L, with levels up to 1000 ng/L in snow and fog) (IPCS, 1998). Recently, it has been reported that urban run-off from asphalt-paved car parks treated with coats of coal tar emulsion seal could account for the majority

of PAHs in many watersheds in the USA. PAH levels in drinking-water are typically much lower.

Food is a major source of intake of PAHs for the general population. Estimates of PAH intake from food vary widely, ranging from a few nanograms to a few micrograms per person per day. Sources of PAHs in the diet include barbecued/grilled/broiled and smoke-cured meats; roasted, baked and fried foods (high temperature heat processing); breads, cereals and grains (at least in part from gas/flame drying of grains); and vegetables grown in contaminated soils or with surface contamination from atmospheric fall-out of PAHs.

The current working group notes there is background exposure to PAH from various sources in the general population. This background level is likely higher in smokers.

Occupational exposure levels

Occupational exposure to PAH mainly occurs in the major PAH-generating industries, such as coal liquefaction, coal gasification, coke production and coke ovens, coal-tar distillation, roofing and paving (involving coal-tar pitch), iron and steel foundries, wood impregnation/preservation with creosote, aluminium production (including anode manufacture), carbon-electrode manufacture, chimney sweeping, and power plants (IARC, 2012). As PAH are present as gaseous compounds (four aromatic rings or less) or bound to airborne particulates (five aromatic rings or more), occupational exposure to PAH occurs primarily through inhalation and via skin contact. Occupational exposure data for several PAH-generating occupational settings was thoroughly reviewed by IARC in 2010 and 2012 (IARC, 2010, 2012). They concluded that the highest levels of occupational exposure to PAH were observed in aluminium production (Söderberg process) with values up to 100 μg BaP/m^3 (Figure 4). Mid-range levels were observed for roofing and paving (10–20 μg BaP /m^3) and the lowest concentrations (at or below 1 μg BaP /m^3) were observed in coal liquefaction, coal-tar distillation, wood impregnation, chimney sweeping and power plants (IARC, 2010, 2012). If calculated as total PAH and not BaP fraction, exposure levels were a magnitude greater in some cases. A tendency towards lowered exposure levels in the more recent studies were observed across all occupational settings, which indicate that newer methods and processes may release less PAH than their older counterparts.

Recently, Petit and colleagues assessed human lung cancer risk in nine different occupational settings, with several subgroups within each setting. For this, they used occupational BaP exposure data collected in the period 2000-2014 in France (Petit et al., 2019). Average BaP exposure levels varied from <0.001 μg/m^3 to 3.4 μg/m^3, whereas maximum levels varied from <0.001 μg/m^3 to 26.2 μg/m^3. The exposure levels in the Petit et al. 2019 study are generally in line with the levels presented by IARC (IARC, 2010, 2012).

Based on the presented data, the present working group notes that historical BaP exposure levels vary more than 10,000-fold between occupational settings, with the highest values around 100 μg BaP/m^3 for historical data and around 26 μg BaP/m^3 for more recently reported data.

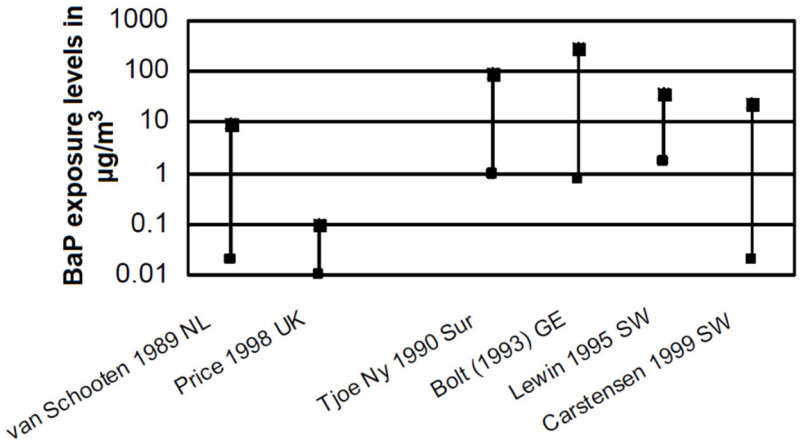

Figure 4. Benzo[a]pyrene exposure range levels in aluminium production (Söderberg process) in µg/m^3 from different studies. Adapted from (IARC, 2010).

Epidemiological cancer studies

The primary toxicological endpoint of PAH, and especially BaP, exposure is cancer. Due to the complexity of PAH exposure, no epidemiological data on BaP exposure alone is available. However, several epidemiological studies have assessed carcinogenesis caused by occupational exposure to complex PAH mixtures. These include cohort and case-control studies in various PAH-emitting industries. It should be noted that in all of the studied industries, workers were exposed to other possibly harmful substances in addition to the PAH exposure. Often, they were co-exposed to substances like organic solvents, nitro-PAH, aromatic amines, metals, and dust particles, the effects of which are important to take into account when assessing hazardous effects caused by PAH exposure alone.

BaP is classified as carcinogenic to humans (IARC category 1) and several other PAH are classified in group 2A (cyclopenta[cd]pyrene, dibenz[a,h]anthracene and dibenzo[a,l]pyrene), and 2B (benz[j]aceanthrylene, benz[a]anthracene, chrysene, 5-methylchrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, dibenzo[a,i]-pyrene, benzo[c]phenanthrene, dibenzo[a,h]pyrene and indeno[1,2,3-cd]pyrene). Cancer and cancerous related changes are therefore the focus of the majority of epidemiological studies. Several case-control, cohort and meta-studies evaluating PAH emission in occupational settings are available in the literature. In this section, the results of three studies will be highlighted. They were selected for different reasons. The first, Armstrong et al. 2003;2004, was selected as it is an extensive, well-performed meta-study comprising a broad variety of studies available at the time of publication. In addition, it has been used in several reports as basis for setting OELs (AGS, 2011; DECOS, 2006). The second study, Rota et al. 2014, updated previously published systematic reviews of cohort studies from 1997 and 2007, which assessed cancer in workers employed in selected industries with potential PAH exposure (Rota et al., 2014). The last study, Petit et al. 2019, is a recent comprehensive cohort study that was selected due to its harmonized and well-defined data collection (Petit et al., 2019). Collectively, the three selected, well-performed studies represent a broad time period and the present working group considers them as representative of the overall available data.

Armstrong et al. 2003;2004

In 2003, Armstrong and colleagues conducted a highly credited meta-study on the available published occupational epidemiological studies on PAH exposure-response relationships in the form of a research report for the British Health and Safety Executive (Armstrong, 2003). In 2004, they published the meta-study as a scientific, peer-reviewed article (Armstrong et al., 2004). The meta-analysis was based on published cohort studies, in which the relationship between occupational, airborne PAH exposure and lung and bladder cancer were quantitatively investigated. For the study selection, the authors established the following inclusion criteria:

- Original epidemiological studies of occupational PAH exposure by inhalation.
- Studies of workplaces in which PAH was considered the predominant carcinogen (this meant exclusion of rubber industry, diesel exhaust, foundries and part of steel works).
- Studies in which misclassification of exposure was unlikely.
- Only the most recently reported results from the same workforce reported in several papers.

Thirty-nine distinct cohort studies met the inclusion criteria and were reviewed by the authors (Table 3). These papers covered 39 distinct cohorts (35 cohorts, 1 case-cohort sampling from within the cohort, and 3 nested case-control samples). These 39 cohorts comprised 2891 cancer cases (total number of participants was not provided). For comparison across cohorts, it is essential that the individual PAH exposures were determined or estimated by a common exposure parameter. Thus, the authors converted total PAH and BSM concentrations to BaP concentrations using conversion ratios (for details see the original publications). For studies with no exposure measurements, a job-exposure matrix indicating estimated mean concentrations of BaP exposure for each industry and occupation was established in collaboration with industrial hygienists and by using published reviews on workplace exposure (proxy measures).

For each included cohort, unit relative risks (URRs) were calculated and the URRs were subsequently averaged across studies. This was conducted as follows; first the authors estimated the relative risk (RR) per 100 μg/m^3 years cumulative BaP for each study. They used the log-linear model: RR = exp(bx), where RR is relative risk, x is cumulative exposure in μg/m^3 and b is the slope of the exposure–response relationship. The relative risk therefore represents the risk of lung cancer at a specified exposure (x) relative to that at zero exposure. For example, RR = 1.40 at x = 100 μg/m^3 years BaP exposure implies that at this exposure, lung cancer risk is 1.4 times that of an unexposed person—a 40% excess.

Table 3. Unit relative risk for lung cancer in various industrial sectors or occupations with PAH exposure. Modified from (Armstrong et al., 2004).

Group	Studies (*n*)	Mean URR[a] (95% CI)	Significance tests[b]
All cohorts	39	1.20 (1.11–1.29)	*p*(het) = 0.007
Excluding less precise URR estimates			
Restricted to URRs with SE < 10	31	1.20 (1.11–1.30)	*p*(het) = 0.002
Restricted to URRs with SE < 1	19	1.18 (1.12–1.23)	*p*(het) = 0.19
By industry			*p* = 0.002
Coke ovens	10	1.17 (1.12–1.22)	
Gasworks	4	1.15 (1.11–1.20)	
Aluminum	8	1.16 (1.05–1.28)	
(above three combined)	22	1.17 (1.12–1.22)	*p*(het) > 0.20]
Carbon	4	4.30 (0.81–22.79)	
Asphalt	3	17.50 (4.21–72.78)	
Tar distillery	3	12.28 (0.48–314.4)	
Chimney sweep	2	16.24 (1.64–160.7)	
Power	3	> 1,000 (0 to > 1,000)	
Carbon black	2	0 (0 to > 1,000)	
By exposure information from authors			*p* > 0.20
BaP	10	1.29 (1.11–1.49)	
Proxy	6	1.16 (1.11–1.21)	
None	23	1.17 (1.03–1.33)	
By smoking adjustment			*p* = 0.05
No	35	1.16 (1.11–1.21)	
Yes	4	1.31 (1.16–1.48)	

a: RR at 100 µg/m^3 BaP years. Adjusted for differences across industries by including industry indicator in a meta-regression. Means are scaled to show fitted values for coke ovens, although ratios would apply to any industry. *b*: Generally, the Wald test was used for significance of variation in mean URRs across the groups; "p(het)" indicates the test for heterogeneity across all studies.

The URR was defined as follows:

"Unit relative risks were defined as those predicted by the models at 100 µg/m^3 years BaP [i.e., exp(100b)]. The exposure of 100 µg/m^3 years BaP is close to the mean of the maximum exposures in included studies and corresponds to a concentration of 2.5 µg/m^3 BaP over 40 years." (Armstrong et al., 2004; Armstrong, 2003)

The average URR of all 39 studies was 1.20 (95% CI, 1.11-1.29; p< 0.001; log-linear model). Although none of the studies dominated the average estimate, significant differences across industries were found (Table 3). These differences could be due to errors in exposure estimation, especially in the studies that rely on a job-exposure matrix estimations and in those with small numbers of cases. Another source of variation could be caused by differences in control for confounders, e.g. smoking status, which was only reported in some case studies. Lastly, the carcinogenic potential of PAH mixtures may also vary across industries. PAH mixtures in different industries may have various PAH profiles, depending, for example, on raw material composition, which may lead to variations in carcinogenicity.

However, previous reports establishing OELs concluded that taking both the uncertainties and the strength of the meta-analysis by Armstrong et al. into account, they found no serious constraints to exclude certain cohorts with no or limited exposure

data (AGS, 2011; DECOS, 2006). The present working group agrees with this assessment. The meta-analysis concluded that overall, PAH exposure significantly increased risk of lung cancer (Table 3).

The meta-study by Armstrong and colleagues also assessed bladder cancer. Twenty-seven cohorts reported bladder cancer, with an overall mean URR was 1.33 (95% CI: 1.16-1.52). No statistically significant variation by industry was observed; however, the mean URR was strongly dependent on results from two studies on large aluminium production industries. In contrast, little evidence of a relationship between bladder cancer and PAH exposure was found in coke ovens and other industries. The authors therefore considered the causal relationship between PAH exposure and bladder cancer as weak, despite the larger URR for bladder cancer compared to lung cancer. This was mainly due to less cases limiting the statistical power, lower mortality of bladder cancer in the general population, and the fact that bladder cancer is induced by substances (not PAH), which normally are found in the types of industries included in the meta-study.

The lung dose-response relationship from the meta-analysis by Armstrong and colleagues was used as foundation for subsequent reports establishing OEL levels (AGS, 2011; DECOS, 2006; SCOEL, 2016).

Rota et al. 2014

The aim of this study was to update a previously published systematic review of cohort studies, which assessed cancer (mortality and incidences) in workers employed in selected industries with potential PAH exposure (Boffetta et al., 1997). Such update had also been conducted in 2007 (Bosetti et al., 2007), but in the 2014 publication, Rota and colleagues extended this period to 2014, resulting in a review of available cohort studies published between 1958 and 2014 (Rota et al., 2014). Several of the cohort studies included in the Rota et al. 2014 meta-study were also included in the Armstrong meta-study; however, only studies published prior to 2001 were commonly present in both meta-studies. This inclusion of more recent studies, compared to the Armstrong meta-study, increases the relevance of the Rota et al. study and is a cardinal reason for its inclusion in the present report.

Rota and colleagues conducted a literature search, which resulted in the inclusion of 13 individual cohort studies since the last update (Bosetti et al., 2007). These included assessments in aluminium production industries (seven studies), iron and steel foundries (two studies), carbon black production (two studies) and for asphalt workers (two studies). From each of these studies, the authors extracted information related to the production type, worker employment, duration of follow-up, outcome, investigated cancers, observed and expected number of death or cases, and standardized mortality ratio (SMR) or standardized incidence ratio (SIR). No papers were published between January 1, 2006, and January 31, 2014, for the following industries: Coal gasification, coke production and carbon electrode manufacturing. The results from the previous update were therefore still up to date: Coal gasification (251 cases, SMR: 2.40), coke production (762 cases, SMR: 1.49) and carbon electrode manufacture (130 cases, SMR: 0.93)(Bosetti et al., 2007).

The authors first calculated the SMRs for each cancer type of interest, and then computed the pooled relative risks (RRs) and corresponding 95% confidence intervals (CIs) for each industry using random-effects models. Their meta-analysis revealed an excess risk of cancers in the respiratory tract (mainly lung cancer) in iron and steel foundries (pooled RR: 1.31, CI: 1.08–1.59, from 14 studies), as well as a weak excess risk (pooled RR: 1.08, CI: 0.95–1.23 from 11 studies) in aluminium production (Table 4). In addition, Bosetti et al. also found excess risk of respiratory tract cancers for coal gasification and coke production workers in the previous meta-analysis update from 2007 (Bosetti et al., 2007). The updated meta-study from 2014 revealed no increase in respiratory tract cancer risk in asphalt or carbon black production workers (Table 4).

Bladder cancer risk was borderline increased in aluminium production (pooled RR: 1.28, 95 % CI 0.98–1.68 from 10 studies) and in iron and steel foundries (pooled RR 1.38, 95 % CI 1.00–1.91 from 9 studies). Increased risk of urinary tract cancers was also reported for coke production workers in the previous update (Bosetti et al., 2007). No increased risk was observed for the other PAH-emitting industries included in the meta-study.

Table 4. Overall standardized mortality ratios (SMRs) and pooled relative risks (RRs) with 95% confidence intervals (CIs) for selected cancer sites for workers exposed to PAH in various industries and occupations. From (Rota et al., 2014).

Industry, cancer site	No. of cohorts	Obs/Exp	SMR	Pooled RR[a] (95 % CI)	*p* value for heterogeneity
Aluminum production					
Larynx	7	71/63.4	1.12	1.15 (0.91–1.45)	0.700
Lung	10	1,314/1,154.7	1.14	1.07 (0.93–1.23)	<0.0001
Respiratory tract[b]	11	1,349/1,183.9	1.14	1.08 (0.95–1.23)	<0.0001
Bladder	10	279/202.2	1.38	1.28 (0.98–1.68)	0.002
Kidney	8	131/126.4	1.04	1.06 (0.89–1.25)	0.728
Iron and steel foundry					
Larynx	5	59/41.2	1.43	1.48 (1.14–1.91)	0.537
Lung	13	2,903/2,762.4	1.05	1.31 (1.07–1.61)	<0.0001
Respiratory tract[b]	14	2,932/2,784.7	1.05	1.31 (1.08–1.59)	<0.0001
Bladder	9	151/127.7	1.18	1.38 (1.00–1.91)	0.001
Kidney	6	68/69.4	0.98	1.03 (0.78–1.35)	0.304
Asphalt workers					
Larynx	2	45/42.7	1.05	1.89 (0.45–7.95)	0.013
Lung	3	827/735.7	1.12	1.59 (0.68–3.76)	<0.0001
Bladder	2	109/107.1	1.02	1.03 (0.82–1.30)	0.305
Carbon black production					
Lung	3	249/201.1	1.24	1.52 (0.91–2.52)	<0.0001
Respiratory tract[b]	4	283/243.6	1.16	1.30 (0.84–2.01)	<0.0001
Bladder	3	15/14.8	1.02	1.10 (0.61–2.00)	0.288

Obs/Exp observed/expected number of cancer cases/deaths

[a] Calculated as a weighted average of the SMRs through random-effects models

[b] Including lung and other respiratory cancers not specified

This updated review and meta-analysis by Rota et al. confirm the previous observed increased risk of respiratory tract and bladder cancers in PAH-emitting occupations, i.e. aluminium production industries, iron and steel foundries, coal gasification and coke production. Significant heterogeneity between the cohorts in the individual groups (occupational setting/cancer type) was observed, which the authors believe reflects

variable exposure patterns across different cohorts and time periods. Also, some level of bias or confounding is to be expected, as the workers included in the meta-study may be exposed to other occupational carcinogens, as well as to tobacco smoking, which was not adjusted for in all of the included cohort studies.

Petit et al. 2019

In this recent study, Petit and colleagues assessed PAH-induced occupational lung cancer risks in various industries in France (Petit et al., 2019). The data used were from the Exporisq-HAP database (E-HAP), which included atmospheric PAH measurements performed from 2000 to 2014, consistently using the same strategy for sampling and analysis. The E-HAP database contained personal and area air samplings comprising the concentrations (in ng/m^3) of 17 PAH over short-term sampling (≤2 h) and long-term sampling (> 2 h) durations. However, for assessing the lung cancer risk, only personal samplings carried out for long-term durations were included. As with previous studies and risk assessments, the authors chose to use BaP concentrations for the exposure assessment. BaPeq concentrations were also provided for comparative purposes, but they were very similar to BaP concentrations, and as the authors did not discuss the BaPeq results, neither will the present working group.

The E-HAP organized the industrial landscape into three overlapping hierarchical levels of increasing detail. Each sampling was assigned a level 1 (L1G), level 2 (L2G) and level 3 (L3G) exposure group, which were the least, intermediate and most accurate levels of description, respectively. Samplings were performed in nine L1Gs: aluminium production, silicon production, coke production, manufacturing of carbon products, foundries, combustion processes, use of lubricating oils, engine exhaust emissions, and bitumen (road paving). Each measurement was associated with additional information, such as the sampling time or use of protective equipment. Samplings with a missing measurement or with no duration were excluded, and only exposure groups (L1Gs, L2Gs, L3Gs) with at least six samplings were included. The exposure was considered as being representative, as only long-term personal samplings were used and because these samplings were not worst-case samplings or regulatory samplings (Petit et al., 2017).

The authors conducted two evaluations: First, they estimated risk of lung cancer for all exposure groups (L1G-L3G) divided into five risk ranking groups from the determined exposure levels. Detailed description can be found in the original article (Petit et al., 2017). Then, they calculated the number of additional lung cancers at the current exposure levels. The present working group will focus on the latter results. Prior to the risk assessment, the French population of workers potentially exposed to PAH was estimated in each L1G and in total, through national statistics, national studies and grey literature. The exposed population (N) was determined and the authors assumed that the BaP concentrations (C) were identical for all exposure durations (15, 30 or 45 years). Expected number of additional lung cancer was not estimated for the L2Gs and L3Gs, as it was not possible to estimate the total number of workers within these groups. The expected number of additional lung cancers E(τ) after an exposure of duration τ was derived as follows:

$$E(\tau) = N(\tau) \times P(\tau) = N(\tau) \times [1 - \exp(-\beta C \tau (\tau/T))]$$

in which P(τ) denotes the probability of the occurrence of lung cancer after an exposure of duration τ. N(τ) is the number of individuals who were exposed to the BaP for a time period. β denotes the fallout factor, which is defined mathematically in the article (Petit et al., 2017). Cτ is the (constant) mean concentration of BaP that the population has been exposed to over the exposure of duration τ, and T=45 years is the maximal occupational exposure duration.

The calculated E(τ) for the 9 L1G are presented in Table 5. A total of 27 (95% CI: 0.14–54) additional lung cancers could be expected per year in France among the 3.7 million workers estimated to be exposed to PAH, if all workers had been exposed for 45 years. The calculations are based on the risk estimates determined previously in the article. The E(τ) for the individual L1G varied about a 200-fold, with the largest number of E(τ) found in coke production, followed by combustion processes and engine exhaust emissions (Table 5). Although lung cancer risks were considered high or very high for L1Gs that used products derived from coal (Petit et al., 2019), the exposed population in these groups was far smaller (1000–40,000 workers) than for L1Gs using products derived from petroleum (100,000 to 2 millions) where lung cancer risks were considered low or very low.

The results of this study suggest that exposure to PAH at the current occupational exposure levels in selected industries in France will lead to ca. 27 excess lung cancer incidences per year among 3.7 million exposed workers, roughly corresponding to 1: 100,000. The OEL for BaP in France is 150 ng/m^3 (Maitre et al., 2018). In line with the older studies, the study by Petit et al. 2019 only assessed inhalation exposure and did not consider skin absorption. Also, the E(τ) reported are to be considered as rough estimates, due to uncertainties in risk assessment (e.g. measurement of uncertainties and individual factors) and because the precise number of exposed workers was not accurately known. Although this study is comprehensive and well powered, the data presented are not readily usable for setting a health-based exposure limit. However, overall it supports the conclusions drawn in the older studies, thereby highlighting the continuing issue with PAH-induced lung cancers in occupational settings.

Table 5. Exposed population and number of additional lung cancer cases per year in France. Adapted from (Petit et al., 2019).

Exposed population and number of additional lung cancer cases per year in France			
L1G	Exposure duration τ	Exposed population N(τ)	Additional lung cancer E(τ)_2000–2014 BaP: P90 (95% CI)
Coke production	45 years	4100	11 (0.05, 22)
	>30 years	2050	5.6 (0.03, 11)
	15–30 years	1700	3.1 (0.01, 6.2)
	≤15 years	350	0.32 (0.002, 0.63)
Silicon production	45 years	1500	1.6 (0.001, 3.8)
	>30 years	1000	1.1 (0.001, 2.5)
	15–30 years	350	0.25 (2×10−4, 0.59)
	≤15 years	150	0.05 (5×10−5, 0.13)
Aluminium production	45 years	10700	1.1 (0.02, 1.8)
	>30 years	7800	0.80 (0.01, 1.3)
	15–30 years	2600	0.18 (0.003, 0.29)
	≤15 years	300	0.01 (2×10−4, 0.02)
Manufacturing of carbon products	45 years	3300	0.83 (0.002, 1.8)
	>30 years	2100	0.53 (0.001, 1.2)
	15–30 years	1200	0.20 (5×10−4, 0.44)
	≤15 years	0	0 (0, 0)
Foundries	45 years	32830	0.60 (0.002, 1.3)
	>30 years	6600	0.12 (3×10−4, 0.25)
	15–30 years	24400	0.30 (7×10−4, 0.62)
	≤15 years	1830	0.01 (3×10−5, 0.02)
Combustion	45 years	400000	6.2 (0.02, 13)
	>30 years	165000	2.5 (0.008, 5.3)
	15–30 years	180000	1.8 (0.006, 3.8)
	≤15 years	55000	0.28 (9×10−4, 0.59)
Engine exhaust emissions	45 years	2000000	3.8 (0.02, 7.0)
	>30 years	890000	1.7 (0.01, 3.1)
	15–30 years	857000	1.1 (0.006, 2.0)
	≤15 years	253000	0.16 (9×10−4, 0.30)
Use of lubrication oils	45 years	1130000	1.9 (0.02, 3.3)
	>30 years	632000	1.1 (0.01, 1.8)
	15–30 years	430000	0.48 (0.006, 0.83)
	≤15 years	68000	0.04 (4×10−4, 0.07)
Bitumen/road paving	45 years	110000	0.06 (7×10−4, 0.10)
	>30 years	60000	0.03 (4×10−4, 0.05)
	15–30 years	38000	0.01 (2×10−4, 0.02)
	≤15 years	12000	0.002 (3×10−5, 0.003)
Total	45 years	3692430	27 (0.14, 54)
	>30 years	1766550	13 (0.07, 27)
	15–30 years	1535250	7.5 (0.04, 15)
	≤15 years	390630	0.88 (0.004, 1.8)

95% CI: 95% confidence interval. E(τ): expected number of additional lung cancers in France after an exposure of duration τ. P90: 90th percentile.

Summary

Epidemiological studies investigating relations between human exposure to PAH and cancer risks are numerous in the literature. The three selected studies described in the present report comprise cohort data ranging from the 1970s to 2014, and use different approaches for calculating and describing their data. The overall conclusion based on the three studies is that human exposure to PAH mixtures at levels observed occupationally leads to increased risk of developing lung cancer, and to a degree bladder cancer. This is in line with the conclusions made in case-control studies and other cohort studies in the literature (DECOS, 2006; IARC, 2010, 2012). As stated by IARC:

"Based on the best available, consistent and strong experimental and human mechanistic evidence it is concluded that benzo[a]pyrene contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAH mixtures that contain benzo[a]pyrene." (IARC, 2012)

Differences in the risk of lung cancer observed both between and within industries are probably related to factors such as ventilation, raw material composition, personal protective equipment use, distance from the emission source or process type and temperature. The availability of cohort data ranging from the 1970s to 2014 allows for evaluation of differences in lung cancer risk across time periods, albeit the differences in reported risk level metrics renders comparison less straightforward. In general, occupational exposure in the major PAH-producing industries, such as aluminium production, coke production and manufacturing of carbon products, all induced significantly increased risk of PAH-induced lung cancer across all time periods. This supports the use of the older meta-study by Armstrong et al. 2004 as basis for setting a health-based OEL for PAH and BaP exposure. However, large differences in bitumen/asphalt workers were observed across the three studies, with large risk levels observed in the meta-study by Armstrong et al. 2004, whereas negligible levels were observed for the two newer studies. In addition, large variation in the exposure levels for asphalt workers were also observed within the individual meta-studies. This highlights the essentiality of exposure condition estimations (e.g. ventilation, temperature, distance) in general, but especially for this industry, if exposure levels are to be compared across studies.

Based on the epidemiological evidence, the current working group considers lung and bladder cancer as critical effects of BaP and PAH exposure.

Skin cancer

Scrotal cancer, a squamous cell skin cancer, has historically been connected to occupational exposure in English chimney sweeps. The culprit was later determined to be dermal PAH exposure (Brown & Thornton, 1957). However, more recent studies were unable to detect increases in non-melanoma skin cancer incidence in Nordic chimney sweeps (Evanoff et al., 1993; Hogstedt et al., 2013; Pukkala et al., 2009). This is most likely due to reduced exposure from better occupational hygiene (IARC, 2012). A review from 1997 exploring incidences of different cancers after occupational PAH exposure concluded that increased risk of skin cancer generally is restricted to settings

with substantial dermal exposure, such as for roofers and asphalt workers (Boffetta et al., 1997). Indeed, a study among asphalt workers reported an increased risk of mortality from non-melanoma skin cancer in this occupational setting (SMR of 4.0 (95% CI 1.0, 10.9) for workers employed ≥20 years)(Hammond et al., 1976). Similarly, two studies reported increased skin cancer standardized incidence ratios (1.5 (95% CI 0.7, 2.6) based on 5 exposed cases and 2.37 (95% CI 1.08, 4.50) based on 9 cases) in occupations with expected dermal exposure to creosote (i.e. timber workers and brick makers)(Karlehagen et al., 1992; Tornqvist et al., 1986).

It should be noted, that the available occupational studies and cancer registries may underestimate the risk of squamous cell carcinoma, because non-melanoma skin cancers rarely are fatal when caught early due to preventative excision of precancerous lesions (EPA, 2017). In conclusion, the current working group considers cancerous changes in the skin induced by dermal exposure to BaP and PAH as a critical effect.

Other toxicological effects

Toxicological effects other than cancer or genotoxicity have been reported after PAH exposure. However, similar to epidemiological cancer studies, non-carcinogenic human toxicity studies of single PAH compounds are very limited in number, and thus most findings are reported after exposure to complex PAH emissions. In addition, only very limited human data are available on PAH-induced systemic effects, such as cardiovascular, gastrointestinal, hepatic, immunotoxic, and dermal effects. However, developmental and reproductive toxicity are documented in humans, which this section will focus on.

In regards to acute effects, these have primarily been reported after exposure to naphthalene caused by sucking or ingestion of mothballs. This resulted in nausea, vomiting, convulsions, and diarrhoea a few days after exposure, followed by acute haemolytic anaemia (DECOS, 2006; SCOEL, 2016).

Reproductive toxicity

PAH and BaP have been reported to induce reproductive toxicity in both men and women in several studies in humans (EPA, 2017). In men, this included effects on sperm quality and fertility in populations exposed to mixtures of PAH either occupationally or through smoking (Hsu et al., 2006; Soares & Melo, 2008). Coke oven workers showed a higher frequency of low sperm count and twice the number of morphologically abnormal sperm compared to controls (Hsu et al., 2006). In addition, increased levels of benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) DNA adducts in sperm cells were measured in men exposed to PAH occupationally (Gaspari et al., 2003) and through cigarette smoke (Phillips, 2002; Zenzes et al., 1999). As a possible consequence of these changes, lowered male fertility was demonstrated. Spermatozoa from smokers showed reduced fertilizing capacity, and embryos displayed lower implantation rates (Soares & Melo, 2008). In addition, men with higher urinary levels of PAH metabolites were shown to have a higher risk of being infertile (Xia et al., 2009).

In women, epidemiological studies have linked cigarette smoking with reduced fertility (EPA, 2017). However, only few studies have specifically examined BaP exposure levels and female reproductive outcomes. The ability to conceive was investigated among women undergoing *in vitro* fertilization (Neal et al., 2008). Women who did not conceive had significantly higher follicular fluid BaP levels, compared to women who did get pregnant. However, this association was not observed for serum levels of BaP and conception success. Maternal blood BaP-DNA adduct levels were also shown to be strongly associated with increased risk of early foetal death (i.e., before 14 weeks of gestation)(Wu et al., 2010).

Developmental toxicity

BaP, in conjunction with dibenzo(a,c)anthracene and chrysene were found in the placenta and in the umbilical cord of pregnant Indian women, indicating that the foetuses were exposed to these PAH through the environmental exposure of their mothers (Madhavan & Naidu, 1995). In addition, BaP have also been detected in human breast milk, especially in smokers (Lapole et al., 2007; Yu et al., 2011; Zanieri et al., 2007).

Developmental effects following *in utero* exposure to PAH mixtures have been reported in human studies and were summarized by EPA (EPA, 2017). EPA reports that exposure to mixtures of PAH lead to decreased head circumference, decreased birth weight, and decreased postnatal weight, as well as increased frequency of miscarriage. In addition, two birth cohort studies of different populations (New York City and China) reported neurotoxic effects of prenatal exposure after environmental PAH exposure (Perera et al., 2009; Perera et al., 2004; Perera et al., 2005; Perera et al., 2012; Tang et al., 2006; Tang et al., 2008). The effects included reduced head circumference, impaired cognitive ability, impaired neuromuscular function, and increased attention problems and anxious/depressed behaviour. Both studies used specific BaP measures (i.e., adduct levels measured in cord blood samples)(Perera et al., 2009; Perera et al., 2004; Perera et al., 2005; Perera et al., 2012; Tang et al., 2006; Tang et al., 2008).

Animal studies

Since epidemiological data is available on PAH exposure and cancer risk, data from animal studies are primarily included to strengthen the conclusions made from the human data and to evaluate BaP as a single substance, which is not possible using epidemiological data. This section will therefore only include animal studies investigating BaP-induced toxicity. Although BaP has been reported to induce cancer in various organs upon localized exposure in all species tested (Table 6)(IARC, 2012), this report will focus on effects solely after lung and dermal exposure, which are considered the most relevant occupational exposure routes. In addition to cancer, the present report will evaluate other hazardous effects related to PAH and BaP exposure.

Table 6. Summary of report of malignant tumours clearly induced in experimental animals by BaP. From (IARC, 2012).

Organ site/ species	Lung	Trachea	Larynx	Forestomach	Liver	Lymphoid tissue (lymphoma)	Sarcoma (injection site)	Skin	Mammary gland
Mouse	x			x	x	x	x	x	
Rat	x						x		x
Hamster	x	x	x	x			x		

Exposure routes. Lung: Oral administration, intraperitoneal injection, inhalation, intrapulmonary injection, intratracheal administration, intrafetal injection. Trachea: Inhalation. Larynx: Inhalation. Forestomach: Oral administration, intraperitoneal injection, inhalation, intratracheal administration, buccal pouch application, intracolonic instillation. Liver: Oral administration, intraperitoneal injection. Lymphoid tissue: Oral administration. Sarcoma: Subcutaneous injection, intraperitoneal injection. Skin: Skin application. Mammary gland: Oral administration, intramammilary administration.

Cancer

Lung exposure

Only one lifetime inhalation study has been identified in laboratory animals (Thyssen et al., 1981). In this study, male Syrian hamsters were exposed to BaP on sodium chloride particles by nose only inhalation (0, 2.2, 9.5, 46.5 mg/m^3, 4.5 hours per day, 7 days per week for 10 weeks; thereafter 3 hours per day, 7 days per week (total average doses: 0, 29, 127, 383 mg/animal)). BaP exposure induced dose-related increases in the incidence of papillomas and squamous-cell carcinomas in both the upper respiratory tract (nose, larynx and trachea) and the upper digestive tract (pharynx, esophagus and forestomach)(Thyssen et al., 1981). Tumour rates were 0%, 25-35%, and 50-60% for the low, middle and high dose groups, respectively. The authors reported that tumour rates of all other organs generally corresponded to the rates in controls. The present working group therefore notes that BaP exposure induced dose-related increases in the incidence of cancer in the airways of Syrian hamsters.

Non-inhalation pulmonary exposure to BaP has been evaluated in several animal studies. Single pulmonary injection of BaP into the lung of rats resulted in dose-related increases in the incidence of malignant lung tumours (IARC, 2012). Doses varied from 0.03 to 1.0 mg/animal, with observation periods ranging from 100-140 weeks after exposure. Tumour rates for the high dose exposure groups per study were: 94.3% (1.0 mg/animal)(Deutsch-Wenzel et al., 1983), 69% (1.0 mg/animal)(Iwagawa et al., 1989),

77.1% (0.3 mg/animal)(Wenzel-Hartung et al., 1990), and 44.4% (0.3 mg/animal)(Horikawa et al., 1991). The carcinogenic potential of BaP suspended in saline (alone or mixed with particulates) after intratracheal instillation has also been investigated in several studies using mice, rats and hamsters (IARC, 2010, 2012). The exposures resulted in benign and malignant respiratory tumours, and additionally forestomach tumours in hamsters. Tumour rates varied from 10-95% across the studies described in monograph 100F by IARC (IARC, 2012). Larger BaP particles were generally more carcinogenic than smaller ones.

Although differences in patterns of deposition and conversion of dose should be kept in mind when comparing inhalation with non-inhalation pulmonary exposure, the result of the non-inhalation studies support the carcinogenicity of BaP identified in the inhalation study, as well as in epidemiological studies.

In summary, the present working group notes that results obtained from animal studies confirm the carcinogenic potential of PAH and, in particular, BaP observed in occupational settings after pulmonary exposure.

Dermal exposure

IARC summarized animal data on dermal exposure (skin and subcutaneous) to BaP exposure as follows (IARC, 2012):

"In several studies in which benzo[a]pyrene was applied to the skin of different strains of mice, benign (squamous cell papillomas and keratoacanthomas) and malignant (mainly squamous cell carcinomas) skin tumours were observed. No skin-tumour development was seen in $AhR^{-/-}$ mice that lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice developed squamous-cell carcinomas of the skin. In a large number of initiation–promotion studies in mice, benzo[a]pyrene was active as an initiator (mainly of squamous-cell papillomas) when applied to the skin.

In subcutaneous injection studies of benzo[a]pyrene, malignant tumours (mainly fibrosarcomas) were observed at the injection site in mice and rats. No fibrosarcomas were observed in $AhR^{-/-}$ mice that lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice did develop these tumours. In another study, male and female newborn Swiss mice that were given benzo[a]pyrene subcutaneously showed a significant increase in lung-adenoma incidence and multiplicity. A single study in 12 strains of hamsters resulted in sarcomas at the site of injection in both sexes of all 12 strains." (IARC, 2012)

In summary, the current working group concludes that there is strong evidence of BaP induced cancer in laboratory animals when applied to the skin or injected subcutaneous. The aryl hydrocarbon receptor appears important for the promotion of dermal cancer.

Other adverse health effects

Exposure to BaP has also been linked to non-cancerous effects in animals. The most well-documented effects relate to reproductive and developmental effects. These effects were thoroughly reviewed by the U.S. Environmental Protection Agency (EPA) in 2017, and

their summarized findings are presented below (EPA, 2017). Effects in the liver, kidney, cardiovascular, immune, and nervous systems in animals exposed as adults have also been reported; however, there is less robust and consistent evidence for these effects (EPA, 2017). They are therefore not included in the present report.

Reproductive toxicity

Toxicological changes to the reproductive system after BaP exposure have previously been reported in rodents (DECOS, 2006; DFG, 2012; EPA, 2017; SCOEL, 2016). A large proportion of the reproductive toxicity studies used intraperitoneal injection as exposure route, which is not relevant for the present report. Thus, only inhalation, dermal and oral exposure routes are considered. DECOS reported that PAH-induced reproductive and embryotoxic effects depend on the genotype of the mice (induction of the cytochrome P450 monooxygenase receptor) and the ability to transform PAH into active PAH metabolites (DECOS, 2006).

Male effects

In 2017, EPA summarized the reported BaP-induced male reproductive toxicity in animals as follows:

"Exposure to benzo[a]pyrene in laboratory animals induces male reproductive effects including decreased levels of testosterone and increased levels of luteinizing hormone, decreased sperm count and motility, histological changes in the testis, and decreased reproductive success. Male reproductive toxicity has been observed after oral and inhalation exposure to rats or mice. The male reproductive effects associated with benzo[a]pyrene exposure are considered to be biologically plausible and adverse.
In conclusion, EPA identified male reproductive system effects as a human hazard of benzo[a]pyrene exposure." (EPA, 2017)

Of the above described male reproductive changes in animals, inhalation was the exposure route in two of the studies (Archibong et al., 2008; Ramesh et al., 2008). Both studies were derived from the same experimental setup, in which male F344 rats (12–13 weeks old) were exposed via nose-only inhalation to 0 or 75 μg BaP/m^3 for 4 hours a day for 60 days. Each group contained 10 adult males. BaP was absorbed to carbon black particles (CB), which was used as a carrier. Control rats were constrained in similar fashion as exposed rats, but received neither BaP-CB nor CB. The authors based the decision of not including a CB control on studies showing lack of effect of 10- and 60-days exposure to CB on the endocrine and reproductive characteristics of rats (Accardi-Dey & Gschwend, 2003; Inyang et al., 2003). The exposed group displayed all the male reproductive effects described in EPA's summary of effects in animals cited above. These findings were supported by similar observations in studies using the oral exposure route (EPA, 2017).

Female effects

For female reproductive effects, EPA summarized the reported BaP-induced toxicity in animals as follows:

"Studies in multiple strains of rats and mice indicate fertility-related effects including decreases in ovarian follicle populations and decreased fecundity. Decreased serum estradiol has also been noted in two different strains of rats exposed by oral or inhalation exposure. The reproductive effects associated with benzo[a]pyrene exposure are biologically supported and relevant to humans.

In conclusion, EPA identified female reproductive effects as a human hazard of benzo[a]pyrene exposure." (EPA, 2017)

Of the above-summarized findings on female reproductive toxicity, two studies were inhalation studies (Archibong et al., 2002; Archibong et al., 2012). The 2002 study investigated hormone changes in pregnant Fisher 344 rats exposed to 25 or 75 μg BaP/m^3 for 4 hours a day for 10 days (gestation days 11–20) by nose-only inhalation (Archibong et al., 2002). Each group contained 10 females. CB was used as a carrier for BaP. Control rats were exposed to either CB to control for potential inert BaP carrier effects or remained as unexposed controls (UNC). The authors reported decreased estradiol and prolactin levels and increased progesterone levels at gestation day 17 after exposure to 75 μg BaP/m^3. These hormonal changes may have an important effect on pregnancy outcomes, which were also reported to be affected in the study, as decreased litter size and pup survival were observed already at the 25 μg BaP/m^3 exposure level (Archibong et al., 2002). No differences in any endpoint was observed between the UNC and the CB carrier groups.

In the 2012 study from the same group, adult female Fisher-344 rats were exposed to 50, 75 or 100 μg BaP/m^3 via nose-only inhalation for 4 hours a day for 14 days (Archibong et al., 2012). Each group contained 20 females. CB was used as a carrier for BaP. Control (UNC) rats were constrained in similar fashion as exposed rats, but did not receive neither BaP-CB nor CB. This choice of not including a CB control was supported by their own previous results (Archibong et al., 2002) and of results found in the literature, showing no differences between CB and unexposed rats on the investigated outcomes (Accardi-Dey & Gschwend, 2003; Inyang et al., 2003). BaP metabolites were identified in ovary, lung and liver tissue after exposure to 100 μg BaP/m^3, indicating widespread distribution of BaP after pulmonary exposure. The authors reported increased estrous cycle length (+24 hours) in rats exposed at the highest level compared to the other exposure groups. A closer look at hormone levels throughout the different stages of the estrous cycle revealed differences between exposed rats at the highest level and UNC rats. This included decreased serum estradiol and LH levels in the proestrus fase, decreased serum progesterone levels in the diestrus I fase, and increased serum FSH at all stages of the estrous cycle. In addition to these changes, the ovulation rate was also decreased in the 100 μg BaP/m^3 dose group compared to the UNC group.

The results of the two inhalation studies were supported by similar observations in studies using the oral exposure route (for overview, see (EPA, 2017).

Based on the available information in both human and animal reproductive toxicology studies, the current working group considers both male and female reproductive endpoints as critical effects of BaP exposure. A lowest observed adverse effect level

(LOAEL) of 75 μg BaP/m^3 for 4 hours a day for 60 days was identified for male reproductive effects (Archibong et al., 2008; Ramesh et al., 2008) and a no observed adverse effect level (NOAEL) of 75 μg BaP/m^3 for 4 hours a day for 14 days was identified for female reproductive effects (Archibong et al., 2012).

Developmental toxicity

Animal studies have shown that BaP and BaP metabolites are widely distributed in both maternal and foetal tissues after exposure, which indicate that they are able to translocate the placenta (Neubert & Tapken, 1988; Shendrikova & Aleksandrov, 1974; Withey et al., 1993). Indeed, developmental toxicity was observed in several animal studies. These effects have been summarized by EPA in 2017 as follows:

"In summary, it has been consistently demonstrated that developmental exposure to benzo[a]pyrene, particularly during late gestation or early postnatal development, causes persistent neurobehavioral effects that have been observed across two species, multiple strains, and both sexes of experimental animals, and across several behavioral domains. In addition, molecular changes in experimental animals have been observed, which are consistent with altered central nervous system function. While not every endpoint tested was affected to the same extent, or in the same manner, across studies and species, all of the identified studies reported at least one nervous system effect of developmental exposure, demonstrating a high level of consistency within the available database. In conclusion, although significant exposure gaps remain to be tested (most notably, benzo[a]pyrene exposures spanning gestation and lactation), EPA identified developmental toxicity (including developmental neurotoxicity) as a human hazard of benzo[a]pyrene exposure." (EPA, 2017)

The above mentioned effects were all observed in studies using the oral exposure route, with the exception of one inhalation study (Archibong et al., 2002). This study was also described above in the reproductive toxicology section. In short, the study investigated developmental changes following exposure of pregnant Fisher 344 rat to 25, 75 or 100 μg BaP/m^3 for 4 hours a day for 10 days (gestation days 11–20), nose-only inhalation (Archibong et al., 2002). Each group contained 10 females. CB was used as carrier of BaP. Control rats were exposed to either CB to control for potential inert BaP carrier effects or remained unexposed (UNC). Adverse pregnancy outcomes included decreased litter size and pup survival already at the 25 μg BaP/m^3 exposure level (Archibong et al., 2002). No differences in these endpoints were observed between the UNC and the CB carrier groups. This is in accordance with findings in newer studies investigating airway exposure to CB during gestation (Jackson et al., 2011; Skovmand et al., 2019).

The result of the oral studies included in the 2017 report from EPA are summarized in Figure 4. Only one oral study found similar decrease in litter size as in the inhalation study by Archibong and colleagues from 2002 (MacKenzie & Angevine, 1981). However, this study used doses that were more than a magnitude greater than the other oral studies investigating litter size, and they used the mouse animal model compared to rats in the other studies (Figure 4). Similar inconsistencies in effects on offspring body weight were reported across oral studies, whereas BaP oral exposure more greatly and more consistently affected reproductive effects in the offspring (Figure 4). For the majority of the studies, an oral LOAEL of 10 mg/kg-day was identified.

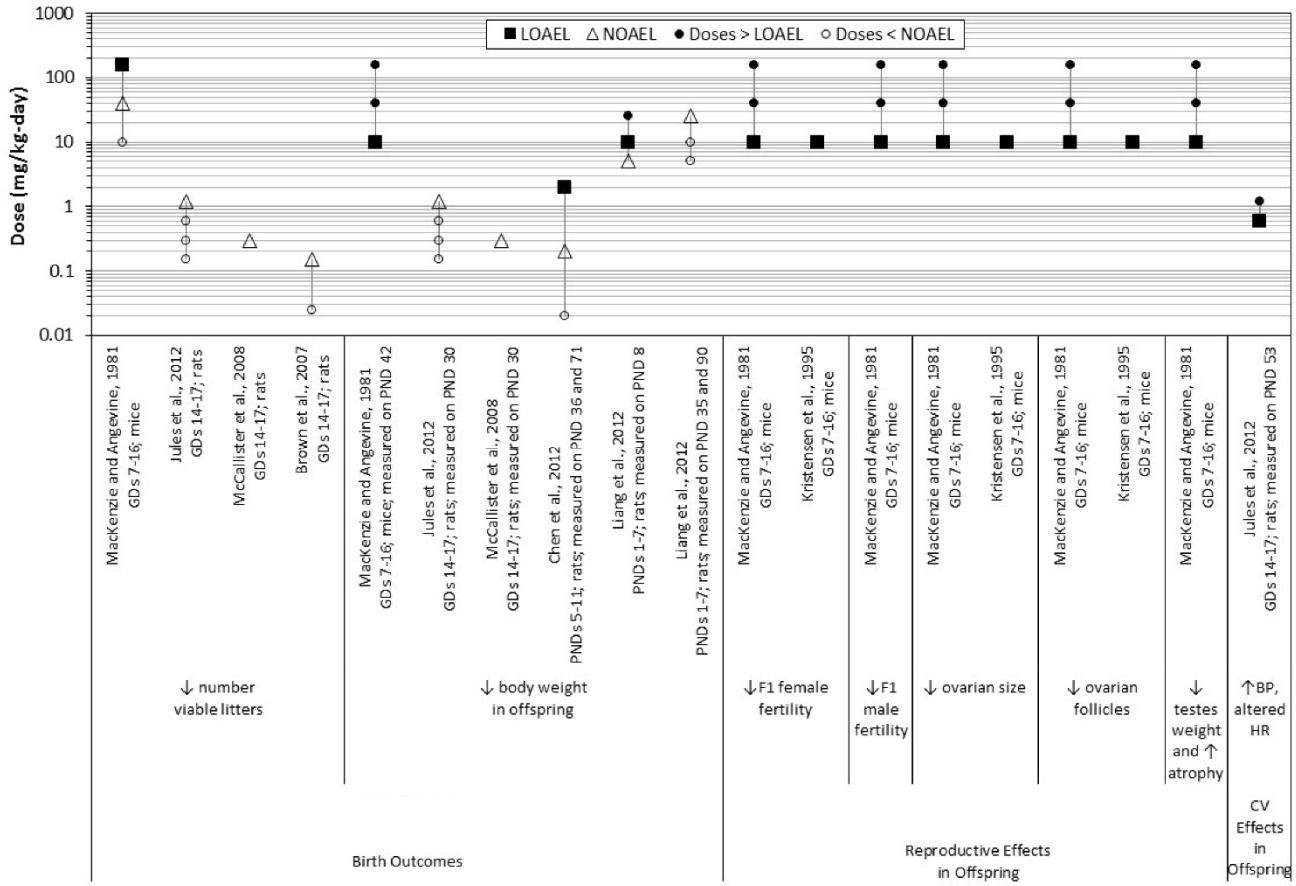

Figure 4. Overview of developmental effects and identified exposure-responses following oral exposure to BaP. From (EPA, 2017).

Based on the available information in both human and animal developmental toxicology studies, the current working group considers developmental toxicity a critical effect of BaP exposure. A LOAEL for developmental effects was set at 25 μg BaP/m^3 for exposure for 4 hours a day for 10 days (gestation days 11–20)(Archibong et al., 2002).

Mechanisms of toxicity

This section will focus on the primary hazards identified after PAH, and specifically BaP exposure, namely cancer, reproductive toxicity and developmental toxicity.

Cancer

This section will focus primarily on BaP, as the mechanisms of BaP induced carcinogenicity is well documented in animal models. BaP primarily induces carcinogenicity by a mutagenic mode of action, which is presumed to apply to all tumour types and is relevant for all exposure routes. Key events in the mode of action for BaP carcinogenicity is presented in Figure 5 (EPA, 2017).

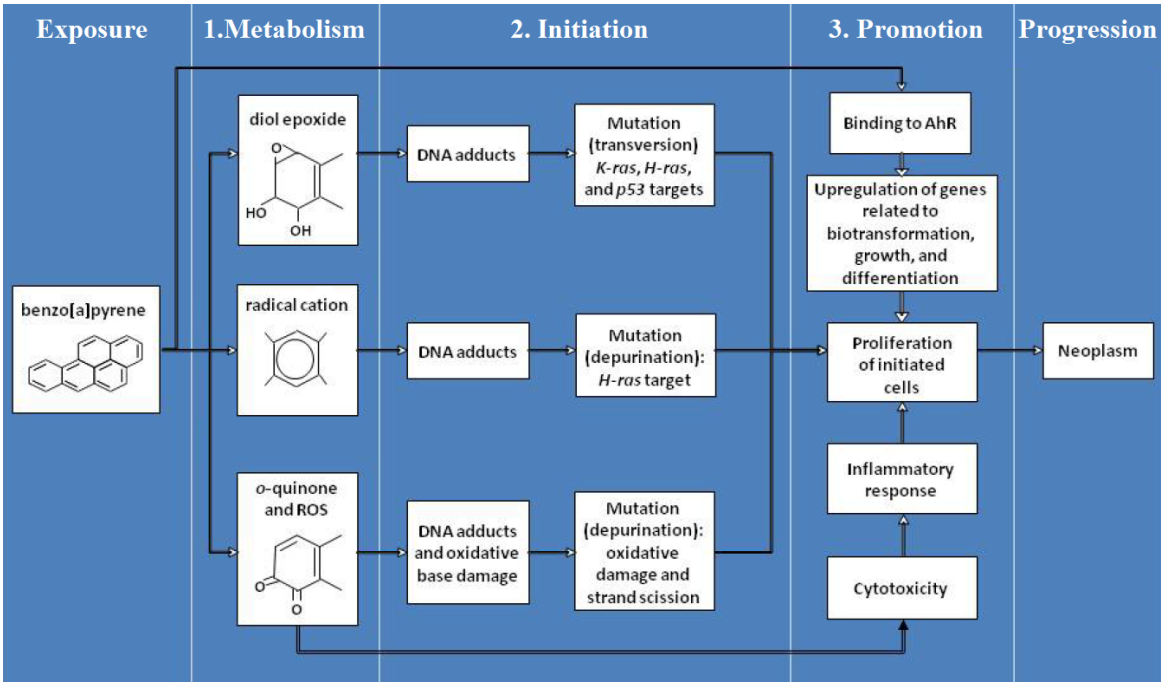

Figure 5. Overview of key events in the mode of action for BaP carcinogenicity. Modified from (EPA, 2017).

There are several steps involved in BaPs mutagenic mode of action:
1. Metabolism of BaP leads to bio-activated DNA-reactive chemicals: The diol epoxide pathway, the radical cation pathway, and the *o*-quinone and reactive oxygen species (ROS) pathway (Figure 5: Metabolism).
2A. Direct DNA damage by reactive metabolites, including the formation of DNA adducts and ROS-mediated damage (Figure 5: Initiation).
2B. Formation and fixation of DNA mutations, particularly in tumour suppressor genes or oncogenes associated with tumour initiation (Figure 5: Initiation).
3. Clonal expansion of mutated cells during the promotion and progression phases of cancer development (Figure 5: Promotion)(EPA, 2017).

In addition, BaP may induce cancer through AhR binding, which leads to an upregulation of genes related to biotransformation, growth, and differentiation, and from cell proliferation resulting from cytotoxicity and a sustained inflammatory response (Figure 5: Promotion). However, there is still insufficient evidence that these contributing mechanisms act independently of the DNA damage and mutations induced

by BaP metabolites to produce neoplasms (EPA, 2017). The present working group will therefore focus on the metabolite-induced mutagenesis.

Metabolite-induced mutagenesis

As stated above, the three main metabolic pathways are the diol epoxide pathway, the radical cation pathway, and the o-quinone and ROS pathway (Figure 6).

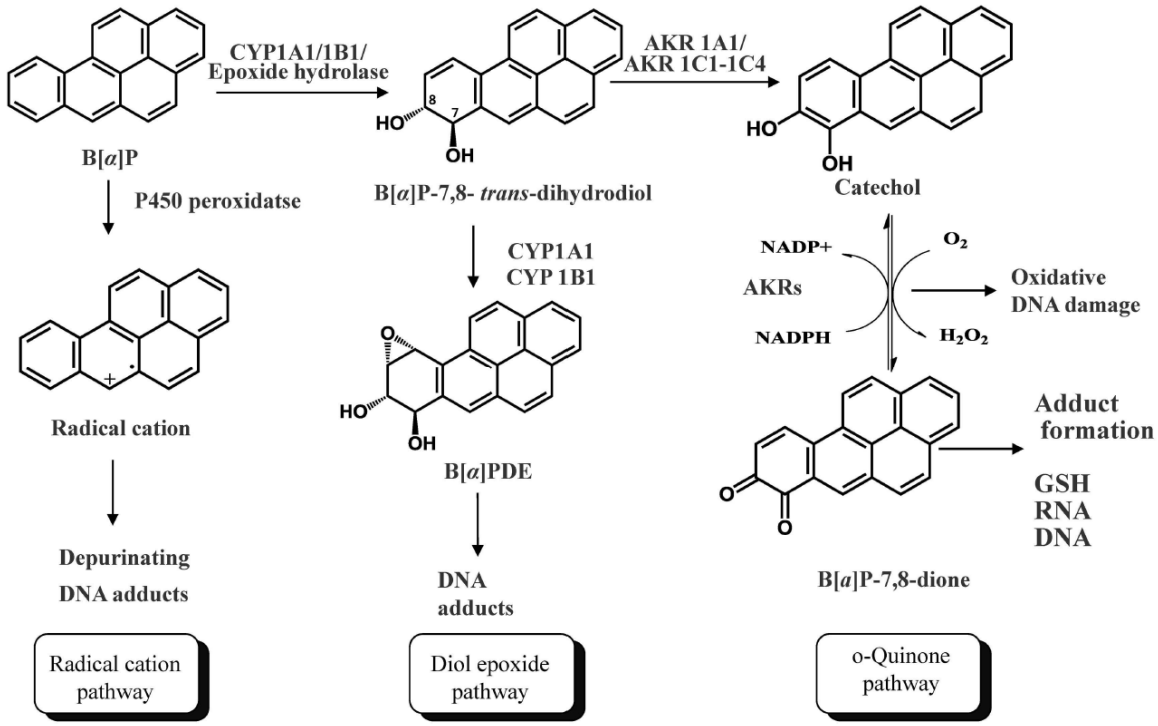

Figure 6. Three pathways of metabolic activation of BaP. AKR: Aldo-keto reductase. GSH: Glutathione. From (Zhang et al., 2012).

The diol epoxide pathway was briefly described in the metabolism section of the present report (Figure 2). This pathway has been highlighted as the main pathway in BaP-induced mouse-lung tumorigenesis (IARC, 2012), and EPA summarized the diol epoxide pathway and its effects as follows:

"Benzo[a]pyrene diol epoxide metabolites, believed to be the most potent DNA-binding metabolites of benzo[a]pyrene, are formed through a series of Phase I metabolic reactions. The initial metabolism is carried out primarily by the inducible activities of CYP enzymes including CYP1A1, CYP1B1, and CYP1A2, producing four benzo[a]pyrene epoxides. Further metabolism by epoxide hydrolase and the mixed function oxidase system yields trans-dihydrodiols, one of which, benzo[a]pyrene-7,8-diol (formed from benzo[a]pyrene-7,8-oxide), is the metabolic precursor to the potent DNA-binding metabolite benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE). The stereochemical nature of the diol epoxide metabolite (i.e., anti- versus syn-diol epoxides) affects the number and type of adducts and mutation that occurs; the enantiomer (+)-benzo[a]pyrene-7R,8S-diol-9S,10R-epoxide [(+)-anti-BPDE] is the most potent DNA-binding metabolite of benzo[a]pyrene. Benzo[a]pyrene diol epoxide metabolites interact preferentially with the exocyclic amino groups of deoxyguanine and deoxyadenine. Adducts may give rise to mutations unless these adducts are removed by DNA repair processes prior to replication.

Transversion mutations (e.g., GC→TA or AT→TA) are the most common type of mutation found in mammalian cells following diol epoxide exposure." (EPA, 2017)

In addition to adduct formation, BaP and its stable metabolites of the diol epoxide pathway were shown to induce gene expression changes related to hepatocellular carcinoma in HepG2 cells (Souza et al., 2016). This indicates that the metabolites are multifunctional in their ability to enact cancerous changes.

The molecular composition of the PAH has been linked to their carcinogenic potential through the diol epoxide pathway. PAH can be categorized based on the arrangement of their aromatic rings into the following categories: Non-bay-region (e.g., naphthalene), bay-region (e.g., benzo[a]pyrene (BaP)), and fjord-region (e.g., benzo[g]chrysene)(Figure 7). Bay-region PAH, which contain an indentation due to an angular benzene ring, have been reported in the literature to be severe carcinogens (SCOEL, 2016). Furthermore, fjord-region PAH are considered even more carcinogenic. This is primarily due to differences in the DNA binding efficiency of their carcinogenic diol epoxide intermediates, which are created through the diol epoxide pathway. Differences in structural features of the created DNA adducts and differences in DNA adduct recognition by nucleotide excision repair also play a part in the total carcinogenic potential (Dreij et al., 2004).

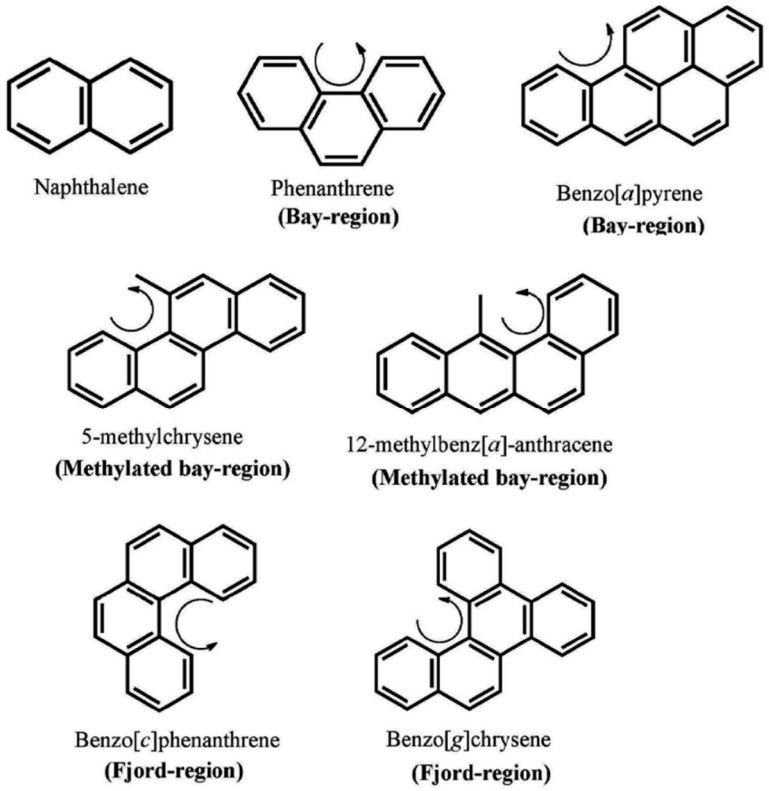

Figure 7. Chemical structures of PAH. The curly arrow denotes the presence of a bay-region, a methylated bay-region or a fjord-region. Modified from (Zhang et al., 2012).

Another mutagenic metabolic pathway of BaP is the radical cation pathway, which involves a one-electron oxidation of BaP by CYP or peroxidase enzymes that produce

electrophilic radical cation intermediates. These may in turn be further metabolized to phenols and quinones or they can form unstable adducts with DNA, which ultimately leads to depurination (Cavalieri et al., 1988a; Cavalieri & Rogan, 1995; Cavalieri et al., 1988b). The radical cation pathway has been shown to be highly involved in BaP-induced mouse-skin carcinogenesis (IARC, 2012).

The last of the main metabolic pathways is the o-Quinone and ROS pathway. The pathway and its effects were summarized by EPA as follows:

"The o-quinone metabolites of PAHs are formed by enzymatic dehydrogenation of dihydrodiols. Dihydrodiol dehydrogenase enzymes are members of the α-keto reductase gene superfamily. o-Quinone metabolites are potent cytotoxins, weakly mutagenic, and capable of producing a broad spectrum of DNA damage. These metabolites can interact directly with DNA as well as result in the production of ROS (i.e., hydroxyl and superoxide radicals) that may produce further cytotoxicity and DNA damage. The o-quinone/ROS pathway also can produce depurinated DNA adducts from benzo[a]pyrene metabolites. In this pathway, and in the presence of NAD(P)+, aldo-keto reductase oxidizes benzo[a]pyrene-7,8-diol to a ketol, which subsequently forms benzo[a]pyrene-7,8-dione. This and other PAH o-quinones react with DNA to form unstable, depurinating DNA adducts. In the presence of cellular reducing equivalents, o-quinones can also activate redox cycles, which produce ROS." (EPA, 2017)

This mechanism has mainly been studied in *in vitro* systems (IARC, 2012).

The current working group concludes that BaP metabolites induce DNA adduct formation and mutations. The current working group considers metabolite-induced mutagenesis as the primary mode of action and as a non-threshold mechanism leading to genotoxicity, mutagenicity and cancerous changes.

Reproductive toxicity

Male effects

Several studies in rodents have shown that BaP exposure induces male reproductive effects that include decreased sperm quality, decreased levels of testosterone, increased levels of LH, and histological changes in the testis (Archibong et al., 2008; Chen et al., 2011; Chung et al., 2011; Mohamed el et al., 2010; Ramesh et al., 2008; Zheng et al., 2010). These findings are supported by epidemiological studies reporting decreased sperm quality in populations highly exposed to PAH mixtures (Hsu et al., 2006; Soares & Melo, 2008). Proposed mechanisms of action include: BaP-mediated DNA damage to male germ cells, altered Leydig cell function, and oxidative stress.

BaP and its metabolites are genotoxic and mutagenic. In addition to their carcinogenic ability, studies have reported that BaP-induced DNA damage in sperm cells potentially could lead to cytotoxicity and apoptosis (Chung et al., 2011; Olsen et al., 2010; Perrin et al., 2011a; Perrin et al., 2011b; Revel et al., 2001), mutations (Xu et al., 2014), and decreased embryo viability post-fertilization (Borini et al., 2006; Seli et al., 2004). In addition, direct DNA damage from the reactive BaP metabolite, BPDE, may decrease

sperm motility (Perrin et al., 2011a). This was reported in a study in smokers, which showed that motile sperm had significantly fewer BPDE-adducts than less motile sperm.

Several studies have reported reduced testosterone levels in adult animals following oral or inhalation BaP exposure (Archibong et al., 2008; Chung et al., 2011; Ramesh et al., 2008; Zheng et al., 2010). This could be a subsequent effect of altered Leydig cell function. In addition to a reduction in testicular testosterone in rats after oral exposure to BaP, the authors also observed decreased testosterone production in isolated, stimulated Leydig cells (Chung et al., 2011). The decrease in testosterone levels and production may also be attributed to activated testicular macrophages, which is hypothesized to regulate Leydig cell function (Hales, 2002). Activated testicular macrophages produce inflammatory mediators, which inhibit Leydig cell testosterone production. This hypothesis was supported by a rat study showing that oral BaP exposure significantly increased ED-1 type testicular macrophages and decreased intratesticular testosterone.

Another possible driving factor in BaP-induced male reproductive effects may be oxidative stress. A study in rats showed that male reproductive effects, such as decreased sperm count, motility, and production, observed after 10-day oral administration of BaP could be ameliorated by antioxidant pre-treatment (Arafa et al., 2009). Citrus flavonoid hesperidin pre-treatment protected the rats from all effects except the decrease in sperm motility.

The current working group concludes that BaP exposure induces male reproductive toxicity, primarily related to genotoxicity, altered Leydig cell function and endocrine disruption, and oxidative stress. Male reproductive toxicity results in lowered sperm quality, which is considered a threshold effect.

Female effects

Both epidemiological and animal studies link BaP and PAH to female reproductive toxicity. In humans, significantly higher levels of BaP were found in the follicular fluid of women who did not conceive with *in vitro* fertilization (Neal et al., 2008). Interestingly, the same relative BaP level was demonstrated to inhibit *in vitro* growth of isolated rat follicles (Neal et al., 2008). In animals, BaP-induced depletion of follicles by destruction or deprivation of the primordial/primary follicle pool was demonstrated (Hoyer & Sipes, 1996; Lim et al., 2013; Mattison, 1982; Stedman, 1968). In addition to BaP, the PAHs 9,10-dimethylbenz-[a]-anthracene (DMBA) and 3-methylcholanthrene (3-MC) induced similar effects. EPA summarized that "*Studies in multiple strains of rats and mice indicate fertility-related effects including decreases in ovarian follicle populations and decreased fecundity"(EPA, 2017).* The current working group therefore highlights depletion of follicles as an important effect of BaP and PAH exposure on female reproductive toxicity.

The underlying mechanisms for BaP-induced follicle depletion are not fully established. However, stimulation of apoptosis is a potential mechanism. BaP have been shown to induce apoptosis in ovarian granulosa cells after exposure in rats (Xu et al., 2010), possibly through activation of AhR in oocytes and granulosa cells, with subsequent expression of pro-apoptotic genes (Kee et al., 2010; Matikainen et al., 2001; Matikainen et

al., 2002; Neal et al., 2010; Pru et al., 2009; Robles et al., 2000; Sadeu & Foster, 2011). AhR is expressed in rodent oocytes and granulosa cells at all stages of development (Bussmann & Baranao, 2006; Robles et al., 2000). A study showed that the harmful effect on folliculogenesis induced by BaP could be reversed by co-treatment with AhR antagonist (Neal et al., 2010). *In vitro* studies in cultured neonatal mouse ovaries confirmed the upregulation of AhR signaling after exposure to BaP, DMBA and 3-MC (Sobinoff et al., 2012a; Sobinoff et al., 2012b).

BaP may also induce female reproductive effects by alterations in the estrous cycle and the balance of reproductive hormones. Female rats exposed to BaP by inhalation showed alterations in their reproductive hormones, their estrous cycle length, and a decreased ovulation rate (Archibong et al., 2012). Similar observations were reported in a study with i.p. administration of BaP (Zhao et al., 2014). Here, female mice exposed to BaP after mating but prior to implantation (gestation days 1−5) had increased plasma estrogen and progesterone, altered endometrium morphology, decreased number of implantation sites, and an overall decreased pregnancy rate (Zhao et al., 2014).

Finally, BaP-DNA adducts have been detected in the ovarian tissue of female rats exposed to a single oral dose of BaP (5 mg BaP/kg)(Ramesh et al., 2010). In support of this, both BPDE-DNA oocyte adducts and DNA strand breaks in the cumulous cells were found in female mice exposed to a single oral dose of 13 mg/kg BaP (Einaudi et al., 2014). These findings indicate that genotoxicity is a possible mechanism for BaP induced female reproductive toxicity, which may ultimately result in cellular damage, reduced oocyte and embryo viability, and potentially transmission of mutations to surviving offspring.

The current working group concludes that BaP exposure induces female reproductive toxicity by a threshold mechanism, primarily through stimulation of apoptosis (resulting in follicle depletion), alterations in the estrous cycle, hormone imbalance and genotoxicity.

Developmental toxicity

Mode of action for PAH and BaP-induced developmental toxicity is still largely unknown. However, possible modes of action include: genotoxicity and mutagenicity, altered cell signalling, and oxidative stress.

As BaP and its metabolites are known mutagens, it is possible that observed developmental effects result from mutations in male and female germ cells, as well as foetal tissues, which could lead to decreased viability, birth defects, and altered development in offspring. This is supported by studies showing that genotoxicity in male germ cells may lead to decreased embryo viability post-fertilization (Borini et al., 2006; Seli et al., 2004).

BaP is a ligand for the AhR, and AhR signalling has been proposed as mode of action for other BaP-induced effects (e.g. cancer and reproductive effects). It is therefore plausible that altered cell signalling through the AhR also plays a role in BaP-induced

developmental toxicity. The association between AhR signalling and developmental toxicity was reviewed by EPA in 2017:

"Benzo[a]pyrene is a ligand for the AhR, and activation of this receptor regulates downstream gene expression including the induction of cytochrome (CYP) enzymes important in the conversion of benzo[a]pyrene into reactive metabolites. Studies in AhR knock-out mice indicate that AhR signaling during embryogenesis is essential for normal liver, kidney, vascular, hematopoietic, and immune development. In experiments in AhR-responsive and less-responsive mice, the mice with the less-responsive AhR were protected from renal injury as adults following gavage treatment with 0.1 or 0.5 mg/kg-day benzo[a]pyrene from GD 10 to 13. Renal injury was indicated by an increase in urinary albumin and a decrease in glomerular number. Another study at much higher doses (200 mg/kg-day by i.p. on GD 7, 10, or 12) found increased developmental effects in AhR-responsive C57BL/6 mice as compared to nonresponsive AhR AKR mice. Specifically, resorptions, malformations, and congenital abnormalities as well as decreased fetal body weight were observed more commonly in AhR-responsive mice. Similar findings were observed in a developmental toxicity study of two PAHs (3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene) in mice, with increases in stillborns, resorptions, and malformations in AH-responsive strains, indicating that mechanisms of developmental toxicity may be related to AhR signaling." (EPA, 2017)

A third possible mechanism of BaP-induced developmental effects is through oxidative stress, leading to oxidized DNA and protein damage mainly through the reactive metabolites of BaP (Wells et al., 1997). Studies have indicated that various organs (i.e. reproductive, lung, brain) are affected in offspring exposed prenatally to BaP (Li et al., 2012; Nakamura et al., 2012; Sheng et al., 2010; Thakur et al., 2014).

The current working group concludes that BaP exposure induces developmental effects primarily through genotoxicity and mutagenicity, altered cell signalling, and oxidative stress. Due to the barrier effect of the placenta, developmental effects are considered threshold effects.

In summary, the current working group considers both cancer, reproductive toxicity and developmental toxicity as critical effects of BaP exposure.

Previous evaluations of PAH and BaP

The most recent and most relevant risk assessments of PAH and BaP are presented in this section.

Benzo[a]pyrene as a risk indicator for PAH exposure

The present report uses BaP as a risk indicator for total PAH exposure, similar to that of other reports (AGS, 2011; DECOS, 2006; IARC, 2010; SCOEL, 2016). SCOEL 2016 summarized the reasoning as follows:

"Although it might be desirable to monitor total PAH or a selection of PAH, considering the vast and consistent amount of data presented for benzo[a]pyrene and the fact that benzo[a]pyrene is considered as one of the more potent PAH carcinogens, most available studies have preferred the use of benzo[a]pyrene as a marker substance for overall airborne PAH exposure for practical reasons. Validated analytic techniques are available to measure benzo[a]pyrene in air. Therefore, SCOEL considers benzo[a]pyrene as a quantitative indicator for general airborne PAH exposure to be an acceptable procedure in practice." (SCOEL, 2016)

The proposed OEL presented in this report will also be based on the concentration of BaP in the exposure.

Lung cancer

OELs can be calculated using different approaches. The approach of choice depends on the mode of action of the substance, and can fundamentally be split up in two: 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 is associated with a risk for adverse effects. Based on these assumptions, non-threshold effects are considered more critical than threshold effects. Cancer and mutagenicity via DNA adducts are considered as non-threshold mechanisms.

BaP has been classified as carcinogenic to humans by IARC (class 1) and as Carc. category 1B by the EU (Table 2). Some PAH have been shown to more carcinogenic than BaP; however, due to limited data these are currently evaluated as group 2A/B carcinogens (IARC, 2010, 2012). In addition, cancer has been identified as the critical effect in the literature and the main mode of toxicity for PAH and BaP (AGS, 2011; DECOS, 2006; IARC, 2010, 2012; SCOEL, 2016). All previous risk assessments have been based on lung cancer and morbidity (AGS, 2011; DECOS, 2006; SCOEL, 2016). As human exposure to PAH and BaP is well documented, previous risk assessments have been conducted based on epidemiogical data.

Previous reports

Dutch Expert Committee on Occupational Safety (DECOS), 2006

DECOS has presented health-based calculated - occupational cancer risk values (HBC-OCRVs), which they defined as the additional lifetime cancer risk value, usually expressed per ng/m^3, under occupational conditions. The authors calculated the HBC-

OCRV based on URR estimates presented in the meta-study by Armstrong et al. 2003, 2004, and they incorporated the following assumptions for occupational exposure: The average man lives 75 years, is exposed 8 hours per day, 5 days per week, 48 weeks per year, for a total of 40 years. Air inhalation was set at 10 m^3 air per 8-hour-working day. Their analyses showed that a log-linear model best described the relationship between exposure and cancer risk compared to a linear model. DECOS used the formula obtained from this log-linear model to derive HBC-OCRVs.
The log-linear equation was expressed as follows:

$URR_{cum\ exp\ X} = [URR_{cum\ exp\ 100}]^{(X/100)}$
$X= 100 \times \ln(URR_{cum\ exp\ X})/\ln(URR_{cum\ exp100})$

in which X represents the exposure concentration of BaP, 100 the benchmark of 100 μg/m^3 BaP years, the $URR_{cum\ exp\ X}$ the relative risk on exposure to X, and $URR_{cum\ exp\ 100}$ the relative risk of 1.20 for exposure to 100 μg/m^3 BaP years (2.5 μg BaP /m^3 x 40 years), as identified in Armstrong et al. 2003, 2004.

DECOS provides risk estimates at the risk levels $4x10^{-3}$ (prohibition risk level) and $4x10^{-5}$ (target risk level). The excess lifetime cancer risk depends on the background rate of lung cancer. In the Netherlands, lung cancer caused on average 24.21 deaths per 250 deaths (age>15 years). For the derivation of an HBC-OCRV, an additional risk of one extra cancer death due to occupational exposure per 250 death cases is taken into account ($4x10^{-3}$, corresponding to 4 excess death per 1000).

Unit relative risk ($URR_{cum\ exp\ X}$) was therefore calculated to: (24.21+1)/24.21 = 1.041. Using the log-linear equation, $X= 100 \times \ln(1.041)/\ln(1.20)$, this corresponds to a concentration of 22.03 μg benzo[a]pyrene/m^3 per 40 years. This corresponds to an average exposure of 551 ng/m^3 benzo[a]pyrene during a period of 40 working years (22.03 μg/40 years). For an additional risk of one cancer death per 25,000 death cases, the unit relative risk ($URR_{cum\ exp\ X}$) was 1.0004131 [(2421+1)/2421]. Using the log-linear equation, $X= 100 \times \ln(1.0004131)/\ln(1.20)$, this corresponds to a concentration of 0.227 μg benzo[a]pyrene/m^3 per 40 years, corresponding to an average exposure of 5.7 ng/m^3 benzo[a]pyrene during 40 working years.

In conclusion, DECOS derived HBC-OCRVs corresponding to an excess cancer mortality level of:
- 4 per 1,000 ($4x10^{-3}$) for 40 years of occupational exposure to benzo[a]pyrene and polycyclic aromatic hydrocarbons from coal-derived sources of 551 ng benzo[a]pyrene/m^3 (prohibition level).
- 4 per 100,000 ($4x10^{-5}$) for 40 years of occupational exposure to benzo[a]pyrene and polycyclic aromatic hydrocarbons from coal-derived sources of 5.7 ng benzo[a]pyrene/m^3 (target risk level).

However, as these HBC-OCRVs are calculated based on a work-life of 40 years instead of 45 as used in Denmark, they need to be transformed for comparison with the risk assessment for the Danish working environment calculated in the present report:

- 4 per 1,000 ($4x10^{-3}$) for 40 years: 550 ng BaP/m^3.
  550 ng BaP/m^3 x 40/45 = 488.9 ng BaP/m^3.
- 4 per 100,000 ($4x10^{-5}$) for 40 years: 5.7 ng BaP/m^3.
  5,7 ng BaP/m^3 x 40/45 = 5.07 ng BaP/m^3.

Ausschuss für Gefahrstoffe (AGS), 2011

Similar to the hazard evaluation from DECOS, AGS (2011) used the URR of 1.20 identified in the meta-study from Armstrong et al. 2003, 2004 for deriving their exposure-risk relationship. The lung cancer rate in Germany (2007) for men was 7.4% or 18.61 deaths per 250 deaths. The authors used the same log-linear equation as in DECOS 2006: $URR_{cum\ exp\ X} = [URR_{cum\ exp\ 100}]^{(X/100)}$. The unit relative risk ($URR_{cum\ exp\ X}$) was calculated to: (18.61+1)/ 18.61 = 1.0537. Using the log-linear equation, X= 100 x ln(1.0537)/ln(1.20), this corresponds to a concentration of 28.7 μg benzo[a]pyrene/m^3 per 40 years. This corresponds to an average exposure of 720 ng/m^3 benzo[a]pyrene over a period of 40 working years (28.7 μg/40 years). For an additional risk of one cancer death per 25,000 death cases, the unit relative risk ($URR_{cum\ exp\ X}$) was 1.00054 [(1861+1)/1861]. Using the log-linear equation, X= 100 x ln(1.00054)/ln(1.20), this corresponds to a concentration of 0.29 μg benzo[a]pyrene/m^3 per 40 years, corresponding to an average exposure of 7.4 ng/m^3 benzo[a]pyrene over 40 working years.

In conclusion, AGS derived accept risks corresponding to an excess cancer mortality level of:
- 4 per 1,000 ($4x10^{-3}$) for 40 years of occupational exposure to benzo[a]pyrene and polycyclic aromatic hydrocarbons from coal-derived sources of 720 ng benzo[a]pyrene/m^3 (prohibition level).
- 4 per 100,000 ($4x10^{-5}$) for 40 years of occupational exposure to benzo[a]pyrene and polycyclic aromatic hydrocarbons from coal-derived sources of 7.4 ng benzo[a]pyrene/m^3 (target risk level).

However, as the excess mortality risks are calculated based on a work-life of 40 years instead of 45 as used in Denmark, they need to be transformed for comparison with the risk assessment for the Danish working environment calculated in the present report:
- 4 per 1,000 ($4x10^{-3}$) for 40 years: 720 ng BaP/m^3.
  720 ng BaP/m^3 x 40/45 = 640 ng BaP/m^3.
- 4 per 100,000 ($4x10^{-5}$) for 40 years: 7.4 ng BaP/m^3.
  7.4 ng BaP/m^3 x 40/45 = 6.58 ng BaP/m^3.

The Scientific Committee on Occupational Exposure Limits (SCOEL), 2016

SCOEL evaluated PAH mixtures containing BaP in 2016 in regards to exposure, monitoring, health effects and possible exposure limits. As PAH are considered genotoxic carcinogens (SCOEL Group A), no safe health-based exposure limit can be derived. Therefore, SCOEL does not recommend an OEL. Instead, they assessed carcinogenic risk, with BaP as indicator substance, based on the two previous risk assessment reports from DECOS and AGS (AGS, 2011; DECOS, 2006).

The report by SCOEL concluded that a mean airborne 8h-TWA PAH exposure in the order of 6 ng benzo[a]pyrene per m^3 during a 40 year work-life would lead to an excess lung cancer mortality rate of 4×10^{-5}.

Scientific basis for setting an occupational exposure limit

Similar to that of previous hazard-assessment reports (AGS, 2011; DECOS, 2006; SCOEL, 2016), the present working group considers BaP as a quantitative indicator for general airborne PAH exposure, and OELs will therefore be calculated based on BaP air concentrations.

Health-based exposure limit based on epidemiological cancer data

The present working group considers PAH and BaP-induced mutagenesis and cancer as non-threshold mechanisms, in line with previous risk assessments (AGS, 2011; DECOS, 2006; SCOEL, 2016). Calculated proposed OELs for lung cancer will therefore be based on non-threshold effects in the present report, and calculations will be performed based on data from epidemiological studies. Inhalation is used as a proxy for the total exposure.

The present working group will calculate and provide health-based OELs for BaP (as a proxy for total PAH content) in the Danish working environment based on the previously described meta-analysis by Armstrong et al. 2003, 2004. Thus, we use the same approach as previous reports (AGS, 2011; DECOS, 2006; SCOEL, 2016). In the comprehensive meta-analysis, the association between lung cancer mortality and morbidity, and occupational exposure to BaP was modelled. This association is described as a log-linear equation (Armstrong et al., 2004; Armstrong, 2003). URR refers to increases in relative lung cancer risk per 100 μg/m^3 benzo[a]pyrene-years, in which 100 μg/m^3 benzo[a]pyrene-years corresponds to a concentration of 2.5 μg/m^3 benzo[a]pyrene over 40 years.

$$URR_{cum\ exp\ X} = [URR_{cum\ exp\ 100}]^{(X/100)}$$

Exposure was measured as BaP in μg/m^3 years.
Mean URR for 39 distinct cohorts: 1.20.
URR= 1.20 exposure to 100 μg/m^3 years (2.5 ug BaP/m^3 40 years).
URR= 1.20 exposure to 100 μg/m^3 years (2.22 ug BaP/m^3 45 years).

In Denmark, the life time risk of developing lung cancer (0-74 years) is 4.9% for men and 4.5% for women (NORDCAN, 2019). 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 7.

Table 7. 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 (NORDCAN, 2019).

	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 = 1.0002	RR = (4500+1)/4500 = 1.0002

One extra cancer incident due to occupational exposure per 1000 death cases, URR for men: (49+1)/49 = 1.02.

$1.02 = [1.20]^{(X/100)}$
$Ln(1.02) = ln(1.20) \times X/100$
$0.109 = X/100$
$X = 10.9$

BaP concentration in $\mu g/m^3$-years = 10.9 $\mu g/m^3$-years
For a 45-year work-life this would correspond to 10.9 $\mu g/m^3$-years/45 years = 0.24 $\mu g/m^3$

Assuming 1:100 000 excess lung cancer incidences among men, the calculation is:
One extra cancer death due to occupational exposure per 100,000 death cases, URR for men: (4900+1)/4900 = 1.0002.

$1.0002 = [1.20]^{(X/100)}$
$Ln(1.0002) = ln(1.20) \times X/100$
$0.00109 = X/100$
$X = 0.109$

BaP concentration in $\mu g/m^3$-years = 0.109 $\mu g/m^3$-years
For a 45-year work-life this would correspond to 0.109 $\mu g/m^3$-years/45 years = 0.0024 $\mu g/m^3$.

Table 8. Calculated excess lung cancer incidence based on epidemiological data (Armstrong et al. 2003,2004).

Excess lung cancer incidence	BaP air concentration ($\mu g/m^3$)
1:1,000	0.24
1: 10,000	0.024
1: 100,000	0.0024

Bladder cancer is also considered a critical effect of BaP and PAH exposure. However, as described in the section, *Epidemiological cancer studies*, the data foundation is not solid enough to calculate OELs for bladder cancer.

Skin notation

From the available data, the current working group notes the following observations: Skin penetration of PAH occurs in significant amounts and urinary 1-hydroxypyrene levels of workers exposed to PAH clearly indicate that a large amount of the excreted metabolites had entered the body through the skin. Based on these observations, the current working group recommends a skin notation for BaP and other PAH.

Health-based exposure limit based on reproductive toxicology and developmental data in animals

The present working group considers PAH and BaP-induced reproductive and developmental toxicity as threshold effects. As no human studies suitable for reproductive and developmental toxicity risk assessment exist, calculations will be performed based on data from animal studies. The present working group calculates the DNEL as recommended by ECHA for toxicological effects having thresholds (ECHA, 2012).

Reproductive toxicity

Male reproductive effects

Two sub-chronic rat studies originating from the same study setup were identified as critical studies for male reproductive toxicity (Archibong et al., 2008; Ramesh et al., 2008). These studies reported comprehensive reproductive effects in male F344 rats after exposure by nose-only inhalation to 75 μg BaP/m^3 for 4 hours a day for 60 days. The reproductive effects included: decreased epididymal sperm motility and sperm count, increased number of abnormal epididymal sperm, decreased testis weight and intratesticular and serum testosterone levels. Exposure to 75 μg BaP/m^3 for 4 hours/day is considered the LOAEL, as there was no NOAEL. It should be noted that this study used CB as a carrier for BaP, but did not include a CB-only control. However, in ambient air BaP naturally absorbs to carbon particles; therefore the current working group does not consider further adjustments for CB-related effects necessary.

Calculations

Initially, the LOAEL is corrected to take into account that rats were exposed for 4 hours a day, whereas humans are considered to be exposed for 8 hours a day in the occupational setting. In addition, exposed rats were at rest, whereas workers are considered to do light activity. A total breathing volume of 10 m^3 for an 8-hour shift at light activity is therefore assumed, compared to 6.7 m^3 at rest.

Corrected LOAEL:
75 μg BaP/m^3 x (4h/day)/(8h/day) x (6.7 m^3/10 m^3) = 25 μg BaP/m^3

Secondly, the corrected LOAEL is adjusted by a number of assessment factors (of which most are default values suggested by ECHA). When the starting point for the DNEL calculation is a LOAEL instead of a NOAEL, it is suggested to use an assessment factor between 3 (as minimum/majority of cases) and 10 (as maximum/exceptional cases) (ECHA, 2012).

The following default assessment factors are therefore used:
Extrapolation from sub-chronic to chronic exposure: 2
Interspecies extrapolation: 2.5
Intraspecies interpolation (default factor for workers): 5
Use of LOAEL: 3-10

The overall assessment factor, $AF_{overall\ min} = 2 * 2.5 * 5 * 3 = 75$
The overall assessment factor, $AF_{overall\ max} = 2 * 2.5 * 5 * 10 = 250$

This results in a DNEL for chronic inhalation for male reproductive toxicity:
$DNEL_{min} = LOAEL_{corr}/AF_{overall\ min} = 25\ \mu g\ BaP/m^3 / 75 = 0.33\ \mu g/m^3$
$DNEL_{max} = LOAEL_{corr}/AF_{overall\ max} = 25\ \mu g\ BaP/m^3 / 250 = 0.10\ \mu g/m^3$

Female reproductive effects
One study was identified as critical for female reproductive toxicity (Archibong et al., 2012). In this study, adult female Fisher-344 rats were exposed to 50, 75 or 100 μg BaP/m^3 via nose-only inhalation for 4 hours/day for 14 days. Group sizes were 20. CB was used as a carrier for BaP. Control rats were constrained in similar fashion as exposed rats, but did not receive neither BaP-CB nor CB. The authors reported changes in the estrous cycle of the rats belonging to the 100 μg BaP/m^3 dose group. This included decreased serum estradiol and LH levels in the proestrus fase, decreased serum progesterone levels in the diestrus I fase, and increased serum FSH at all stages of the estrous cycle, in addition to a general increase in estrous cycle length (+24 hours). The authors also reported decreased ovulation rate in the rats belonging to the 100 μg BaP/m^3 dose group compared to the control group. Based on the information provided in the study, the current working group considers the 75 μg BaP/m^3 exposure level as the NOAEL. Similar to the male reproductive toxicity, CB was used as a carrier without including a CB-only control. However, also in this case the current working group does not consider further adjustments for CB-related effects as necessary, as BaP naturally absorbs to carbon particles in ambient air.

Calculations
A corrected NOAEL is first calculated, in which exposure duration is taken into account (4 hours vs. 8 hours) and a correction for rest vs. light activity is performed.

Corrected NOAEL:
75 μg BaP/m^3 x (4h/day)/(8h/day) x (6.7 m^3/10 m^3) = 25 μg BaP/m^3

Secondly, the corrected NOAEL is adjusted by a number of assessment factors (most are default values suggested by ECHA).

The following default assessment factors are therefore used:
Extrapolation from sub-acute to chronic exposure: 6
Interspecies extrapolation: 2.5
Intraspecies interpolation (default factor for workers): 5

The overall assessment factor, $AF_{overall} = 6 * 2.5 * 5 = 75$

This results in a DNEL for chronic inhalation for female reproductive toxicity:
$DNEL = NOAEL_{corr}/AF_{overall} = 25\ \mu g\ BaP/m^3 / 75 = 0.33\ \mu g/m^3$

Developmental toxicity

One inhalation study in rats was identified as a critical study for developmental toxicity (Archibong et al., 2002). This study reported decreased litter size and pup survival rate in all exposure groups (25, 75 and 100 μg BaP/m³) after nose-only inhalation exposure for 4 hours/day for 10 days (gestation days 11–20). The inhalation study used CB as a carrier, and included both a CB-only control group and a plain control group (exposure to neither BaP nor CB)(Archibong et al., 2002). No effects were observed in the CB-only group. The dose of 25 μg BaP/m³ for 4 hours a day is considered the LOAEL, as no NOAEL was reported. It should be noted that low dose oral exposure studies in rats were unable to detect similar developmental changes (EPA, 2017).

Calculations
A corrected LOAEL is first calculated, in which that exposure duration is taken into account (4 hours vs. 8 hours). Then a correction for rest vs. light activity is performed.

Corrected LOAEL:
$25\ \mu g\ BaP/m^3 \times (4h/day)/(8h/day) \times (6.7\ m^3/10\ m^3) = 8.4\ \mu g\ BaP/m^3$

The corrected LOAEL is then adjusted by a number of assessment factors (when the starting point for the DNEL calculation is a LOAEL instead of a NOAEL, it is suggested to use an assessment factor between 3 (as minimum/majority of cases) and 10 (as maximum/exceptional cases))(ECHA, 2012). No extrapolation to chronic exposure is performed.

The following default assessment factors are therefore used:
Interspecies extrapolation: 2.5
Intraspecies interpolation (default factor for workers): 5
Use of LOAEL: 3-10

The overall assessment factor, $AF_{overall\ min} = 2.5 * 5 * 3 = 37.5$
The overall assessment factor, $AF_{overall\ max} = 2.5 * 5 * 10 = 125$

This results in a DNEL for developmental toxicity:
$DNEL_{min} = LOAEL_{corr}/AF_{overall\ min} = 8.38\ \mu g\ BaP/m^3 / 37.5 = 0.223\ \mu g/m^3$
$DNEL_{max} = LOAEL_{corr}/AF_{overall\ max} = 8.38\ \mu g\ BaP/m^3 / 125 = 0.067\ \mu g/m^3$

Animal model

The calculated DNELs for reproductive and developmental toxicity are based on studies using the F344 rat as animal model. This model has previously been excluded from the US National Toxicology Program as a cancer bioassay model, due to its higher

background frequency of Leydig cell tumors and mononuclear cell leukemia (Maronpot er al, 2016). This raises some concern about a similar possible sensitivity to BaP and other PAH in this animal model. However, oral exposure to BaP results in increased reproductive and developmental toxicity across animal species and strains (Figure 4)(EPA, 2017; Arafa et al., 2009; Chen et al., 2011; Xu et al., 2010; Zheng et al., 2010). Also, similar to that observed for F344 rats (Archibong et al., 2002), lowered birth index was observed in Sprague Dawley rats exposed nose-only to 100 μg BaP/m^3 for 4 hours a day for 11 days (gestation days 11–21)(Wormley et al. 2004). Taken together, this indicates similar sensitivity to BaP exposure in regards to at least developmental toxicity across rat strains. The working group have therefore decided not to adjust for rat strain in the DNEL calculations.

Sensitive groups

Women are born with a finite number of follicles that gradually diminishes until menopause. It is therefore assumed that the number of follicles at birth reflects human susceptibility to depletion of follicle reserves (premature menopause) by BaP and PAH. Hence, individuals born with low follicle counts, or acquiring low follicle counts due to surgery (one-sided ovariectomy) or medication (chemotherapy) would constitute potential sensitive subpopulations (Kirman & Grant, 2012). The average age for women's birth of the first child is approximately 29 years in Denmark, a time where fertility is already reduced (Baird et al., 2005). Assuming an entry to the labour market at 18 years of age, this leaves several years for exposure before pregnancy is pursued.
For men, BaP exposure induces reproductive toxicity resulting in lowered sperm quality. Therefore, men with low sperm quality prior to exposure are considered a sensitive group. Additionally, PAH are formed during incomplete combustion of tobacco. Hence, smokers might also be a sensitive group relative to additional exposure to PAH at work. These considerations have not been taken into consideration in the intraspecies assessment factor.

Conclusion

PAH are bi-products, formed during combustion and pyrolysis processes of organic materials, primarily through man-made sources. More than 100 single PAH have been identified. PAH exist solely in complex mixtures that contain many different PAH and related compounds. The most extensively studied PAH as surrogate for total PAH exposure is BaP, which is also considered to contribute significantly to the carcinogenic potency of PAH-rich mixtures. BaP is classified as carcinogenic to humans by IARC (class 1), and in the EU the substance is classified for carcinogenicity (Carc 1B), mutagenicity (Muta. 1B), reproductive toxicity (Repr 1B), and for skin sensitisation (Skin Sens. 1). Other PAH have been classified as possibly or probably carcinogenic to humans (class 2A or 2B). National and international authorities have decided to use BaP as an indicator for total PAH exposure. Similarly, the current working group is of the opinion that BaP can be used as a quantitative indicator of airborne PAH exposure in the working environment. The current Danish OEL is 0.2 mg/m^3 for PAH (benzene soluble fraction). No specific OEL exists for BaP in Denmark.

Occupational exposure to PAH mainly occurs in the major PAH-generating industries. The present working group notes that BaP exposure levels vary up to a 1000-fold between occupational settings, with the highest exposure levels ranging from 26 to around 100 µg BaP/m^3. Airborne PAH can be absorbed though inhalation, (secondary) ingestion and skin contact. Metabolism occurs at the site of exposure, and metabolites can subsequently be found in blood and in urine. Furthermore, the current working group notes that PAH and their metabolites are able to pass the placental barrier. PAH metabolites and their conjugates are excreted in urine and faeces. The current working group notes that 1-hydroxypyrene in urine is the most commonly measured biomarker of BaP and PAH exposure, but that urine levels of the BaP metabolite 3-hydroxy-benzo[a]pyrene may prove to be a more suitable biomarker for biomonitoring.

The most critical toxicological endpoint of PAH, and especially BaP, exposure is cancer. Based on the presented epidemiological evidence, the current working group considers lung and bladder cancer as critical effects of BaP and PAH exposure. This is facilitated through metabolism of BaP and PAH leading to bio-activated DNA-reactive metabolites that directly damage the DNA by formation of DNA adducts. The current working group considers this as the primary mode of action and as a non-threshold mechanism leading to genotoxicity, mutagenicity and cancerous changes.

The most well-documented non-cancerous effects of PAH and BaP exposure relate to reproductive and developmental effects. Based on the available information in both human and animal studies, the current working group consider both male and female reproductive toxicity and developmental toxicity as critical effects of BaP exposure via a threshold mechanism.

An OEL based on non-threshold lung cancer data from epidemiological studies and DNELs based on threshold effects from animal reproductive and developmental toxicity studies were therefore calculated.

Prior to the risk assessment calculations, the most recent and relevant risk assessments of PAH and BaP were reviewed. These reports calculated risk levels for lung cancer based on data from the meta-study by Armstrong and colleagues (Armstrong et al., 2004; Armstrong, 2003). Prohibition levels (4 per 1,000) and target risk levels (4 per 100,000) are presented in Table 9.

Table 9. Overview of health-based OELs calculated in previous assessments after 45 work years based on a non-threshold mechanism of action (AGS, 2011; DECOS, 2006).

Lung cancer			
Previous assessments			
DECOS 2006		AGS 2011	
HBC-OCRV	BaP air concentration (μg/m^3)	Excess lung mortality risk	BaP air concentration (μg/m^3)
4 per 1,000	0.454	4: 1,000	0.64
4 per 10,000	0.049	4: 10,000	0.066
4 per 100,000	0.0051	4: 100,000	0.0066
1 per 1,000	0.114	1: 1,000	0.160
1 per 10,000	0.012	1: 10,000	0.017
1 per 100,000	0.0013	1: 100,000	0.0017

The present working group calculated and proposed health-based OELs for BaP (as a proxy for total PAH content) for the Danish working environment based on the lung cancer data from the meta-analysis by (Armstrong et al., 2004; Armstrong, 2003), taking the same approach as previous reports. The calculated excess lung cancer incidences are presented in Table 8 and 10. High comparability with previous risk assessments were observed. In addition to the lung cancer assessment, the current working group also recommends a skin notation for BaP and other PAH.

Table 10. Calculated excess lung cancer incidence based on epidemiological data (Armstrong et al. 2003,2004).

Lung cancer	
Present report	
Excess lung cancer incidence	BaP air concentration (μg/m^3)
1: 1,000	0.24
1: 10,000	0.024
1: 100,000	0.0024

DNELs for male and female reproductive toxicity and developmental toxicity based on animal studies were calculated as recommended by ECHA for toxicological effects having thresholds (ECHA, 2012). A LOAEL of 75 μg BaP/m^3 for 4 hours a day for 60 days was identified for male reproductive effects, and a NOAEL of 75 μg BaP/m^3 for 4 hours a day for 14 days was identified for female reproductive effects. A LOAEL for developmental effects was identified at 25 μg BaP/m^3 for exposure for 4 hours a day for

10 days (gestation days 11–20). Due to the range in assessment factors for LOAEL to NOAEL (3 or 10), $AF_{overall\ min}$ and $AF_{overall\ max}$ were calculated for male reproductive and developmental toxicity. For the conclusion, the current working group has decided to present calculations based on $AF_{overall\ min}$, but notice the lower DNELs when using the $AF_{overall\ max}$. The current working group notes that the severity of the reported reproductive and developmental effects, combined with introduction of sensitive groups, could warrant the use of the more strict assessment factor. DNELs for male and female reproductive toxicity and developmental toxicity are presented in Table 11.

Table 11. Overview of DNEL for BaP air concentration based on a threshold based mechanism of action.

Non-neoplastic toxicity					
Present report					
Developmental toxicity		**Reproductive toxicity**			
		Male		Female	
DNEL	0.223 µg/m^3	DNEL	0.335 µg/m^3	DNEL	0.335 µg/m^3

The current working group considers both cancer and the identified non-neoplastic toxicities as critical effects. Therefore, the current working group recommends that both outcomes are taken into consideration.

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