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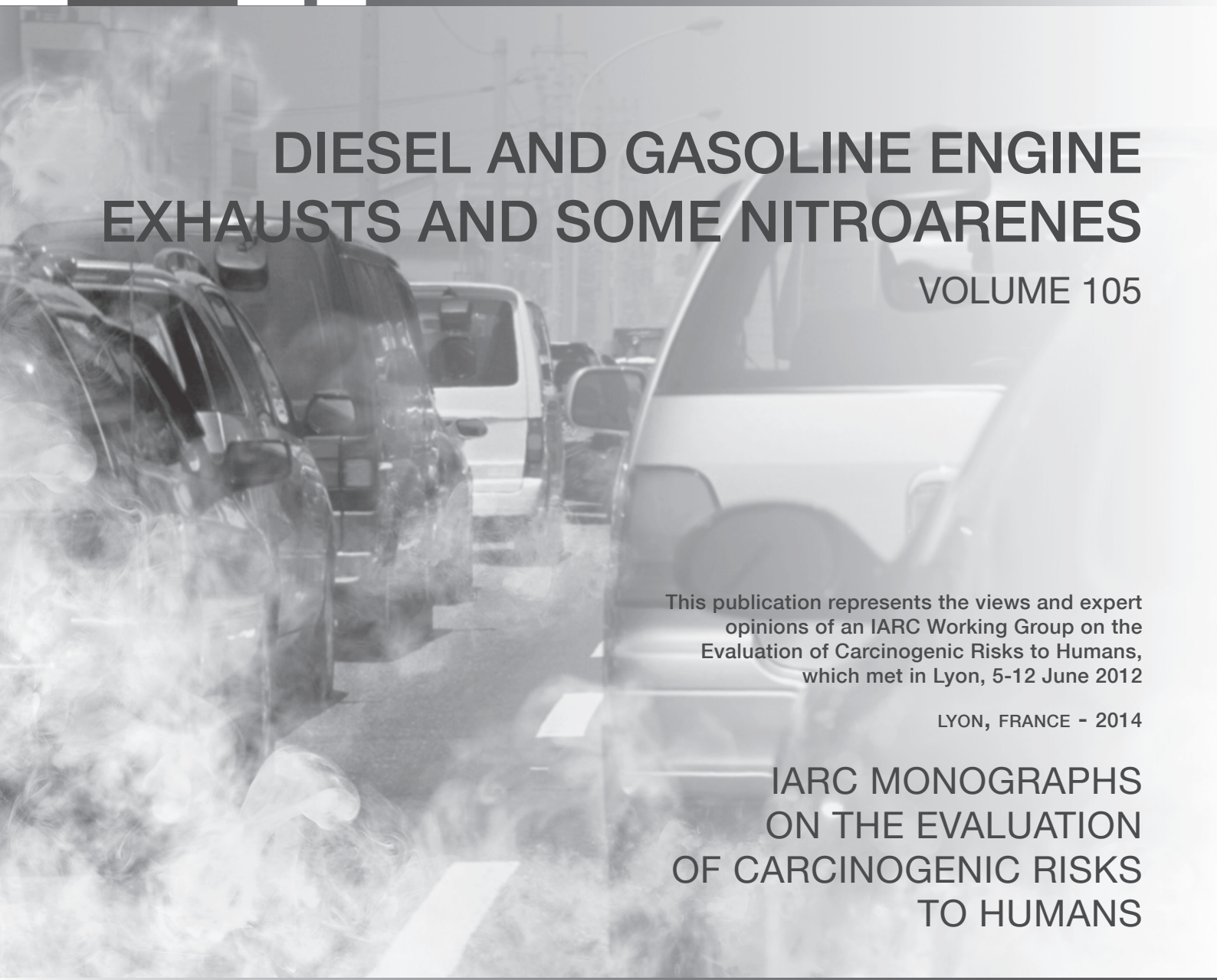

DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

3-NITROBENZANTHRONE

3-Nitrobenzanthrone has not previously been evaluated by an IARC Working Group.

1. Exposure Data

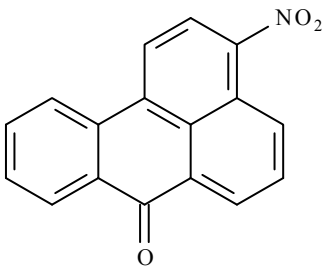
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abst. Serv. Reg. No.: 17117-34-9

Synonym:
3-Nitro-7*H*-benzo[*d*,*e*]anthracen-7-one

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{17}H_9NO_3$

Relative molecular mass: 275.27 g/mol

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow powder (Enya *et al.*, 1997)

Melting-point: 256–257 °C (Enya *et al.*, 1997); 252 °C (Suzuki *et al.*, 1997)

Boiling-point: 506.2 °C at 760 mm Hg

Flash-point: 256.6 °C

Spectrometry data: Infrared and nuclear magnetic resonance data have been reported (Enya *et al.*, 1997; Suzuki *et al.*, 1997).

1.2 Analysis

For the analytical methods of nitro-polycyclic aromatic hydrocarbons (PAHs) in general, the reader is referred to Section 1.2.2(*d*) of the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume.

3-Nitrobenzanthrone is a semi-volatile compound with a high molecular weight, and can therefore be collected on a filter.

Phousongphouang & Arey (2003) measured low concentrations of 3-nitrobenzanthrone in ambient air by mass spectrometry, and Tang *et al.* (2004) developed a method using high-performance liquid chromatography (HPLC) with chemiluminescence detection for the analysis of nitrobenzanthrones in airborne particulates.

1.3 Production and use

1.3.1 Production

3-Nitrobenzanthrone can be produced by the direct nitration of benzanthrone with nitric acid in an organic solvent (Suzuki *et al.*, 1997), or with gaseous nitrogen dioxide and ozone (Enya

et al., 1998). Suzuki *et al.* (1997) synthesized the compound using the modified Ullmann cross-coupling reaction between 4-nitro-substituted 1-iodonaphthalene and methyl-iodo-benzoate, followed by ring closure of the resulting 2-(1-naphthyl)benzoic acid derivative.

1.3.2 Use

No evidence was found that 3-nitrobenzanthrone has been used in commercial applications.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

3-Nitrobenzanthrone was first discovered by Suzuki *et al.* (1997) in organic extracts of both diesel exhaust and airborne particles. It is formed by the combustion of organic material and from reactions of complex PAHs with nitrogen oxides. It has been suggested that 2-nitrobenzanthrone might be formed more specifically by atmospheric processes (Inazu *et al.*, 2008), while 3-nitrobenzanthrone seems to be formed preferentially by combustion processes, such as in a diesel engine (Feilberg *et al.*, 2002; Phousongphouang & Arey, 2003; Tang *et al.*, 2004).

Table 1.1 summarizes the available data on environmental exposure from point sources and on environmental samples of air, soil and rainwater.

(a) Point sources

3-Nitrobenzanthrone has been detected in diesel exhaust particles at concentrations of up to 6.6 μg/g particles (Enya *et al.*, 1997; Murahashi, 2003). Furthermore, it was detected in extracts of particles collected from the chimney of a domestic coal-burning stove at a concentration of 0.23 μg/g particles, suggesting that particles emitted from industrial and domestic coal-burning sources should be considered as possibly minor sources of 3-nitrobenzanthrone in urban air pollution (Taga *et al.*, 2005).

(b) Environmental levels

Airborne concentrations of 3-nitrobenzanthrone in ambient particles have been reported to be in the order of several picgrams per cubic metre (up to > 11.5 pg/m^3) in urban and semi-rural areas, and at sites affected by traffic or industrial emissions. Higher levels (up to > 80 pg/m^3) have been recorded in ambient air at workplaces exposed to high levels of diesel emissions (Seidel *et al.*, 2002).

More recently, Inazu *et al.* (2008) measured airborne levels of 3-nitrobenzanthrone in central Tokyo, Japan, in the range of 0.5–3.5 fmol/m^3 (0.2–1.0 pg/m^3); the highest levels were observed in the winter (average, 0.6 pg/m^3) and the lowest levels were found in the spring (average, 0.2 pg/m^3).

Murahashi *et al.* (2003a) detected 3-nitrobenzanthrone in rainwater samples collected at a residential area in Kyoto, Japan, at levels ranging from 0.07 to 2.6 ng/L. It was also detected in surface soil at levels of up to 1200 pg/g soil (Murahashi *et al.*, 2003b; Watanabe *et al.*, 2003).

Substantially higher concentrations of 2-nitrobenzanthrone than of 3-nitrobenzanthrone have been found in ambient air, with a ratio of 37.5–70:1 (Phousongphouang & Arey, 2003; Inazu *et al.*, 2008).

1.4.2 Exposure of the general population

Exposure to 3-nitrobenzanthrone occurs mainly by inhalation and secondarily by oral intake. Inhalation occurs through contamination in air that is formed by combustion. Oral exposure can occur either by mucociliary clearance and subsequent swallowing of the material that has been inhaled or by the consumption of foods that have been affected by dry or wet deposition of airborne 3-nitrobenzanthrone. Overall, inhalation is considered to be the greatest and perhaps most important source of exposure. On the basis of the reported levels of 3-nitrobenzanthrone in diesel exhaust particles and a daily

Table 1.1 Sources of environmental exposure to 3-nitrobenzanthrone

Reference	Country	Sampling information	Concentration of 3-NBA
Diesel exhaust particles			
Enya *et al.* (1997)	Japan	Isuzu engine Model 6HEL 7127 c (maximum power, 250 sp/2700 rpm) working under various loading conditions	< 0.001–6.61 µg/g particulate
Murahashi (2003)	Japan	Diesel engine vehicles, including heavy- and light-duty vehicles, driven at 80 km/h (n = 3)	0.027–0.056 µg/g particulate
Phousongphouang & Arey (2003)	USA	Standard reference material (SRM) 1975 obtained from the National Institute for Standards and Technology (NIST), Gaithersburg, MD	Identified by GC–MS SIM
Airborne particles			
Enya *et al.* (1997)	Japan	Sampling point was in central Tokyo; sampling time, winter 1994; same day and night time collection	5.2–11.5 pg/m^3
Zhu *et al.* (2001)	USA	2.5 µm particles ($PM_{2.5}$) were collected in Salt Lake City, UT; sampling time, 5 October 1999 (n = 1)	Tentatively identified by MS
Feilberg *et al.* (2002)	Denmark	Sampling site was located at Riso in a semi-rural area about 35 km west of Copenhagen; sampling time, from February 1998 to February 1999 (3-NBA was present ~25% of collected samples; n = 31)	ND–68.4 pg/m^3
Seidel *et al.* (2002)	Germany	Samples were collected at five typical workplaces in an underground salt mine (n = 5)	ND–80 pg/m^3
Phousongphouang & Arey (2003)	USA	Sampling point was at an industrial site affected by emissions in Concord, CA (n = 1); sampling time, January 1987	0.4 pg/m^3
Tang *et al.* (2004)	Japan	Sampling point was a heavy-traffic road in Kanazawa (n = 1)	6.79 pg/m^3
Inazu *et al.* (2008)	Japan	Samples collected in central Tokyo: n = 3 n = 4 n = 10 n = 8	0.13–0.96 pg/m^3 spring: 0.19 pg/m^3 summer: 0.38 pg/m^3 autumn: 0.44 pg/m^3 winter: 0.57 pg/m^3
Rainwater			
Murahashi *et al.* (2003a)	Japan	Sampling point was a building roof in a residential area of Kyoto (n = 6); sampling time, from May to July and from October to December 2001	0.07–2.6 ng/L

Table 1.1 (continued)

Reference	Country	Sampling information	Concentration of 3-NBA
Surface soil			
Murahashi *et al.* (2003b)	Japan	Samples were collected in the Chuba area (n = 6)	1.2–1020 pg/g
Watanabe *et al.* (2003)	Japan	Samples were collected in the Kinki region, particularly in Osaka and neighbouring cities (n = 8); sampling time, between February and December 1999	144–1158 pg/g
Lübcke-von Varel *et al.* (2012)	Germany	Polar fraction of a sediment extract of chemical industrial area of Bitterfeld (n = 1)	60 pg/g SEQ
Coal-burning-derived particles			
Taga *et al.* (2005)	China	Sampling point was the chimney of a domestic coal stove in Shenyang (n = 1)	0.234 µg/g particulate

3-NBA, 3-nitrobenzanthrone; GC–MS SIM, gas chromatography–mass spectroscopy selected ion monitoring; MS, mass spectroscopy; ND, not detected; SEQ, sediment equivalents
Adapted from Arlt (2005)

intake by inhalation of 1 μg of particles per cubic metre of air, Arlt (2005) estimated a human lung dose of approximately 90 pg per day, based on a breath intake of about 15 m^3 air per day, although the value may be exceeded in highly exposed populations.

1.5 Regulations and guidelines

There are currently no regulations or guidelines regarding exposure to 3-nitrobenzanthrone.

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Initiation-promotion

A study was conducted in NMRI mice to evaluate the initiating and carcinogenic potential of 3-nitrobenzanthrone and one of its putative metabolites. Groups of 20 female mice [age unspecified] received a single topical application of 0, 25, 100 or 400 nmol of 3-nitrobenzanthrone or its primary metabolite, *N*-hydroxy-3-aminobenzanthrone, in acetone followed by weekly applications of 0 or 5 nmol of the tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate, in acetone for up to 40 weeks. Another group received topical applications of 3-nitrobenzanthrone twice a week. No tumours were observed in any of the treatment groups except for a positive control group treated with 7,12-dimethylbenz[*a*]anthracene (Schmeiser *et al.*, 2009).

3.2 Rat

Intratracheal administration

A long-term study of the intratracheal instillation of 3-nitrobenzanthrone was conducted to determine the formation of DNA adducts at short-term time-points and to evaluate tumour formation at 24 months after the initiation of treatment. Groups of 25, 21 and 33 female Fischer 344 rats, aged 4 weeks, received intratracheal instillations of 0 (vehicle control) or 0.5 mg of 3-nitrobenzanthrone in 10% propylene glycol/saline three or five times a week (total doses, 1.5 or 2.5 mg/rat [15 or 22 mg/kg body weight (bw)] for the low- and high-dose groups, respectively) for up to 18 months. Animals were killed at interim time-points to evaluate DNA adduct formation, and after 18 months or when the animals were moribund for the study of carcinogenesis, at which time the respiratory tracts were collected and analysed histologically. Animals in the high-dose group started to become moribund after 7 months of treatment. Squamous cell carcinomas were found in the lungs of animals in the high-dose group after 7–9 months (3 out of 33; 9%), and in the low-dose group after 10–12 months (2 out of 33; 6%). The study was terminated at 18 months due to excess mortality in the high-dose group. The incidence of squamous cell carcinoma at 18 months was 3 out of 15 and 11 out of 19 [$P < 0.0001$] low- and high-dose animals, respectively. The sum at all time-points yielded an incidence of 3 out of 21 and 11 out of 33 [$P < 0.0013$] squamous cell carcinomas in the low- and high-dose groups, respectively. A single lung adenocarcinoma developed in each of the low- and high-dose groups, but not dose-dependently. None of the above lesions was observed in controls (0 out of 25) and no other tumour types were observed in the lungs of the treated groups (Nagy *et al.*, 2005a).

Table 3.1 Studies of the carcinogenicity of 3-nitrobenzanthrone in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, NMRI (F) 40 wks Schmeiser *et al.* (2009)	Topical application 0 (control), 25, 100 or 400 nmol/mouse in 0.1 mL acetone, followed 1 wk later by 0 or 5 nmol TPA; DMBA was used as a positive control Groups of 20 mice	No tumours observed except in positive control	No initiation or promotion observed	No tumour initiation or promotion observed; a second part of the study investigated a putative metabolite that also showed no initiation or promotion activity.
Rat, F344 (F) 18 mo or when moribund Nagy *et al.* (2005a)	Intratracheal instillation 0 (control), 15 or 22 mg/kg bw in 0.1 mL propylene glycol/saline (9:1), once/wk for 3 or 5 wks Groups of 21–33 aged 4 wks	Lung (squamous cell carcinoma): 0/25, 3/21 (14%), 11/33 (33%)* Lung (adenocarcinoma): 0/25, 1/21 (5%), 1/33 (3%)	*[$P < 0.0013$]	High dose expected to simulate environmental exposure; tumours were first observed at 7–9 mo in the high-dose group and 10–12 mo in the low-dose group. Study terminated at 18 mo due to excess mortality in the high-dose group. No statistics reported for tumour formation

bw, body weight; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; M, male; mo, month; TPA, 12-O-tetradecanoylphorbol-13-acetate; wk, week

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Seidel *et al.* (2002) examined the urinary levels of PAHs and nitrated PAHs (nitroarenes) in the 24-hour urine of 18 (nine smokers and nine non-smokers) underground salt-mine workers occupationally exposed to diesel engine exhaust. 3-Aminobenzanthrone, the major metabolite of 3-nitrobenzanthrone (Borlak *et al.*, 2000; Arlt *et al.*, 2003a; Hansen *et al.*, 2007), and 1-aminopyrene, a reductive metabolite of 1-nitropyrene (El-Bayoumy *et al.*, 1983; Howard *et al.*, 1985; van Bekkum *et al.*, 1998; Chae *et al.*, 1999), were identified in both groups of workers at similar levels of 1–143 ng/24-hour urine and 2–200 ng/24-hour urine, respectively. These results suggest that 3-nitrobenzanthrone and 1-nitropyrene bind to diesel engine exhaust particles and are ingested/inhaled by humans (Seidel *et al.*, 2002), distributed in different organs, metabolized by various enzymes to more polar products (such as 3-aminobenzanthrone and 1-aminopyrene) and excreted in the urine. Thus, 3-aminobenzanthrone and 1-aminopyrene could be used as urinary biomarkers in humans exposed to diesel engine exhaust (Arlt, 2005; Stiborová *et al.*, 2005).

4.1.2 Experimental systems

Several *in vivo* studies have shown that 3-nitrobenzanthrone is metabolically activated to DNA-binding products in the tissues of animals administered the compound by various routes (Arlt *et al.*, 2001, 2003a, 2004a, 2006a, 2007; Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2005a, 2006, 2007; Schmeiser *et al.*, 2009). These studies have been confirmed by several in-vitro studies which showed that 3-nitrobenzanthrone first requires metabolic activation through nitroreduction by various xenobiotic-metabolizing enzymes,

including nicotinamide adenine dinucleotide phosphate (NADPH)-quinone oxidoreductase (NQO1), xanthine/xanthine oxidase, NADPH-P450 oxidoreductase (POR) or cytochrome P450 (CYP) enzymes, leading to the formation of the DNA-binding reactive intermediate, *N*-hydroxy-3-aminobenzanthrone (Borlak *et al.*, 2000; Arlt *et al.*, 2003a, b, 2004b; Bieler *et al.*, 2003) (Fig. 4.1). *N*-Hydroxy-3-aminobenzanthrone can be further activated by acetylation and sulfation, which are catalysed by *N*-acetyltransferases (NATs) and sulfotransferases (SULTs), to form highly reactive *N*-acetoxy or *S*-sulfoxy esters, respectively, leading to increased DNA binding (Arlt *et al.*, 2002, 2003c). Although 3-aminobenzanthrone has been found to be the reductive metabolite of 3-nitrobenzanthrone in mammalian cells in culture, including 3-nitrobenzanthrone-treated human lung adenocarcinoma A549 cells (Borlak *et al.*, 2000; Hansen *et al.*, 2007), detailed chemical analyses to identify 3-nitrobenzanthrone and its metabolites *in vivo* in experimental animals have been scarce.

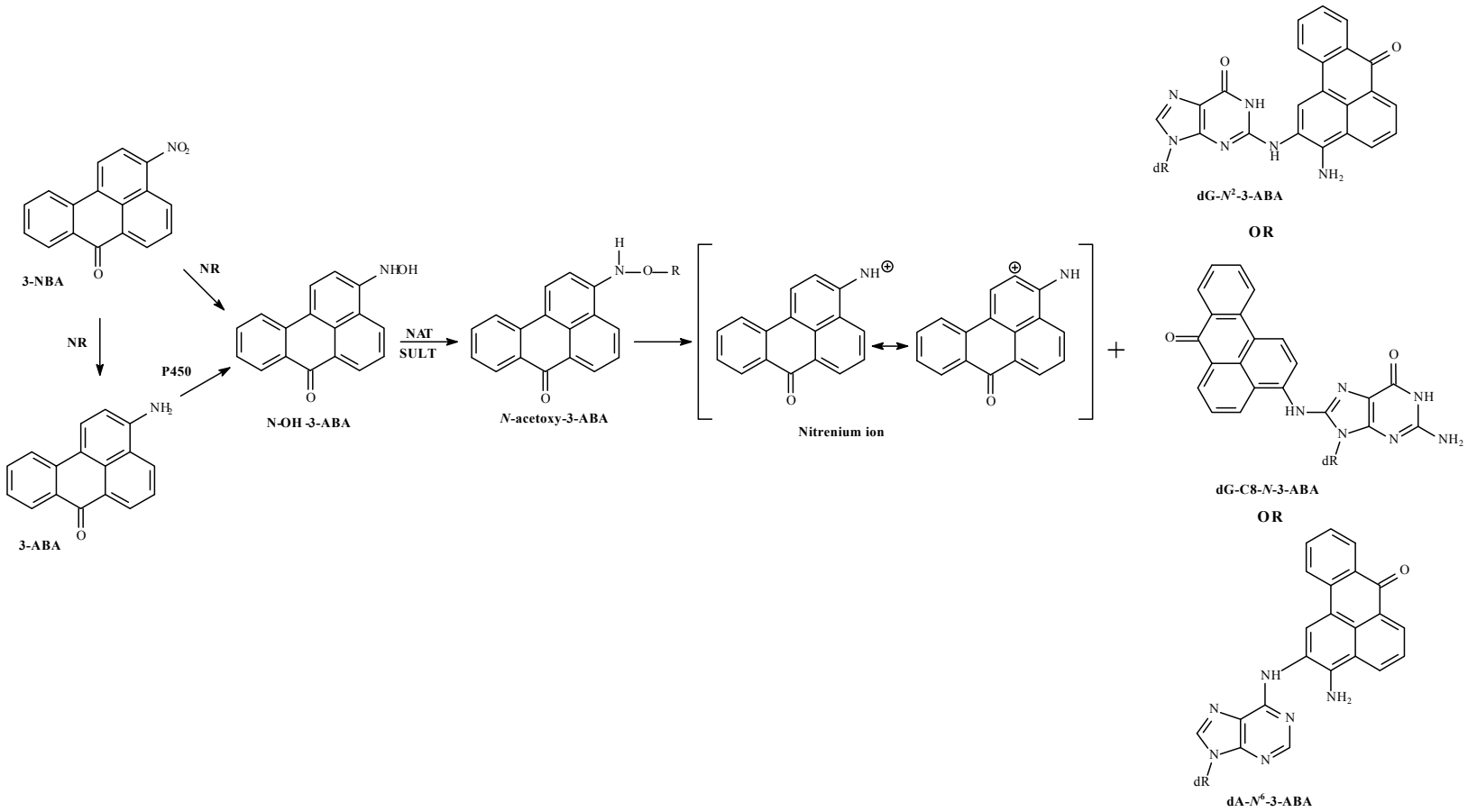
Recently, a mercapturic acid metabolite of 3-aminobenzanthrone, *N*-acetyl-*S*-(3-aminobenzanthrone-2-yl)cysteine, as well as 3-aminobenzanthrone and 3-acetylaminobenzanthrone, have been identified in the urine of rats administered 3-nitrobenzanthrone (2 mg/kg bw) by gavage, and it has been suggested that this metabolite could be used as a biomarker in exposed humans (Linhart *et al.*, 2012). The same authors also showed that 2-nitrobenzanthrone, which is reported to be less active in forming DNA adducts (Arlt *et al.*, 2007; Stiborová *et al.*, 2010), does not produce a mercapturic acid metabolite, suggesting that reactive metabolites of 3-, but not those of 2-nitrobenzanthrone, can be trapped by glutathione *S*-transferase.

4.1.3 Role of xenobiotic-metabolizing enzymes in the metabolic activation of 3-nitrobenzanthrone

Several *in vitro* and *in vivo* studies have indicated that the metabolic conversion of 3-nitrobenzanthrone is catalysed by xenobiotic-metabolizing enzymes to form DNA-binding metabolites in humans and experimental animals (Fig. 4.1) (reviewed by Arlt, 2005). 3-Nitrobenzanthrone is first bio-activated in humans and experimental animals to *N*-hydroxy-3-aminobenzanthrone by various nitroreductases, such as NQO1, xanthine/xanthine oxidase and POR (Arlt *et al.*, 2003b, 2004c, 2005; Bieler *et al.*, 2003). Cytosolic NQO1 is the major enzyme involved in the bio-activation of 3-nitrobenzanthrone, based on experiments with an NQO1 inhibitor (dicoumarol) and human recombinant NQO1 (Arlt *et al.*, 2005; Stiborová *et al.*, 2006, 2010). The role of POR in the nitroreduction of 3-nitrobenzanthrone *in vivo* may be minor, because no difference in the levels of DNA adducts in the liver was observed between 3-nitrobenzanthrone-treated wild-type C57BL/6 and hepatic POR-null mice lacking hepatic CYP enzyme activity (Arlt *et al.*, 2005; Stiborová *et al.*, 2006, 2008). In contrast, the DNA binding induced by 3-aminobenzanthrone in the liver was reduced in POR-null compared with wild-type mice, indicating that the bio-activation of 3-aminobenzanthrone depends on hepatic CYP enzymes (Stiborová *et al.*, 2006).

3-Aminobenzanthrone, the reductive metabolite of 3-nitrobenzanthrone (Borlak *et al.*, 2000; Arlt, 2005; Hansen *et al.*, 2007), can be oxidized by rat CYP enzymes (mainly CYP1A) to *N*-hydroxy-3-aminobenzanthrone, as detected by HPLC analysis (Mizerovská *et al.*, 2008). Human CYP1A1 and -1A2 (Arlt *et al.*, 2004c, 2006b; Stiborová *et al.*, 2008) and other human CYPs, including CYP2A6, -3A4 and -2B6 (Arlt *et al.*, 2003c; Bieler *et al.*, 2003), have also been shown to be involved in this reaction step. Several

Fig. 4.1 Metabolic activation of 3-nitrobenzanthrone and DNA-adduct formation



3-ABA, 3-aminobenzanthrone; 3-NBA, 3-nitrobenzanthrone; dG-N^2-3-ABA, 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone; dG-C8-*N*-3-ABA, *N*-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone; dA-N^6-3-ABA, 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone; dR, 2′-deoxyribose; NAT, *N*-acetyltransferase; NR, nitroreductase; P450, cytochrome P450; R, $-COCH_3$ or SO_3H; SULT, sulfotransferase

peroxidases, such as lactoperoxidase, myeloperoxidase and prostaglandin H synthase, were also able to catalyse the conversion of 3-aminobenzanthrone to *N*-hydroxy-3-aminobenzanthrone (Stiborová *et al.*, 2005; Arlt *et al.*, 2006b). The reactivity of *N*-hydroxy-3-aminobenzanthrone in biological systems has been reported in human cell lines, *in vivo* in rats after intraperitoneal injection (Arlt *et al.*, 2007) and in cultured embryonic fibroblasts from human *TP53* knock-in mice (vom Brocke *et al.*, 2009). Arlt *et al.* (2003b) found that purified POR isolated from rabbit liver microsomes catalysed the activation of 3-nitrobenzanthrone to DNA-binding products in a reconstituted system, indicating that *N*-hydroxy-3-aminobenzanthrone is the DNA-reactive intermediate.

N-Hydroxy-3-aminobenzanthrone has been shown to be further activated by NATs (NAT1 and NAT2) or SULTs (SULT1A1 and SULT1A2) (Arlt *et al.*, 2002, 2003a, c, 2004b, 2005; Stiborová *et al.*, 2006) (Fig. 4.1). Reactive *N*-acetoxy or *N*-sulfoxy esters can bind covalently to DNA (Snyderwine *et al.*, 1988; Arlt, 2005), and evidence has been presented that an aryl nitrenium ion, which is formed through heterolytic cleavage of these ester metabolites, is the ultimate DNA-binding species of 3-nitrobenzanthrone (Arlt, 2005). NATs (i.e. NAT2) may be more important than SULTs in the bio-activation of 3-nitrobenzanthrone (Arlt *et al.*, 2002, 2003c, 2005). By incorporating human SULT1A1, -1A2 and -1A3 into a *Salmonella typhimurium* strain deficient in *O*-acetyltransferase activity, Oda *et al.* (2012) found that the strain that expressed human SULT1A1 was more effective in activating 3-nitrobenzanthrone to genotoxic metabolites than those that harboured SULT1A2 or -1A3, as determined in the *umu* gene expression assay.

NATs have also been reported to be involved in the acetylation of *N*-hydroxy-3-aminobenzanthrone to form *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone, and the resultant metabolite is also activated by NATs to form highly reactive *N*-acetyl-*N*-acetoxy-3-aminobenzanthrone (Enya *et al.*, 1997; Kawanishi *et al.*, 1998, 2000; Arlt *et al.*, 2002, 2003a). However, this activation step has no relevance to the metabolic activation of 3-nitrobenzanthrone in experimental animals, because all of its major DNA adducts identified *in vivo* lack an *N*-acetyl group in the adduct molecules (see Section 4.2.1) (Arlt *et al.*, 2002, 2003a; Kanno *et al.*, 2007; Takamura-Enya *et al.*, 2007); moreover, *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone was weakly mutagenic in the *Salmonella* strain DJ450, which expresses human recombinant NAT2 (Arlt *et al.*, 2002). It has also been reported that *N*-acetyl-*N*-hydroxy-3-aminobenzanthrone is readily deacetylated by microsomal enzymes (Arlt *et al.*, 2002, 2003b, c).

4.1.4 *Induction of xenobiotic-metabolizing enzymes by 3-nitrobenzanthrone and 3-aminobenzanthrone*

3-Nitrobenzanthrone and 3-aminobenzanthrone induce several forms of xenobiotic-metabolizing enzyme in experimental animals *in vivo* (Stiborová *et al.*, 2006, 2009; Mizerovská *et al.*, 2011). Intraperitoneal administration of 3-nitrobenzanthrone or 3-aminobenzanthrone to rats (at a single dose of 0.4, 4 or 40 mg/kg bw) caused increases in cytosolic menadione reduction and 7-ethoxyresorufin *O*-deethylation activities with increased levels of DNA adducts in the liver, indicating that these compounds induced NQO1 and CYP1A; the induction of NQO1, and CYP1A1 and -1A2 protein was confirmed by western blot analysis (Stiborová *et al.*, 2006). Intraperitoneal administration of 3-nitrobenzanthrone and 3-aminobenzanthrone to rats also induced CYP1A1 and NQO1 in the lung and kidney (Stiborová *et al.*, 2008, 2009). Because these enzymes play important roles in both the activation and detoxification of PAHs and nitro-PAHs, it is possible that 3-nitrobenzanthrone and 3-aminobenzanthrone not only

alter their own toxic responses by inducing xenobiotic-metabolizing enzymes but also modulate the metabolism of related PAHs and nitro-PAHs found in diesel-engine exhaust.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) DNA adduct formation

In vivo results in experimental animals show that the levels of 3-nitrobenzanthrone–DNA adducts in different tissues are largely dependent on the route of administration (Arlt *et al.*, 2001, 2003a, 2004a, 2006a, 2007; Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2005a, 2006, 2007; Schmeiser *et al.*, 2009) (Table 4.1). DNA-adduct formation by 3-nitrobenzanthrone has been mainly investigated by ^{32}P-postlabelling using thin-layer chromatography (TLC) or HPLC. Oral administration of 3-nitrobenzanthrone to rats induced DNA adducts in the small intestine, stomach, liver, kidney and urinary bladder, but relatively fewer in the lung (Arlt *et al.*, 2001; Nagy *et al.*, 2006). A single intraperitoneal dose of 3-nitrobenzanthrone resulted in the distribution of DNA adducts in several organs, such as the pancreas, kidney, liver, lung, urinary bladder, heart and colon (Arlt *et al.*, 2003a, 2005, 2006a, 2007; Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2005a). Intratracheal instillation of 3-nitrobenzanthrone to rats, which induced squamous cell carcinomas in the lung of Fischer 344 rats (Nagy *et al.*, 2005a), resulted in DNA-adduct formation in the lung and other tissues, such as the kidney, pancreas, urinary bladder, heart, small intestine and liver (Bieler *et al.*, 2005, 2007; Nagy *et al.*, 2006). The highest levels of DNA adducts in female Fischer 344 rats that received intratracheal instillations of 3-nitrobenzanthrone (10 mg/kg bw) were observed in the lungs (~250 adducts/10^8 nucleotides); adduct levels were also high in the kidney (~200 adducts/108 nucleotides), but low in the liver (~30 adducts/10^8 nucleotides). Although the kidney showed high levels of 3-nitrobenzanthrone–DNA adducts, tumour formation was not reported in this organ (Nagy *et al.*, 2005a). Topical application of 3-nitrobenzanthrone and *N*-hydroxy-3-nitrobenzanthrone to the skin of NMRI mice was found to produce DNA adducts in the epidermis, but adducts in other organs were only found after treatment with 3-nitrobenzanthrone (Schmeiser *et al.*, 2009). These results suggest that 3-nitrobenzanthrone can largely be metabolized by xenobiotic-metabolizing enzymes near the sites of application to produce DNA adducts, and that 3-nitrobenzanthrone and its metabolites may be distributed via the blood to other organs in which further metabolism may occur (i.e. activation or detoxification).

TLC- and HPLC-^{32}P-postlabelling analyses have been used to characterize 3-nitrobenzanthrone-derived DNA adducts *in vitro* and *in vivo* (Kawanishi *et al.*, 1998; Arlt *et al.*, 2001; Bieler *et al.*, 2003; Nagy *et al.*, 2005a; Osborne *et al.*, 2005) (Table 4.1). In TLC, the pattern of DNA adducts induced by 3-nitrobenzanthrone consisted of a characteristic cluster of four major DNA adducts (spots 1, 2, 3 and 4); initial studies showed that all four adducts formed were derived from reductive metabolites bound either to deoxyadenosine (spots 1 and 2) or deoxyguanosine (spots 3 and 4) (Arlt *et al.*, 2001, 2003a, c). Among a series of deoxyguanosine and deoxyadenosine adducts characterized to date, the following have been identified as the major lesions *in vivo*: 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone (spot 3), *N*-(deoxyguanosin-8-yl)-3-aminobenzanthrone (spot 4) and 2-(2'-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (spot 1) (Nagy *et al.*, 2005a, 2006; Osborne *et al.*, 2005; Arlt *et al.*, 2006a, 2008, 2011; Kanno *et al.*, 2007; Takamura-Enya *et al.*, 2007;) (Fig. 4.1). The formation of 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone and

Table 4.1 Detection of DNA adducts in tissues of experimental animals treated with 3-nitrobenzanthrone and *N*-hydroxy-3-aminobenzanthrone by different routes of administration *in vivo*

Species, strain (sex) Reference	*In vivo* conditions for experiments; time of sacrifice of animals for analysis	DNA adducts in various organs (relative adduct levels/10^8 nucleotides)
Rat, SD (F) Arlt *et al.* (2001)	Single oral dose (2 mg/kg bw) of 3-NBA in tricaprylin (1 mg/mL); 4 h after the dose	Small intestine (38), forestomach (33), kidney (13), liver (10), lung (9.5)
Rat, F344 (F) Nagy *et al.* (2006)	Single oral dose (9 mg/kg bw) of 3-NBA in trioctanoin (1 mg/mL); 48 h after the dose	Fore stomach (50), glandular stomach (47), kidney (45), caecum (43), liver (37), small intestine (33), colon (31), lung (30)
Mouse, C57BL/6 (M) Arlt *et al.* (2005)	Single ip dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.5 mg/mL); 24 h after the dose	Liver (200), urinary bladder (50), kidney (27), colon (24), lung (4)
Rat, Wistar (F) Arlt *et al.* (2003a)	Single ip dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.5 mg/mL); 24 h after the dose	Pancreas (300), colon (84), lung (52), kidney (47), heart (45), liver (13)
Rat, Wistar (F) Arlt *et al.* (2007)	Single ip dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.5 mg/mL); 24 h after the dose	Kidney(134), lung (89), colon (87), pancreas (84), liver (28)
	Single ip dose (10 mg/kg bw) of N-OH-3-ABA in DMSO; 24 h after the dose	Kidney (262), lung (312), colon (191), pancreas (430), liver (55)
Rat, SD (F) Bieler *et al.* (2005)	Single it dose (0.2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 48 h after the dose	Pancreas (55). lung (39), kidney (33), urinary bladder (28), heart (26), small intestine (19), liver (12), blood (10)
	Single it dose (2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 48 h after the dose	Pancreas (620), lung (350), kidney (330), heart (220), urinary bladder (215), small intestine (98), liver (59), blood (41)
Rat, SD (F) Bieler *et al.* (2007)	Single it dose (0.2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 2 d after the dose	Pancreas (55), lung (39), kidney (33), urinary bladder (28), heart (26), small intestine (19), liver (12)
	Single it dose (0.2 mg/kg bw) of 3-NBA in tricaprylin (0.4 mg/mL); 36 wks after the dose	Pancreas (11), lung (11), kidney (12), urinary bladder (5), heart (not detected), small intestine (5), liver (4)
Rat, F344 (F) Nagy *et al.* (2005a)	Single it dose (10 mg/kg bw) of 3-NBAin propylene glycol:saline (9:1; 0.1 mL); 24 h after the dose	Lung (250), kidney (170), liver (15)
Rat, F344 (F) Nagy *et al.* (2007)	Single it dose (5 mg/kg bw) of 3-NBA in trioctanoin (5 mg/mL); 48 h after the dose	Lung (55), kidney (45), liver (31), glandular stomach (27), forestomach (24), spleen (20), caecum (17), colon (16), small intestine (15)
Mouse, NMRI (F) Schmeiser *et al.* (2009)	Single or repeated (once daily for 4 d) topical dose (100 nmol) of 3-NBA in acetone; 24 h after the dose	Skin (17 for single dose; 40 for four daily doses)
	Single or repeated (once daily for 4 d) topical dose (100 nmol) of N-OH-3-ABA in acetone; 24 h after the dose	Skin (140 for single dose) or skin (170 for 4 daily doses)

Some of the values in this table were taken from figures in the references.
bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; ip, intraperitoneal; it, intratracheal; M, male; 3-NBA, 3-nitrobenzanthrone; *N*-OH-3-ABA, *N*-hydroxy-3-aminobenzanthrone; wk, week

N-(deoxyguanosin-8-yl)-3-aminobenzanthrone in 3-nitrobenzanthrone-treated rodents has been confirmed by mass spectrometry (Gamboa da Costa *et al.*, 2009). None of the DNA adducts detected *in vivo* contained an *N*-acetyl group, suggesting that there is no important role of *N*-acetyl-*N*-acetoxy-3-aminobenzanthrone in the reaction with DNA (Arlt, 2005; Kanno *et al.*, 2007). Overall, the pattern of 3-nitrobenzanthrone-derived DNA adducts in experimental animals is the same *in vivo* and *in vitro* (Arlt, 2005; Stiborová *et al.*, 2010).

3-Nitrobenzanthrone–DNA adducts have been detected in various organs of rats within 36 weeks after intratracheal instillation of a single dose (0.2 mg/kg bw), including in the target organ, the lung; the most abundant and persistent DNA adduct was 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone (Bieler *et al.*, 2007). Lukin *et al.* (2011) showed that this lesion increased the stability of the damaged DNA duplex, which may provide an explanation for its long persistence *in vivo*.

TP53-dependent adduct formation by 3-nitrobenzanthrone was investigated in two isogenic human colorectal HCT116 cell lines, one that expressed *TP53* (p53-wild-type) and another that had this gene knocked out (p53-null) (Hockley *et al.*, 2008; Simoes *et al.*, 2008). The authors showed that the levels of DNA adducts after treatment with benzo[*a*]pyrene and nitroarene aristolochic acid I was lower in p53-null cells compared with p53-wild-type cells, whereas DNA binding by 3-nitrobenzanthrone was *TP53*-independent. These results suggest that the cellular *TP53* status is linked to the CYP-mediated metabolism of benzo[*a*]pyrene and nitroarene aristolochic acid I, whereas the nitroreduction of 3-nitrobenzanthrone is not *TP53*-dependent. The possible lack of protective functions mediated by p53 after the treatment of p53-null cells with 3-nitrobenzanthrone needs to be explored further.

(b) Mutagenicity of 3-nitrobenzanthrone and its metabolites

(i) In-vivo studies

Arlt *et al.* (2004a) reported that intraperitoneal administration of 3-nitrobenzanthrone to the MutaMouse increased the mutant frequency in the *cII* gene in the colon, liver and urinary bladder, but not in the lung, kidney, spleen or testis. In addition, the percentage of G:C to T:A transversions in the liver was found to be higher in 3-nitrobenzanthrone-treated mice than in untreated mice (49% versus 6%). The authors also showed preferential DNA binding at deoxyguanosine (70–80%), which correlated with the preferential occurrence of G:C to T:A transversions; 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone and *N*-(deoxyguanosin-8-yl)-3-aminobenzanthrone appeared to be the pre-mutagenic lesions. *LacZ* mutant frequencies were also investigated in the MutaMouse strain treated with 3-nitrobenzanthrone and 3-aminobenzanthrone (0, 2 and 5 mg/kg bw per day for 28 days) (Arlt *et al.*, 2008). Dose-related increases in mutant frequency were seen in the liver and bone marrow, but not in the lung; the mutagenic activity of 3-aminobenzanthrone was approximately twofold lower than that of 3-nitrobenzanthrone. Chen *et al.* (2008) showed that 3-nitrobenzanthrone induced *lacZ* mutations in the bone marrow and liver, but not in the lung or intestinal epithelium, in the MutaMouse after single and repeated oral administration. High 3-nitrobenzanthrone nitroreductase activity was found in all tissues examined, whereas no NAT activity was observed in bone marrow.

(ii) In-vitro studies

3-Nitrobenzanthrone was highly mutagenic in *S. typhimurium* strain TA98, only in the absence of metabolic activation. It was less mutagenic in *S. typhimurium* TA100 than in TA98, suggesting that it causes frameshift-type mutations in the bacteria. In strain TA98, the mutagenic potency of

3-nitrosobenzanthrone was comparable with that of 1,8-dinitropyrene, and both compounds were found to be highly mutagenic in *S. typhimurium* YG1024, an *O*-acetyltransferase-overexpressing strain (Enya *et al.*, 1997; Watanabe *et al.*, 2005). It should be noted that 3-nitrosobenzanthrone induced 6.3 million revertants/nmol in strain YG1024, and is thus one of the most potent bacterial mutagens known to date. Takamura-Enya *et al.* (2006) compared the mutagenic activities of several derivatives of mono-, di- and trinitrobenzanthrone in *S. typhimurium* TA98, and found that 3-nitrobenzanthrone was more mutagenic than 1-, 2-, 9- and 11-nitrobenzanthrone, 1,9-, 3,9- and 3,11-dinitrobenzanthrone, and 3,9,11-trinitrobenzanthrone. In comparison, 3-nitrobenzanthrone showed high mutagenic potency, similar to that of 1,3-, 1,6- and 1,8-dinitropyrene, and 3,7- and 3,9-dinitrofluoranthene, in *S. typhimurium* TA98 in the absence of metabolic activation. In general, these nitroarenes have been less active in inducing reverse mutations in *S. typhimurium* TA100. It should be mentioned that the nitrenium ion of 3-nitrobenzanthrone is more stable than comparable ions derived from other nitrated benzanthrone derivatives, which could provide a possible explanation for its potent mutagenic and DNA adduct-forming activities (Arlt *et al.*, 2007, 2011; Reynisson *et al.*, 2008).

Watanabe *et al.* (2005) found that 3-aminobenzanthrone and 3-acetyl-3-aminobenzanthrone, two metabolites of 3-nitrobenzanthrone, were activated by a rat-liver metabolic activation system to reactive metabolites that caused the induction of reverse mutations in *S. typhimurium* TA98, TA100, YG1024 and YG1029; the highest activity was seen in the YG1024 strain, which overexpresses *O*-acetyltransferase. They also showed that 3-nitrobenzanthrone itself was deactivated by the metabolic activation system in this assay, suggesting that enzymes present in this fraction may detoxify 3-nitrobenzanthrone or its metabolites.

Oda *et al.* (2007) reported that a very low concentration (~2 nM) of 3-nitrobenzanthrone induced *umu* gene expression in *S. typhimurium* NM2009 and NM3009, which express *O*-acetyltransferase and exhibit both nitroreductase and *O*-acetyltransferase activities, respectively. They also showed that 3-nitrobenzanthrone was cytotoxic in these tester strains, suggesting the formation of active metabolites. More recently, Oda *et al.* (2012) reported that 3-nitrobenzanthrone induced *umu* gene expression more actively in *S. typhimurium* NM7001, which expresses SULT1A1, than in strains NM7002 and 7003, which express human SULT1A2 and 1A3, respectively.

3-Nitrobenzanthrone induced base-substitution mutations in mammalian cell systems (Phousongphouang *et al.*, 2000; Arlt *et al.*, 2008; Nishida *et al.*, 2008). The induction of base-substitution mutations in mammalian cells compared with frameshift mutations in bacteria is probably due to differences in the target genes or in the mechanisms of mutagenesis between bacterial and mammalian systems. In human B-lymphoblastoid cell lines, 3-nitrobenzanthrone induced mutations at the *thymidine kinase+/–* and *hypoxanthine phosphoribosyltransferase* loci (Phousongphouang *et al.*, 2000). 3-Nitro- and 3-aminobenzanthrone produced a dose-dependent increase in mutant frequency in lung epithelial FE1 cells derived from the MutaMouse (in the presence and absence of metabolic activation), which correlated with an increase in DNA-adduct formation (Arlt *et al.*, 2008).

Nishida *et al.* (2008) determined the mutagenic specificity of *N*-acetoxy-3-aminobenzanthrone, probably the most active metabolite of 3-nitrobenzanthrone, in the *supF* system using human fibroblast cell lines. They found that *N*-acetoxy-3-aminobenzanthrone bound to guanine rather than adenine and preferentially induced G:C to T:A transversions; deoxyadenosine adducts may be repaired more efficiently by

nucleotide excision repair than deoxyguanosine adducts.

3-Nitrobenzanthrone also induced G:C to T:A transversions in the *TP53* gene in immortalized human *TP53* knock-in murine embryonic fibroblasts (vom Brocke *et al.*, 2009; Kucab *et al.*, 2012), suggesting that G:C to T:A transversions in *TP53* could be used as a signature mutation for exposure to 3-nitrobenzanthrone in human lung tumours, when exposure to this carcinogen has been documented (Kucab *et al.*, 2010).

(c) *Other genetic effects of 3-nitrobenzanthrone* in vivo *and* in vitro

3-Nitrobenzanthrone induced micronuclei in mouse peripheral blood reticulocytes after intraperitoneal injection (25 or 50 mg/kg bw) into male ICR mice (Enya *et al.*, 1997). Similar results have been reported by Arlt *et al.* (2004a). Intraperitoneal administration of 3-nitrobenzanthrone (25 mg/kg bw once a week for 4 weeks) increased the frequency of micronuclei in the peripheral reticulocytes of the male MutaMouse.

In vitro induction of micronucleus formation and DNA damage following treatment with 3-nitrobenzanthrone has also been reported in human hepatoma HepG2 cells (Lamy *et al.*, 2004), human B-lymphoblastoid MCL-5 cells (Arlt *et al.*, 2004b) and human A549 lung cells (Nagy *et al.*, 2005b). Both 3-nitrobenzanthrone and 3-aminobenzanthrone increased the tail moment in the alkaline comet assay in human lung epithelial A549 cells and in the liver, kidney, spleen, lung and bone marrow of male ICR mice (Watanabe *et al.*, 2005; Hansen *et al.*, 2007).

N-Hydroxy-3-aminobenzanthrone induced oxidative DNA damage through the formation of 8-hydroxydeoxyguanosine in the presence of divalent copper and nicotinamide adenine dinucleotide *in vitro* (Murata *et al.* 2006; Hansen *et al.*, 2007). Using ^{32}P-labelled DNA fragments from human *TP53*, Murata *et al.* (2006) showed that the DNA damage caused by *N*-hydroxy-3-aminobenzanthrone could be inhibited by catalase and bathocuproline, suggesting the involvement of hydrogen peroxide and monovalent copper in the induction of DNA damage; DNA damage occurred at the cytosine and guanine residues of an ACG sequence complementary to codon 273, which is a well known hotspot for *TP53* mutations (Murata *et al.*, 2006). Shimohara *et al.* (2008) reported that 3-nitrobenzanthrone caused double-strand DNA breaks by analysing the phosphorylation of histone H2AX in human Hela cells.

3-Nitrobenzanthrone induced apoptosis, DNA-adduct formation and DNA damage in human bronchial epithelial BEAS-2B cells (Ovrevik *et al.*, 2010; Oya *et al.*, 2011). In mouse hepatoma Hepa1c1c7 cells, Landvik *et al.* (2010) reported that 3-nitrobenzanthrone caused cell death with increasing levels of DNA adducts, single-strand DNA breaks and oxidative DNA damage (measured in the comet assay).

4.3 Mechanistic considerations

The main metabolite of 3-nitrobenzanthrone, the reductive amino metabolite 3-aminobenzanthrone (Borlak *et al.*, 2000; Hansen *et al.*, 2007; Linhart *et al.*, 2012), has been found in individuals occupationally exposed to diesel engine exhaust, clearly indicating that human exposure to 3-nitrobenzanthrone can be significant and is detectable (Seidel *et al.*, 2002). 3-Nitrobenzanthrone is bio-activated to DNA-binding products by various xenobiotic-metabolizing enzymes *in vivo* in humans and experimental animals as well as *in vitro* (Arlt, 2005). It is first activated by nitroreduction, which is catalysed most efficiently by NQO1, to *N*-hydroxy-3-aminobenzanthrone (Arlt, 2005; Arlt *et al.*, 2005; Stiborová *et al.*, 2010). 3-Aminobenzanthrone is oxidized by CYPs (mainly CYP1A1 and CYP1A2) or by several peroxidases to the same reactive intermediate, *N*-hydroxy-3-aminobenzanthrone (Arlt *et al.*, 2004c, 2006b; Stiborová *et al.*, 2005), which can

be further activated by NATs (NAT1 and NAT2) or SULTS (SULT1A1 and SULT1A2) to form highly reactive *N*-acetoxy or sulfoxy esters (Arlt *et al.*, 2002, 2003a, c, 2004b, 2005; Stiborová *et al.*, 2006). The aryl nitrenium ions formed through the heterolytic cleavage of these ester metabolites are the most reactive with DNA, and lead to the formation of covalent DNA adducts (Arlt, 2005). NATs may be more important in the bio-activation of 3-nitrobenzanthrone than SULTs (Arlt *et al.*, 2002, 2003c, 2005). Collectively, comparable metabolic activation pathways have been found in rodents and humans.

^{32}P-Postlabelling analyses have detected multiple DNA adducts bound to activated 3-nitrobenzanthrone metabolites *in vivo* and *in vitro* (Arlt, 2005). The major purine-based DNA adducts have been structurally identified *in vivo* as 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone, *N*-(deoxyguanosin-8-yl)-3-aminobenzanthrone and 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone (Arlt, 2005; Osborne *et al.*, 2005; Arlt *et al.*, 2006a; Nagy *et al.*, 2006; Kanno *et al.*, 2007; Takamura-Enya *et al.*, 2007; Gamboa da Costa *et al.*, 2009). The deoxyguanosine adducts are found most persistently in the DNA of the target tissue – the lung (Bieler at al., 2007) – and induce G:C to T:A transversions in mammalian cells and in transgenic mouse mutation assays (Arlt *et al.*, 2004b, 2008; Nishida *et al.*, 2008). Such increases in G:C to T:A transversions have also been found in the DNA-binding domain sequence of *TP53* in human *TP53* knock-in murine embryonic fibroblasts exposed to 3-nitrobenzanthrone (vom Brocke *et al.*, 2009). 3-Nitrobenzanthrone was a strong direct-acting mutagen in *S. typhimurium* strain TA98, in which its high mutagenic potency is comparable with that of 1,3-, 1,6- and 1,8-dinitropyrene. It was also a powerful mutagen in *S. typhimurium* YG1024, an *O*-acetyltransferase-overexpressing strain, and induced more than 6 million revertants/nmol (Enya *et al.*, 1997). 3-Nitrobenzanthrone induced *umu* gene expression in *S. typhimurium* tester strains that overexpress nitroreductase and *O*-acetyltransferase activties (Oda *et al.*, 2007), the development of micronuclei in mouse peripheral blood reticulocytes *in vivo* (Enya *et al.*, 1997) and increases in micronuclei in male MutaMouse peripheral reticulocytes, as well as increased mutant frequencies in the *cII* gene in the colon, liver and urinary bladder or in *lacZ* in the liver and bone marrow (Arlt *et al.*, 2004a; 2008; Chen *et al.*, 2008). Many *in vitro* bioassays in human cells showed that 3-nitrobenzanthrone can induce micronuclei (Arlt *et al.*, 2004c; Lamy *et al.*, 2004), DNA strand breaks (Nagy *et al.*, 2005b) and gene mutations (Arlt *et al.*2008; Nishida *et al.*, 2008, Phousongphouang *et al.*, 2000. It induced apoptosis with increasing levels of DNA adducts in human bronchial epithelial BEAS-2B cells and the expression of cytokine/chemokine (Landvik *et al.*, 2010; Ovrevik *et al.*, 2010).

Nagy *et al.* (2005a) showed that intratracheal instillation of 3-nitrobenzanthrone induced lung squamous cell carcinomas in rats after 18 months. Tumour formation was also associated with a high level of DNA-adduct formation in the lungs Nagy *et al.* (2005a); 2-(2′-deoxyguanosin-*N2*-yl)-3-aminobenzanthrone was the most abundant and persistent DNA adduct in the lung (Bieler *et al.*, 2007). No skin tumours were found after topical administration of 3-nitrobenzanthrone or *N*-hydroxy-3-aminobenzanthrone to NMRI mice (Schmeiser *et al.*, 2009). In addition to differences in the various routes of administration, the authors concluded that the bioactivation of 3-nitrobenzanthrone in mouse skin, which has also been shown for some other nitro-PAHs (Nesnow *et al.*, 1984; Möller *et al.*, 1993), may not be sufficient to initiate tumour formation in this model.

Collectively, these data indicate that 3-nitrobenzanthrone, which has been identified in diesel engine exhaust, is a powerful mutagen and rodent carcinogen.

5. Summary of Data Reported

5.1 Exposure data

3-Nitrobenzanthrone is primarily formed as a product of the combustion of fossil fuels. It is a constituent of diesel exhaust emissions and was also detected in particulate matter collected from the chimney of a domestic coal-burning stove. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. 3-Nitrobenzanthrone was detected in the air, soil and water. Concentrations of 3-nitrobenzanthrone in airborne particulate matter collected at urban locations were reported to be in the subpicogram per cubic metre range (up to 70 pg/m^3), and similar values were reported in confined workplaces. Occupational exposure to this substance was reported after urine analysis.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

3-Nitrobenzanthrone was evaluated in one initiation–promotion study in mice and in one study in rats by intratracheal instillation. Intratracheal instillation resulted in an increase in the incidence of squamous cell carcinoma of the lung in rats. The initiation–promotion study in mice was negative.

5.4 Mechanistic and other relevant data

3-Nitrobenzanthrone is a powerful mutagen in bacterial and mammalian assays, and produces lung tumours in rats after intratracheal administration. It induced 6.3 million revertants/nmol in a bacterial strain that overexpresses *O*-acetyltransferase, and is therefore one of the most potent bacterial mutagens known to date. The main reductive metabolite of 3-nitrobenzanthrone, 3-aminobenzanthrone, was found in the urine of salt-mine workers exposed to diesel engine exhaust, indicating that human exposure to 3-nitrobenzanthrone from diesel emissions can be significant and is detectable. 3-Nitrobenzanthrone is first metabolically activated by nitroreduction, leading to the formation of *N*-hydroxy-3-aminobenzanthrone, which can also be formed by the *N*-oxidation of 3-aminobenzanthrone and can be further activated by acetylation or sulfation to form highly reactive *N*-acetoxy- or *S*-sulfoxy esters that can bind to DNA and form adducts. Comparable metabolic activation pathways have been found in rodents and humans. The major DNA adducts identified *in vivo* are deoxyguanosin-N^2-yl-3-aminobenzanthrone, deoxyguanosin-C8-yl-*N*-3-aminobenzanthrone and deoxyadenosin-N^6-yl-3-aminobenzanthrone; the deoxyguanosin-N^2-yl-3-aminobenzanthrone adduct is the most abundant and persistent DNA lesion in the lung – the target organ for carcinogenesis. 3-Nitrobenzanthrone induces predominantly G:C to T:A transversions in mammalian and transgenic rodent mutation assays; the same mutation pattern has been found in the human DNA-binding domain of *TP53*. Both 3-nitrobenzanthrone and 3-aminobenzanthrone can induce oxidative damage to DNA, and 3-nitrobenzanthrone has also been shown to induce micronuclei and DNA strand breaks.

These data provide *strong mechanistic evidence* to support the carcinogenic properties of 3-nitrobenzanthrone in animals and *moderate mechanistic evidence* to support its carcinogenic properties in humans.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of 3-nitrobenzanthrone.

6.3 Overall evaluation

3-Nitrobenzanthrone is *possibly carcinogenic to humans (Group 2B).*

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