

DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 5-12 June 2012

LYON, FRANCE - 2014

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

6-NITROCHRYSENE

6-Nitrochrysene was evaluated by a previous IARC Working Group in 1988 ([IARC, 1989](#)). New data have since become available, and these have been taken into consideration in the present evaluation.

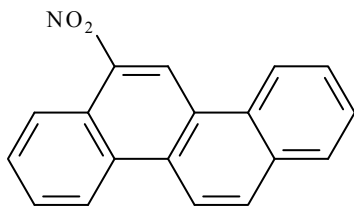
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 7496-02-8

1.1.2 Structural and molecular formulae, and relative molecular mass



$C_{18}H_{11}NO_2$

Relative molecular mass: 273.3

1.1.3 Chemical and physical properties of the pure substance

Description: Chrome-red, thick prismatic crystals ([Prager & Jacobson, 1922](#)); orange-yellow needles ([Boit, 1965](#)); light-yellow needles ([Chemsyn Science Laboratories, 1988](#))

Boiling-point: Sublimes without decomposition ([Prager & Jacobson, 1922](#))

Melting-point: 209 °C ([Boit, 1965](#)); 211.5–212.5 °C ([Chemsyn Science Laboratories, 1988](#))

Spectroscopy data: Mass spectral data have been reported ([Schuetzle & Jensen, 1985](#)).

Solubility: Insoluble in many organic solvents; slightly soluble in cold ethanol, diethyl ether and carbon disulfide; somewhat more soluble in benzene, toluene and acetic acid; soluble in hot nitrobenzene ([Prager & Jacobson, 1922](#); [Chemsyn Science Laboratories, 1988](#)).

Reactivity: Forms 6-aminochrysene on heating with tin and concentrated hydrochloric acid in acetic acid at 100 °C; reacts with bromine to form 12-bromo-6-nitrochrysene; reacts with fuming nitric acid to form 6,12-dinitrochrysene ([Boit, 1965](#)).

1.1.4 Technical products and impurities

6-Nitrochrysene (99% pure) is sold in small quantities for analytical purposes by one company each in Germany, China and the USA ([Chemexper, 2012](#); [Chemical Buyers, 2012](#)). It is also available as a reference material with a certified purity of 98.91% ([Belliaro *et al.*, 1988](#))

Table 1.1 Levels of 6-nitrochrysene in gasoline and diesel engine emissions

Engine	Year of manufacture	Carburant	T ^a (°C)	No. ^b	Emission (µg/km) ^c
Mazda Millenia, Ford Explorer, Nissan Maxima, GMC 1500 Pickup, Mercury Sable	1982–96	Normal gasoline	22	18	0.11
Ford F-150 pick-up	1976	Gasoline black smoker	22	5	0.60
Mitsubishi Montero	1990	Gasoline white smoker	22	2	0.13
Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TDI	1998–2000	Current technology diesel vehicles	22	9	0.24
Dodge Ram 2500 Pickup	1991	Diesel high articulate matter emitter	22	6	1.19
Mazda Millenia, Ford Explorer, Nissan Maxima, GMC 1500 Pickup, Mercury Sable	1982–96	Normal gasoline	–1	12	0.03
Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TD	1998–2000	Current technology diesel vehicles	–1	6	2.08

^a Temperature of the engine when tested, calculated from Fahrenheit by the Working Group

^b Number of test cycles

^c Calculated from µg/mile by the Working Group

From [Zielinska et al. \(2004\)](#)

1.2 Analysis

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

6-Nitrochrysene has been detected in particles that were collected on glass and quartz fibre or Teflon-coated silica fibre membrane filters ([Tokiwa et al., 1990](#); [Schauer et al., 2004](#)). It can be extracted from particulate matter using dichloromethane for extraction by sonification ([Tokiwa et al., 1990](#)). To analyse 6-nitrochrysene, gas chromatography-negative ion chemical ionization-mass spectrometry was used, following pressurized-fluid extraction ([Bamford et al., 2003](#); [Albinet et al., 2006](#)). This substance was also analysed after reduction to 6-aminochrysene with titanium(III) citrate and subsequent detection by fluorescence spectrometry ([Zielinska & Samy, 2006](#)), or, alternatively, by chemiluminescence detection to increase the sensitivity for 6-aminochrysene in airborne particulates ([Murahashi & Hayakawa, 1997](#)).

1.3 Production and use

1.3.1 Production

6-Nitrochrysene was first synthesized in 1890 by heating chrysene with aqueous nitric acid in acetic acid at 100 °C ([Prager & Jacobson, 1922](#)). It can also be synthesized by briefly heating chrysene with nitric acid and concentrated sulfuric acid in acetic acid at 40 °C ([Boit, 1965](#)).

1.3.2 Use

No evidence was found that 6-nitrochrysene has been used in commercial applications.

1.4 Occurrence and exposure

1.4.1 Diesel and gasoline exhaust emissions

Different types of in-use passenger cars were tested on a dynamometer using the California Unified Driving Cycle with commercial fuel and used crankcase oil ‘as received’ ([Zielinska et al., 2004](#)). The model year and testing conditions strongly influenced emission levels ([Table 1.1](#)). The 6-nitrochrysene emissions were one order of magnitude higher in cold diesel-powered cars

Table 1.2 Mean air concentrations of 6-nitrochrysene associated with particulate matter

Reference	Source	No. of samples	Concentration (pg/m ³) [mean ± SD (range)]
Garner et al. (1986)	Urban (Bayreuth, Germany)	2	800; 1500 ^a
Murahashi & Hayakawa (1997)	Urban (Kanazawa, Japan), October 1994	1	1.1 ^b
	Suburban (Kanazawa, Japan), October 1994	1	0.13 ^b
Schauer et al. (2004)	Urban (Münich, Germany), 2002	10	54 ± 8
	Rural (Münich, Germany)	5	18 ± 13
	Rural (alpine site)	9	2.1 ± 1.7
Albinet et al. (2006)	Rural (Maurienne Valley, France), 2002–03	13	0.6 (0.2–2.4)
Albinet et al. (2007)	Urban (Marseille, France)	12	33.1 (0.1–147.5)
	Suburban (La Penne sur Huveaune, France)	14	0.7 (ND–4.4)
	Remote (Plan d'Aups, France)	14	0.3 (ND–2.5)
Araki et al. (2009)	Urban road side (Kanazawa, Japan), winter 2007	5	13.4 ± 5.2 (8.0–21.8)

^a Estimated from a graph (no numbers presented)

^b Calculated from fmol/m³ by the Working Group
ND, not detected; SD, standard deviation

relative to warm engines. Such a change was not observed in the gasoline-powered cars.

In three types of heavy-duty diesel engine, different fuels were tested in the heavy-duty transient Federal Test Procedure: neat biodiesel fuel (B100), a blend of 20% biodiesel with 80% normal diesel fuel by volume (B20) and neat diesel fuel (2D). Following the use of B100, only traces of 6-nitrochrysene were detected in the exhaust. When fuelled with B20 and 2D, the amounts of 6-nitrochrysene detected in the exhaust of two of the three engines (both 1997 models) were in the range of 0.6–0.9 ng/horse power (hp)–h. After the addition of an oxidation catalyst, 6-nitrochrysene was detected at 1.8 ng/hp–h in the exhaust of one of the two engines that used B100 fuel. In the exhaust from the third engine (a 1995 model), levels of 6-nitrochrysene without a catalyst were 1.9 and 11 ng/hp–h for B20 and 2D, respectively. After the addition of an oxidation catalyst, the production of 6-nitrochrysene increased to 58 and 56 ng/hp–h for B20 and 2D, respectively ([Sharp et al., 2000](#)).

1.4.2 Ambient air and dust

[Garner et al. \(1986\)](#) first identified 6-nitrochrysene in the nanogram per cubic metre range in airborne particulate matter ([Table 1.2](#)). More recent reports reported values in the subnanogram per cubic metre range in urban areas, and in the lower and subpicogram per cubic metre range (or lower) at rural or remote locations ([Murahashi & Hayakawa, 1997](#); [Schauer et al., 2004](#); [Albinet et al., 2006, 2007](#)).

1.4.3 Biomonitoring of the general population

Blood samples were analysed for the presence of haemoglobin adducts of 6-aminochrysene, a metabolite of 6-nitrochrysene ([Zwirner-Baier & Neumann, 1999](#)). The study comprised groups of garage workers, inhabitants of Southampton and inhabitants of small villages in the region (for details, see [Scheepers et al., 1999](#)). The proportion of blood samples that contained haemoglobin adducts of 6-aminochrysene was 2/29, 3/20 and 0/20 in the three groups, respectively. Adduct

levels were all below 0.27 pmol/g haemoglobin, with a median of 0.03 pmol/g haemoglobin in all three groups. The method of analysis was based on gas chromatography-negative ion chemical ionization-mass spectrometry (see Section 1.2 of the *Monograph* on Diesel and Gasoline Engine Exhaust in this Volume).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

No lifetime bioassays on the carcinogenesis of 6-nitrochrysene have been carried out, but a few selected studies have shown tumour formation in rodents.

3.1 Mouse

See [Table 3.1](#)

3.1.1 Intraperitoneal administration

Groups of 21–29 male and female newborn Swiss-Webster BLU-Ha mice were administered intraperitoneal injections of 0 (control), 38 or 189 µg/mouse of 6-nitrochrysene in dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth. Mice were necropsied at 26 weeks of age and were analysed histologically. A 100% incidence of tumours was observed in the lung for all groups treated with 6-nitrochrysene compared with 3 out of 22 (14%) and 1 out of 15 (7%) male and female controls. The increased number of tumours (compared with controls) was highly significant ($P < 0.001$) ([Busby et al., 1985](#)).

Groups of 45 or 33 male and 34 or 40 female newborn CD-1 mice received intraperitoneal injections of 0 (control) or 2800 (total dose) nmol

of 6-nitrochrysene in DMSO on days 1, 8 and 15 after birth. A further group of 33 males and 40 females received a single injection of 700 nmol of 6-nitrochrysene 10 weeks after birth. Mice were observed for up to 1 year, and those that were moribund and died in the interim were evaluated histologically. In the single 700 nmol-dose group, 25 out of 33 males (76%; one adenoma, 24 carcinomas; $P < 0.05$) and 9 out of 40 females (23%; five adenomas, four carcinomas; $P < 0.005$) developed liver tumours. The 2800-nmol dose produced liver tumours in 3 out of 9 males (33%; carcinomas; $P < 0.05$) and 3 out of 11 females (27%; two adenomas, one carcinoma; $P < 0.05$); hepatic tumours were observed in 5 out of 45 (11%) male and 0 out of 34 female vehicle controls. In the 700-nmol group, lung tumours were observed in 28 out of 33 males (85%; 11 adenomas, 17 carcinomas; $P < 0.05$) and 36 out of 40 females (90%; 19 adenomas, 17 carcinomas; $P < 0.05$). At 2800 nmol, 7 out of 9 (78%) males and 9 out of 11 (82%) females developed lung tumours, while vehicle controls had an incidence of 4 out of 45 males (9%; two adenomas, two carcinomas) and 2 out of 34 females (6%; one adenoma, one carcinoma). The incidence of malignant lymphoma was also increased in treated males (at 700 nmol: 6 out of 33, 18%; at 2800 nmol: 3 out of 9, 33%) and females (at 700 nmol: 9 out of 40, 23%; at 2800 nmol: 4 out of 11, 36%) compared with their respective controls ([Wislocki et al., 1986](#)).

Groups of 91 and 26 male and 101 and 22 female newborn Swiss-Webster BLU-Ha mice received intraperitoneal injections of 0 (control) and 7 µg/mouse 6-nitrochrysene, respectively, in DMSO on days 1, 8 and 15 days after birth, and were necropsied at 26 weeks of age, when their lungs were analysed histologically. Male mice had an incidence of 11 out of 26 (42%) lung adenomas and 8 out of 26 (31%) lung adenocarcinomas. The incidence for females was 8 out of 22 (36%) and 5 out of 22 (28%), respectively, and that in the vehicle-treated groups was 12 out of 91 (13%) and 1 out of 91 (1%) for males and 7 out of 101

Table 3.1 Studies of the carcinogenicity of 6-nitrochrysene in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Swiss-Webster BLU:ha (ICR) (M, F) 26 wks Busby et al. (1985)	Intraperitoneal injection 0 (control), 38.5 or 189 µg in DMSO 1, 8 and 15 d after birth; animals killed and analysed 23 wks later Newborn; 26 M, 22 F	Lung (all tumours): M-3/22 (14%), 26/26 (100%), 26/26 (100%) F-1/15 (7%), 22/22 (100%), 22/22 (100%)	$P < 0.001$	70% of tumours were adenocarcinomas
CD-1 (M, F) 1 yr Wislocki et al. (1986)	Intraperitoneal injection 0 (control) or 2800 nmol (total dose) in DMSO on 1, 8 and 15 d after birth, or 700 nmol (once) at 10 wks Newborn; Vehicle control: 45 M, 34 F; 2800 nmol: 9 M, 11 F 700 nmol: 33 M, 40 F	Liver (adenoma and carcinoma): M-5/45 (11%), 3/9 (33%), 25/33 (76%) F-0/34, 3/11 (27%), 9/40 (23%) Lung (adenomas and carcinomas): M-4/45 (9%), 28/33 (85%), 7/9 (78%) F-2/34 (6%), 9/11 (82%), 36/40 (90%) Malignant lymphoma: M-0/45, 3/9 (33%), 6/33 (18%) F-0/34, 4/11 (36%), 9/40 (23%)	$P < 0.05$ for all treated groups	Significant mortality led study to be stopped at 1 year.
Swiss-Webster BLU:ha (ICR) (M, F) 26 wks Busby et al. (1989)	Intraperitoneal injection 0 (control) or 7.7 µg in DMSO 1, 8 and 15 d after birth; animals killed and analysed 23 wks later Newborn; 26 M, 22 F	Lung (adenoma): M-12/91 (13%), 11/26 (42%) F-7/101 (7%), 8/22 (36%) Lung (adenocarcinoma): M-1/91 (1%), 8/26 (31%) F-0/26, 5/22 (23%)	$P < 0.001$ for total lung tumours when genders combined	Compared with other nitroarenes in this study, 6-nitrochrysene was the most potent at producing tumours.
Swiss-Webster BLU:ha (M, F) 30 wks El-Bayoumy et al. (1989a)	Intraperitoneal injection 0 (control), 100 or 700 nmol (total doses) in DMSO on 1, 8 and 15 d after birth or 100 nmol (once) on day 1 Newborn; Vehicle control: 38 M, 28 F 100 nmol: 23 M, 24 F 700 nmol: 37 M, 46 F; 100 nmol (one injection): 25 M, 21 F	Lung (all tumours): M-18%, 100%, 100%, 100% F-11%, 100%, 100%, 100% Liver (all tumours): M-0%, 84%*, 84%*, 65%* F-0%, 10%, 3%, 4%	* $P < 0.01$	Metabolites also evaluated, and the putative metabolite 1,2-dihydro-1,2-dihydroxy-6-aminochrysene showed similar or greater tumour production, suggesting 6-nitrochrysene is metabolically activated

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
HSD:ICR (M, F) 30 wks El-Bayoumy et al. (1992)	Intraperitoneal injection 0 (control) or 100 nmol (total dose) in DMSO on 1, 8 and 15 d after birth; animals killed and analysed 23 wks later Newborn; 38 M, 24 F	Lung (all tumours): M–12%, 100% F–3%, 96% Liver (all tumours): M–8%, 64% F– 0%, 0%	$P < 0.001$ for lung tumours, and liver tumours in males	Study aimed at comparing isomers; 6-nitrochrysene was the most potent and 1,2- and 3-nitrochrysene did not differ from controls.
Crj:CD-1(ICR) (M, F) 24 wks Imaida et al. (1992)	Intraperitoneal injection 0 (control) or 1.4 µmol (total dose) in DMSO on 1, 8 and 15 d after birth; animals killed analysed 24 wks later Newborn; Control: 22 M, 26 F 1.4 µmol: 9 M, 11 F	Lung (adenoma): M–0/22, 9/9 (100%) F–0/26, 11/11 (100%)	$P < 0.001$	Study to determine potential for colon tumours observed in rats; no colon tumours observed in mice
B6C3F1 (M) 1 yr Fu et al. (1994)	Intraperitoneal injection 0 or 400 nmol in DMSO on 1, 8 and 15 d after birth (treatment (T) 1: 1/7, 2/7 and 4/7; or treatment (T) 2: 0/7, 3/7 and 4/7 of the dose); fed ad-libitum diet or calorie-restricted diet at 14 wks of age; killed and analysed at 1 yr Newborn: ~20/group	Liver (adenoma): Ad-libitum–2/18 (11%), 19/19 (100%)* T1, 21/21 (100%)* T2 Calorie-restricted–0/21, 4/23 (17%) T1, 0/21 T2 Liver (carcinoma): Ad-libitum–0/18, 14/19 (74%)* T1, 21/21 (100%)* T2 Calorie-restricted: 0/21, 1/23 (4%) T1, 0/21 T2 Ad libitum Lung (adenoma): Ad-libitum–1/18 (6%), 11/19 (58%)* T1, 3/21 (14%) T2 Calorie-restricted– 1/19 (5%), 0/23 T1, 0/21 T2	* $P < 0.01$ versus control and versus ad-libitum diet	Purity > 99%; diet had a significant impact on tumour formation; liver tumours more prevalent than lung tumours

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD-1 (M) 11 mo Li et al. (1994)	Intraperitoneal injection 0 (control) or 250 nmol in DMSO on 1, 8 and 15 d after birth; animals killed at 11 mo and analysed Newborn; 21 or 27/group	Lung (adenoma): 3/27 (11%), 21/21 (100%)* Lung (carcinoma) 0/27, 5/21 (24%)	* $P < 0.01$	Comparative treatment with trans 1,2 dihydro-1,2-dihydroxy- 6-aminochrysene, a putative metabolite, showed similar tumour and DNA-adduct formation as 6-nitrochrysene; other metabolites were less potent.
Crl/CD-1(ICR) BR (F) 25 wks El-Bayoumy et al. (1982)	Skin application 0 (control) or 0.1 mg in acetone on alternate d for 20 d; 10 d later, 2.5 µg TPA 3 ×/wk for 25 wks 20/group	Skin (predominantly squamous cell papillomas): 1/20 (5%), 12/20 (60%)	$P < 0.01$	Purity > 99%

d, day; DMSO, dimethyl sulfoxide; F, female; M, male; mo, month; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week; yr, year

(7%) and 0 out of 101 for females, respectively. The increased number of tumours (compared with controls) in males and females combined was highly significant when analysed for total tumours (32 out of 48; $P < 0.001$), but not when analysed by gender ([Busby et al., 1989](#)).

Groups of newborn Swiss-Webster BLU-Ha mice received intraperitoneal injections of 0 (control; 38 males and 28 females), 100 (total dose; 23 males and 24 females) or 700 (total dose; 37 males and 46 females) nmol/mouse of 6-nitrochrysene in DMSO on days 1, 8 and 15 days after birth, or a single injection on day 1 of 100 nmol/mouse (25 males and 21 females) of 6-nitrochrysene in DMSO. Mice were necropsied at 30 weeks of age and their liver and lungs were analysed histologically. All male mice treated with 6-nitrochrysene had a 100% incidence of lung tumours. The incidence of liver tumours was 65%, 84% and 84% in males and 4%, 3% and 10% in females treated with 100 (three doses), 700 (three doses) and 100 (single dose) nmol of 6-nitrochrysene, respectively. The incidence of lung and liver tumours in all treated males was statistically significant compared with that in vehicle controls [type of tumours unspecified]. These results confirm the effects in the lungs, and show a gender-related sensitivity. Metabolites of 6-nitrochrysene were also evaluated in the study, and the putative metabolite, 1,2-dihydro-1,2-dihydroxy-6-amino chrysene, showed carcinogenicity similar to or greater than that of 6-nitrochrysene ([El-Bayoumy et al., 1989a](#); see also [Table 3.2](#)).

An assay was conducted in male and female newborn HSD:ICR mice to compare the potency of 6-nitrochrysene with that of isomers of nitrochrysene in the induction of tumour formation. A total dose of 100 nmol/mouse of 6-nitrochrysene in DMSO was administered by intraperitoneal injection to 38 males and 24 females on days 1, 8 and 15 after birth. A group that received DMSO alone served as a vehicle control. Animals were killed and analysed histologically

for lung and liver tumours 30 weeks after the last dose. The lung tumour incidence was 100% in males and 96% in females, and was statistically significantly increased compared with that in controls (12% and 3%, respectively; $P < 0.001$). Liver tumours were observed in 64% ($P < 0.001$) of the treated males, none of the treated females, 8% of the control males and none of the control females. The types of tumour were not specified. 6-Nitrochrysene was significantly more potent than the isomers of nitrochrysene, which did not show a significant induction of tumours ([El-Bayoumy et al., 1992](#)).

Groups of 22 or 9 male and 26 or 11 female newborn ICR mice received intraperitoneal injections of 0 (control) or 1.4 $\mu\text{mol/mouse}$ (total dose) of 6-nitrochrysene in DMSO on days 1, 8 and 15 days after birth, were killed 24 weeks after the last dose and were analysed histologically. The incidence of lung tumours (adenoma) was 9 out of 9 (100%) treated males and 11 out of 11 (100%) treated females lung tumours versus none in the vehicle controls. No tumours were observed in the colon or the liver ([Imaida et al., 1992](#)).

The effects of diet and modality of treatment on the formation of liver and lung tumours were investigated in groups of ~20 male newborn B6C3F1 mice that received intraperitoneal injections of a total dose of 0 (control) or 400 nmol/mouse of 6-nitrochrysene (purity, > 99%) in DMSO on days 1, 8 and 15 after birth; the three injections of 6-nitrochrysene contained 1/7th, 2/7th and 4/7th (treatment 1) or 0/7th, 3/7th and 4/7th (treatment 2) of the total dose, respectively. The two treatment groups were then separated at 14 weeks of age and either received the standard diet *ad libitum* or received a 70% calorie-restricted diet. The incidence of liver tumours in animals that received the standard diet *ad libitum* was 19 out of 19 (100%; adenomas) 14 out of 19 (74%; carcinomas) in treatment 1 group and 21 out of 21 (100%; adenomas) and 21 out of 21 (100%; carcinomas) in treatment 2 group. The calorie-restricted diet decreased the tumour

Table 3.2 Induction of lung and liver tumours in newborn mice treated with 6-nitrochrysene and its metabolites

Group Compound	Total dose (nmol)	Mice with lung tumours (%)	No. of lung tumours per mouse	Mice with liver tumours (%)	No. of liver tumours per mouse
1 DMSO	–	M – 18.4 F – 10.7	M – 0.2 ± 0.5 F – 0.1 ± 0.3	M – 0 F – 0	M – 0 F – 0
2 6-NC	700	M – 100 ^a F – 100 ^a	M – 50.6 ± 29.6 ^{a,b} F – 47.8 ± 26.3 ^{a,b}	M – 84 ^a F – 3	M – 15.4 ± 15.1 ^{a,b} F – 0.1 ± 0.5
3 6-NC	100	M – 100 ^a F – 100 ^a	M – 11.4 ± 6.9 ^a F – 13.2 ± 9.2 ^a	M – 65 ^a F – 4	M – 5.4 ± 8.6 ^a F – 0.1 ± 0.19
4 1,2-DHD-6-NC	100	M – 100 ^a F – 100 ^a	M – 14.1 ± 9.9 ^a F – 15.8 ± 12.3	M – 81 ^a F – 10.0	M – 17.6 ± 18.9 ^{a,b,c} F – 0.1 ± 0.3
5 1,2-DHD-6-AC	100	M – 89 ^a F – 89 ^a	M – 12.2 ± 12.1 ^a F – 13.4 ± 11.8 ^a	M – 83 ^a F – 5	M – 7.7 ± 7.5 ^a F – 0.3 ± 1.2

^a Significantly different from group 1, $P < 0.01$

^b Significantly different from group 3, $P < 0.01$

^c Significantly different from group 6, $P < 0.05$

1,2-DHD-6-AC, 1,2-dihydroxy-6-aminochrysene; 1,2-DHD-6-NC, 1,2-dihydroxy-6-nitrochrysene; 6-NC, 6-nitrochrysene; DMSO, dimethyl sulfoxide; F, female; M, male

From [El-Bayoumy et al. \(1989a\)](#)

incidence to 4 out of 23 (17%; adenomas) and 1 out of 23 (4%; carcinomas) in treatment 1 mice, but did not decrease that in treatment 2 group. The ad-libitum diet had a statistically significant effect ($P < 0.01$) on tumour incidence in comparison with both controls and the calorie-restricted diet. The incidence of lung tumour was 11 out of 19 (58%; adenomas) in the treatment 1 group and 3 out of 21 (14%; adenomas) in the treatment 2 group fed the ad-libitum diet, whereas no lung tumours were observed in mice fed the calorie-restricted diet ([Fu et al., 1994](#)).

In a study to compare the relative DNA adducts, mutagenicity and tumour formation induced by 6-nitrochrysene and several of its major metabolites in the lungs, groups of 21–27 newborn male CD-1 mice were administered intraperitoneal injections of a total dose of 0 (control) or 250 nmol/mouse of 6-nitrochrysene on days 1, 8 and 15 after birth (1/7th, 2/7th and 4/7th of the total dose, respectively). In parallel, several metabolites of 6-nitrochrysene were evaluated at under similar experimental conditions. Tumour formation was investigated 11 months after the last dose of 6-nitrochrysene. A

statistically significant increase in the incidence of lung adenoma (21 out of 21; 100%) and adenocarcinoma (5 out of 21; 24%) was observed in the 6-nitrochrysene-treated animals compared with controls (adenomas only; 3 out of 27; 11%) ([Li et al., 1994](#)).

3.1.2 Initiation–promotion

Groups of 20 female CD-1 mice, aged 50–55 days, received 10 topical applications of 0 (control) or 0.1 mg/mouse of 6-nitrochrysene (purity, > 99%) in 0.1 mL acetone onto the shaved back every other day for 20 days (total dose, 1 mg/mouse). Ten days later, 2.5 µg of the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate were applied three times a week for an additional 25 weeks, after which the animals were killed and analysed histologically. An increase in the incidence of squamous cell papilloma was observed in the treated group; 12 out of 20 (60%) treated mice developed skin tumours (primarily papillomas; $P < 0.01$) compared with 1 out of 20 (5%) controls. No systemic tumours were observed ([El-Bayoumy et al., 1982](#)).

3.2 Rat

See [Table 3.3](#)

3.2.1 Oral administration

In a study to evaluate the formation of mammary tumours that had previously been demonstrated in female CD1 rats and to compare the effects of several metabolites of 6-nitrochrysene that had also been reported to cause tumours, groups of 20 female Sprague-Dawley rats, aged 30 days, received intragastric intubations of 0 (control), 100 (low dose), 200 (mid-dose) or 400 (high dose) $\mu\text{mol}/\text{rat}$ of 6-nitrochrysene [purity unspecified] in trioctanoin once a week for 8 weeks, and were killed 23 weeks after the last dose, when mammary tissue were analysed by histopathology. An increase in the incidence of mammary adenocarcinomas was observed: 3 out of 30 (10%) vehicle-control, 25 out of 30 (83%) low-dose ($P < 0.0001$), 25 out of 30 (83%) mid-dose ($P < 0.0001$) and 27 out of 30 (90%) high-dose ($P < 0.0001$) animals. A statistically significant increase in the incidence of fibroadenomas occurred in the low-dose group (5 out of 30 (17%) versus 0 out of 30; $P = 0.02$) ([El-Bayoumy et al., 2002](#)).

Groups of transgenic (BBR \times CD)F1 rats (developed by mating Big Blue (BBR) and Sprague-Dawley (CD) strains), aged 30 days, received intragastric intubations of 0 (control), 100 or 200 μmol (total doses) of 6-nitrochrysene in trioctanoin once a week for 8 weeks. The rats were killed 32 weeks after the last dose, and mammary tissue were analysed histologically. An increase in the incidence of mammary tumours was observed: fibroadenomas – 0 out of 33 control, 29 out of 31 (29%) low-dose ($P < 0.01$) and 2 out of 14 (15%) high-dose ($P < 0.05$) animals; adenomas – 0 out of 33 control, 1 out of 31 (3%) low-dose and 1/14 (7%) high-dose animals; adenocarcinomas – 1 out of 33 (3%) control, 5 out of 31 (16%) low-dose and 5 out of 31 (36%)

high-dose ($P < 0.01$) animals; and fibromas – 0 out of 33 control, 1 out of 31 (3%) low-dose and 0 out of 14 high-dose females ([Boyiri et al., 2004](#)).

3.2.2 Intraperitoneal administration

Groups of 31 male and 32 female newborn Crj:CG rats received intraperitoneal injection of 0 (control) or 14.8 $\mu\text{mol}/\text{rat}$ (total dose) of 6-nitrochrysene in DMSO on days 1, 8 and 15 after birth. The animals were killed 32 weeks after the last administration and analysed histologically. The incidence of colon adenomas/dysplasias and adenocarcinomas was 23% and 16% in males ($P < 0.01$) and 44% and 6% in females ($P < 0.01$ for adenomas/dysplasias only), respectively. No colon tumours were observed in the controls, and no lung or liver tumours were observed in the treated or control rats ([Imaida et al., 1992](#)).

3.2.3 Intramammary administration

Groups of 30 female CD1 rats, aged 30 days, received intramammary injection of 0 (control) or 12.3 $\mu\text{mol}/\text{rat}$ (total dose) of 6-nitrochrysene in DMSO over 2 days. Animals were killed and analysed grossly and histologically for tumours 43 weeks after the last injection. Fibroadenomas, but no other tumours, developed in 5 out of 30 (16%) vehicle controls. The incidence of tumours in the 6-nitrochrysene group was 24 out of 30 (80%; $P < 0.0001$) fibroadenomas, 15 out of 30 (50%; $P < 0.0001$) adenocarcinomas and 10 out of 30 (33%; $P < 0.001$) spindle cell carcinomas ([El-Bayoumy et al., 1993](#)).

Table 3.3 Studies of the carcinogenicity of 6-nitrochrysene in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 23 wks El-Bayoumy et al. (2002)	Oral administration 0 (control), 100, 200 or 400 µmol in trioctanoin once/wk for 8 wks; animals killed and analysed 23 wks later Newborn; 20/group	Mammary (adenoma): 0/30, 0/30, 3/30 (10%), 1/30 (3%) Mammary (fibroadenoma): 0/30, 5/30 (17%)*, 3/30 (10%), 3/30 (3%) Mammary (adenocarcinoma): 3/30 (10%), 25/30 (83%)*, 25/30 (83%)*, 27/30 (90%)*	* <i>P</i> < 0.0001 ** <i>P</i> = 0.02	High doses; not exposed by expected route in the environment; also evaluated tumour-forming potential of putative metabolites, and found that several metabolites also cause mammary tumours, none as potent as 6-nitrochrysene
Transgenic (Big Blue F344 × Sprague-Dawley) (F) 32 wks Boyiri et al. 2004	Oral administration 0 (control), 100 µmol or 200 µmol (total dose) in trioctanoin once/wk for 8 wk Aged 30 d; 33 (0), 31 (100 µmol) and 14 (400 µmol)	Mammary (fibroadenoma): 0/33, 9/31 (29%)*, 2/14 (14%)* Mammary (adenoma): 0/33, 1/31 (3%), 5/14 (7%) Mammary (fibroma): 0/33, 1/31 (3%), 0/14 Mammary (adenocarcinoma): 1/33 (3%), 5/31 (16%), 5/14 (36%)**	* <i>P</i> < 0.05 ** <i>P</i> < 0.01	Oral administration induced mammary tumours but intraperitoneal administration in other studies produced colon tumours
Crj:CD (CD) (M, F) 32 wks Imaida et al. (1992)	Intraperitoneal injection 0 (control) or 14.8 µmol (total dose) in DMSO on 1, 8, 15, 22 and 29 d after birth; animals killed and analysed 32 wks later 31 M, 31 F (0); 31 M, 32 F (14.8 µmol)	Colon (dysplasia/adenoma): M–0/31, 7/31 (23%)* F–0/31, 14/32 (44%)* Colon (adenocarcinoma): M–0/31, 5/31 (16%) F–0/31, 2/32 (6%)	* <i>P</i> < 0.01	No lung or liver tumours observed
CD (F) 43 wks El-Bayoumy et al. (1993)	Intramammary injection 0 (control) or 12.3 µmol/rat (total dose) in DMSO on 2 d; animals palpated every 2 wks 3 mo after treatment, and killed and analysed after 43 wks Aged 30 d; 30/group	Mammary (fibroadenoma): 5/30 (17%), 24/30 (80%)** Mammary (spindle cell carcinoma): 0/30, 10/30 (33%)* Mammary (adenocarcinoma): 0/30, 15/30 (50%)**	* <i>P</i> < 0.001 ** <i>P</i> < 0.0001	Not exposed by expected route in the environment

d, day; DMSO, dimethyl sulfoxide; F, female; M, male; mo, month; wk, week

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

(a) *In-vivo* studies

In CD rats treated intraperitoneally with a single dose of [3,4,9,10-³H] 6-nitrochrysene (³H]6-nitrochrysene; 9 µmol/rat), only 1.3% of the dose was excreted in the urine and 23% in the faeces after 24 hours (Chae *et al.*, 1996). The extent of metabolism was extremely limited; 6-nitrochrysene was the major component found in the faeces after 24 hours, accounting for 98% of the radioactivity. In the faeces, *trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene (1,2-DHD-6-NC), chrysene-5,6-quinone and 6-aminochrysene were identified as 6-nitrochrysene metabolites; the major free products (unconjugated) identified in urine were 6-aminochrysene, 1,2-DHD-6-NC and *trans*-9,10-dihydro-9,10-dihydroxy-6-nitrochrysene (9,10-DHD-6-NC) (Chae *et al.*, 1996). The same metabolites were detected in extracts from whole Blu:Ha (ICR) mice after intraperitoneal injection of ³H]6-nitrochrysene (Delclos *et al.*, 1988). The role of intestinal microflora on the metabolism of 6-nitrochrysene was investigated in Balb/c mice after treatment with a single intraperitoneal injection of 0.03 µmol/5 µL/g body weight (bw) ³H]6-nitrochrysene (Delclos *et al.*, 1990). The amount of 6-aminochrysene excreted in the faeces of germ-free mice within 48 hours after treatment was approximately 25% of that excreted in identically treated conventional mice.

6-Nitrochrysene induced hepatic cytochrome P450 (CYP) expression after oral treatment of Sprague-Dawley rats with 2.5, 5 or 25 mg/kg bw for 3 days (Chou *et al.*, 1987). 6-Nitrochrysene was significantly more active than its parent compound, chrysene. In Sprague-Dawley rats treated with 5 mg/kg bw for 3 days, 6-nitrochrysene significantly increased the enzyme activities of uridine diphosphate-glucuronosyl-transferase and glutathione S-transferase whereas chrysene did not (Pegram & Chou, 1989).

(b) *In-vitro* studies

The metabolism of 6-nitrochrysene ([12-³H]6-nitrochrysene) was studied in primary cultures of human breast cells prepared from tissues obtained from reduction mammaplasty (Boyiri *et al.*, 2002). 1,2-DHD-6-NC and 6-aminochrysene were identified as the major metabolites; chrysene-5,6-quinone was also detected. Similar results were found in cultured, immortalized human mammary epithelial MCF-10A cells, as well as estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-435) human breast cancer cell lines, in which *trans*-1,2-dihydro-1,2-dihydroxy-6-aminochrysene (1,2-DHD-6-AC) was also found (Boyiri *et al.*, 2002).

In microsomes prepared from human liver and lung tissue and incubated with ³H]6-nitrochrysene, 1,2-DHD-6-NC, 9,10-DHD-6-NC, 6-aminochrysene and chrysene-5,6-quinone were identified as metabolites but with quantitative differences (Chae *et al.*, 1993). The levels of the major metabolite, 1,2-DHD-6-NC, ranged from 33 to 132 pmol/mg protein/hour using numerous human hepatic microsomal samples; the corresponding levels using human pulmonary microsomes ranged from non-detectable to 33 pmol/mg protein/hour. The remaining metabolites were detected at much lower levels.

The oxidation of 1,2-DHD-6-NC was catalysed by *CYP1A2* in human liver and *CYP1A1*

in human lung; CYP3A4 catalysed the reduction of 6-nitrochrysene to 6-aminochrysene. In human hepatoma HepG2 cells, 6-nitrochrysene induced the expression of *CYP1A1* protein and mRNA levels that may be an important factor to assess the metabolism of 6-nitrochrysene in the liver ([Chen et al., 2000](#)). 6-Nitrochrysene also induced *CYP1A1* mRNA in human lung carcinoma NCI-H322 cells. The induction of *CYP1A1*, *CYP1A2* and *CYP1B1* mRNAs by 6-nitrochrysene was investigated in various human-derived cell lines ([Iwanari et al., 2002](#)). The induction of all three *CYP* genes by 6-nitrochrysene was observed in HepG2 (hepatocellular carcinoma), MCF-7 (breast carcinoma), LS-180 (colon carcinoma), OMC-3 (ovarian carcinoma) and NEC14 (testis embryonal carcinoma) cells; a strong induction of *CYP1B1* was observed in A549 (lung carcinoma) cells.

Incubation of 6-nitrochrysene with an exogenous metabolic activation system from rat liver resulted in the formation of 1,2-DHD-6-NC and 6-aminochrysene as the major metabolites ([El-Bayoumy & Hecht, 1984](#)). Similar results were obtained in incubations containing [³H]6-nitrochrysene and an exogenous metabolic activation system from the liver of 1- or 8-day-old BLU:Ha (ICR) mice; the formation of 1,2-DHD-6-NC (approximately twofold), 9,10-DHD-6-NC (~1.5-fold) and 6-aminochrysene (approximately fourfold) was greater in younger animals ([Delclos et al., 1988](#)). Further metabolism of [³H]1,2-DHD-6-NC by rat liver metabolic activation yielded 1,2-DHD-6-AC as the major product.

Mixed cultures of rat and mouse intestinal bacteria and pure cultures of anaerobic bacteria reduced 6-nitrochrysene to 6-aminochrysene. In the fungus, *Cunninghamella elegans*, 74% of the [³H]6-nitrochrysene added was metabolized within 6 days to form two isomeric sulfate conjugates ([Pothuluri et al., 1998](#)). These conjugates were identified as 6-nitrochrysene-1-sulfate and 6-nitrochrysene-2-sulfate.

4.2 Genetic and related effects

The genetic and related effects of 6-nitrochrysene and some of its metabolites have been reviewed ([Rosenkranz & Mermelstein, 1983](#); [Tokiwa & Ohnishi, 1986](#); [Purohit & Basu, 2000](#); [IPCS, 2003](#))

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

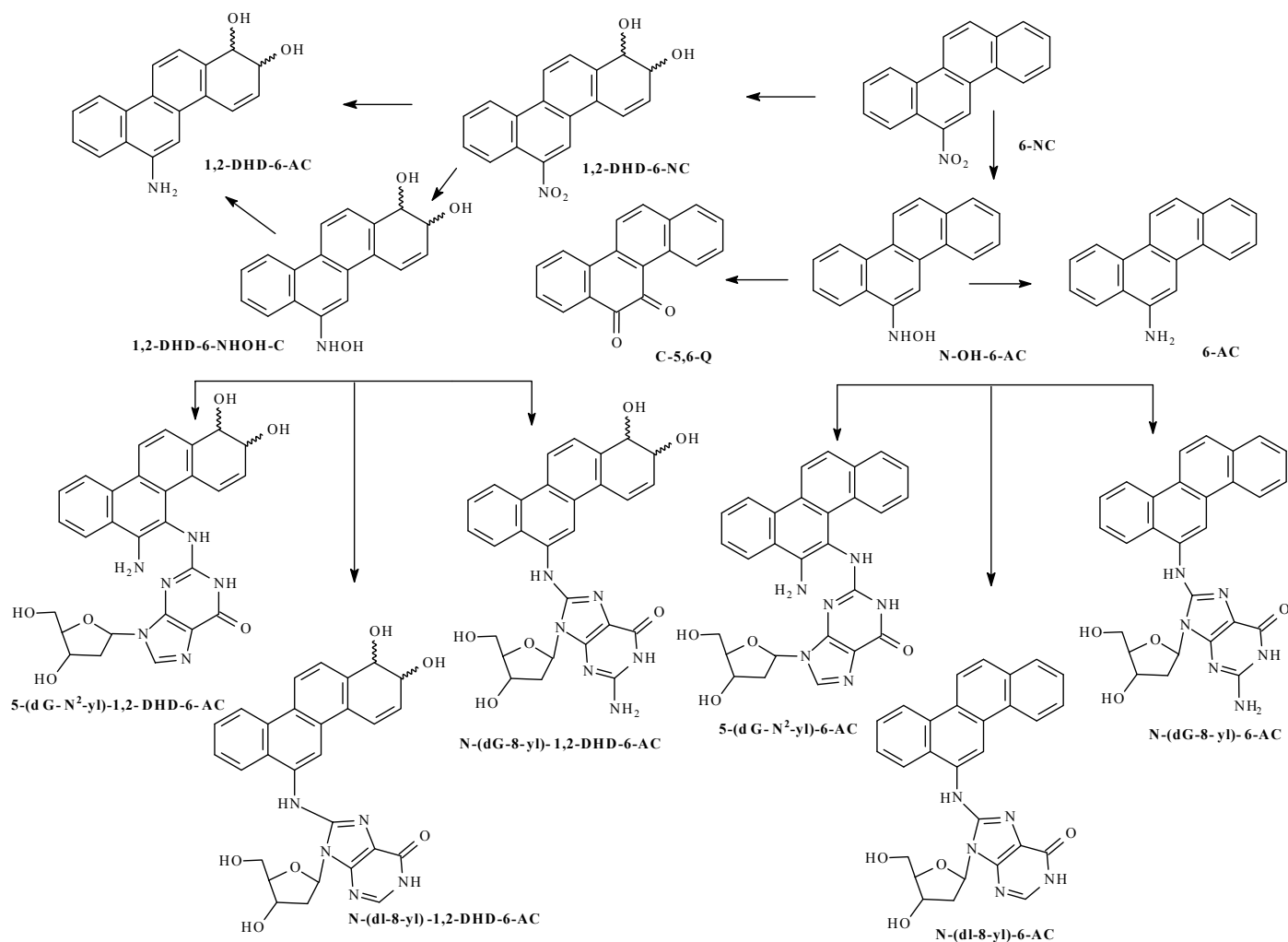
(a) DNA adduct formation

See [Fig. 4.1](#).

(i) *In-vivo* studies

The formation of DNA adduct by 6-nitrochrysene was predominantly measured using the ³²P-postlabelling method and studied in rats. The intraperitoneal injection of CD rats with 14.8 μmol/rat of 6-nitrochrysene on days 1, 8, 15, 22 and 29 after birth resulted in the formation of DNA adducts, 24 hours after the last administration, in the colon (the target organ for carcinogenesis) as well as in the liver and lung (non-target organs for carcinogenesis) ([Chae et al., 1996](#)). Two major DNA adducts were detected (adducts 3 and 4). DNA adduct 3 was chromatographically indistinguishable from 5-(deoxyguanosin-*N*²-yl)-6-aminochrysene [5-(dG-*N*²-yl)-6-AC]. Adduct 4 was proposed by the authors to be derived from 1,2-DHD-6-AC and to be a deoxyguanosine adduct modified by 1,2-DHD-6-AC-3,4-epoxide ([Chae et al., 1996](#)), but was later shown to be derived from 1,2-DHD-6-hydroxylamino-chrysene and was structurally identified as 5-(deoxyguanosin-*N*²-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene [5-(dG-*N*²-yl)-1,2-DHD-6-AC] ([El-Bayoumy et al., 2004](#)). *N*-(Deoxyguanosin-8-yl)-6-aminochrysene [*N*-(dG-8-yl)-6-AC; adduct 1] and *N*-(deoxyinosin-8-yl)-6-aminochrysene

Fig. 4.1 Metabolism and DNA-adduct formation of 6-nitrochrysene



6-AC, 6-aminochrysene; C-5,6-Q, chrysene-5,6-quinone; 5-(dG-N²-yl)-6-AC, 5-(deoxyguanosin-N²-yl)-6-aminochrysene; 5-(dG-N²-yl)-1,2-DHD-6-AC, 5-(deoxyguanosin-N²-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene; 1,2-DHD-6-AC, 1,2-dihydroxy-1,2-dihydro-6-aminochrysene; 1,2-DHD-6-NC, 1,2-dihydroxy-1,2-dihydro-6-nitrochrysene; 1,2-DHD-6-NHOH-C, *trans*-1,2-dihydroxy-1,2-dihydro-*N*-hydroxy-6-aminochrysene; 6-NC, 6-nitrochrysene; N-(dG-8-yl)-6-AC, *N*-(deoxyguanosin-8-yl)-6-aminochrysene; N-(dG-8-yl)-1,2-DHD-6-AC, *N*-(deoxyguanosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene; N-(dI-8-yl)-6-AC, *N*-(deoxyinosin-8-yl)-6-aminochrysene; N-(dI-8-yl)-1,2-DHD-6-AC, *N*-(deoxyinosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene; N-OH-6-AC, *N*-hydroxy-6-aminochrysene

[*N*-(dI-8-yl)-6-AC; adduct 2] were not detectable *in vivo*.

The role of intestinal microflora on the formation of 6-nitrochrysene-induced DNA adducts was investigated in Balb/c mice after a single intraperitoneal injection of [³H]6-nitrochrysene (0.03 μmol/5 μL/g bw) (Delclos *et al.*, 1990). The levels of DNA adducts were similar in conventional and germ-free mice; however, the adducts in the liver were derived from both *N*-hydroxy-6-aminochrysene and 1,2-DHD-6-AC, whereas only adducts derived from 1,2-DHD-6-AC were detected in the lung. These findings are in contrast to other findings in preweanling mice (Delclos *et al.*, 1987a), in which the single major adduct detected in both the liver and the lung was derived from 1,2-DHD-6-AC.

As part of the newborn mouse lung adenoma bioassay, newborn CD-1 mice received intraperitoneal injections of total doses of 250 nmol of 6-nitrochrysene, 6-aminochrysene, *N*-hydroxy-6-aminochrysene or 6-nitrosochrysene or 80 nmol of 1,2-DHD-6-AC within 24 hours, and on days 8 and 15 after birth (Li *et al.*, 1994). In the lung, 6-nitrochrysene, 6-aminochrysene and 1,2-DHD-6-AC produced predominantly a single major DNA adduct, which was subsequently identified as 5-(dG-*N*²-yl)-1,2-DHD-6-AC (El-Bayoumy *et al.*, 2004). 6-Nitrosochrysene induced a single major adduct that was most probably derived from reaction at the C8 position of deoxyadenosine (Li *et al.*, 1994).

(ii) *In-vitro studies*

N-Hydroxy-6-aminochrysene was reacted *in vitro* with DNA. The three major DNA adducts identified, *N*-(dG-8-yl)-6-AC, *N*-(dI-8-yl)-6-AC and 5-(dG-*N*²-yl)-6-AC, accounted for 22%, 32% and 28%, respectively, of the total DNA adducts formed (Delclos *et al.*, 1987b). The deoxyinosine adduct probably results from the oxidative deamination of the corresponding deoxyadenosine adduct. *Anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-6-NC was shown to react with DNA, as

well as with deoxyguanosine or deoxyadenosine, *in vitro* but these DNA adducts did not correspond to adducts found in 6-nitrochrysene-treated rats *in vivo* (Krzeminski *et al.*, 2000). The reaction of *trans*-1,2-dihydroxy-1,2-dihydro-6-hydroxyaminochrysene (1,2-DHD-6-NHOH-C) with DNA *in vitro* resulted in the formation of three major DNA adducts, namely 5-(dG-*N*²-yl)-1,2-DHD-6-AC, *N*-(deoxyguanosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene and *N*-(deoxyinosin-8-yl)-1,2-dihydroxy-1,2-dihydro-6-aminochrysene (El-Bayoumy *et al.*, 2004).

N-(dG-8-yl)-6-AC and 5-(dG-*N*²-yl)-6-AC were repaired to similar extents by nucleotide excision repair, because the *N*²-deoxyguanosine adduct formed by benzo[*a*]pyrene-diol-epoxide [i.e. (+)-*trans*-benzo[*a*]pyrene-diol-epoxide-*N*²-deoxyguanosine] in DNA duplexes constructs incubated with nucleotide excision repair-competent nuclear extracts from human HeLa cells (Krzeminski *et al.*, 2011). As (+)-*trans*-benzo[*a*]pyrene-diol-epoxide-*N*²-deoxyguanosine is considered to be a poorer substrate for nucleotide excision repair, *N*-(dG-8-yl)-6-AC and 5-(dG-*N*²-yl)-6-AC lesions may be repaired slowly and may thus persist in mammalian tissues.

In rat hepatocytes treated with [³H]6-nitrochrysene or [³H]6-aminochrysene, a high degree of DNA binding was observed (0.2–4 adducts per 10⁶ nucleotides); the two major DNA adducts formed were identified as *N*-(dG-8-yl)6-AC and *N*-(dI-8-yl)-6-AC (Delclos *et al.*, 1987a). Similarly, 6-nitrochrysene formed one major adduct by ³²P-postlabelling in primary rat hepatocytes, NCI-H322 derived human lung cells and Chinese hamster lung V79NH cells with endogenous acetyltransferase activity (Topinka *et al.*, 1998). In human MCF-7 cells treated with [12-³H]6-nitrochrysene and [³H]1,2-DHD-6-NC, one major DNA adduct was detected which was chromatographically indistinguishable from a major DNA adduct found in the [12-³H]6-nitrochrysene-treated mammary

glands of rats and a deoxyguanosine adduct derived from the nitroreduction of [^3H]1,2-DHD-6-NC in the presence of xanthine oxidase (Boyiri *et al.*, 2002).

(b) *Mutagenicity of 6-nitrochrysene*

(i) *Mutagenesis and chromosomal instability in vivo*

K-ras mutations in codon 12, 13 or 61 were found in 64 (88%) of 73 lung adenomas and all 15 adenocarcinomas analysed from newborn CD-1 mice treated intraperitoneally with 6-nitrochrysene (Li *et al.*, 1994). All of the mutations in codon 12 and 13 involved a G:C base pair. Liver tumours were induced by the intraperitoneal administration of 6-nitrochrysene to preweaning B6C3F₁ (total dose, 300 nmol/mouse) and CD-1 (total dose 250 nmol/mouse) mice and analysed for *ras* gene mutations (Manjanatha *et al.*, 1996). The frequency of H- and K-*ras* mutations in 6-nitrochrysene-induced tumours was only 4% in B6C3F₁ mice, while 19 liver tumours (90%) from CD-1 mice had the identical CAA to AAA mutation in H-*ras* codon 61. Transgenic mice have been used as powerful toxicological models to study the role of specific genetic alterations as predisposing factors for chemical carcinogenesis. In a previous study, transgenic mice carrying a prototype human c-Ha-*ras* gene was susceptible to lung carcinogens such as 6-nitrochrysene; the authors stated that this susceptibility may be due to somatic mutations in the *ras* transgene (Ogawa *et al.*, 1996).

The mutagenicity of 6-nitrochrysene in the mammary glands was examined in the *cII* gene of transgenic (Big Blue F344 × Sprague-Dawley) F1 rats treated once a week for 8 weeks with 100 or 200 $\mu\text{mol/rat}$ 6-nitrochrysene (Boyiri *et al.*, 2004). The mutant frequency measured 32 weeks after the last administration was comparable in normal, non-involved and tumour tissue from the mammary glands of 6-nitrochrysene-treated rats but was significantly higher than that observed

in control rats. 6-Nitrochrysene induced G:C to T:A, G:C to C:G, A:T to G:C and A:T to T:A mutations, whereas G:C to A:T transitions were primarily found in control rats.

Injection of Balb/c mice with 0–500 mg/kg bw 6-nitrochrysene led to a significant increase in micronuclei in the bone marrow (Sakitani & Suzuki, 1986).

(ii) *Mutagenesis and DNA damage in bacteria*

Since the previous review of 6-nitrochrysene (IARC, 1989), additional studies have shown that 6-nitrochrysene was mutagenic to *Salmonella typhimurium* TA98, TA100 and TA1538 in the presence of absence of an exogenous metabolic activation system (El-Bayoumy *et al.*, 1989b; Jung *et al.*, 1991; Chen *et al.*, 2000). The mutagenicity of 6-nitrochrysene, 1,2-DHD-6-NC and 6-aminochrysene was compared in *S. typhimurium* TA100 and 1,2-DHD-6-NC was identified as the major proximate mutagen of 6-nitrochrysene. Bay region methyl substitution has been shown to either inhibit (5-position) or enhance (11-position) the mutagenic activity of 6-nitrochrysene in *S. typhimurium* (El-Bayoumy *et al.*, 1989b).

6-Nitrochrysene induced DNA damage in *S. typhimurium* (SOS umu test) (Shimada *et al.*, 1989; Rafi *et al.*, 1994; Shimada *et al.*, 1996; Yamazaki *et al.*, 2000); human CYP1B1 was required to activate 6-nitrochrysene in this assay, whereas human CYP1A1 and CYP1A2 did not activate the compound (Shimada *et al.*, 1996). 6-Nitrochrysene inhibited the growth of DNA repair-deficient *Bacillus subtilis* (Tokiwa *et al.*, 1987).

(iii) *Mutagenesis and cellular transformation in mammalian cells*

6-Nitrochrysene induced mutations in the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) forward mutation assay in Chinese hamster ovary cells in the presence of a liver homogenate from Aroclor 1254-pretreated rats (Delclos & Heflich, 1992). The mutation

frequency was higher in DNA repair-deficient Chinese hamster ovary cells than in repair-proficient cells. In contrast, exposure to 6-nitrochrysene and 6-aminochrysene did not result in a concentration-dependent increase in mutation frequency at the *HPRT* locus in human lymphoblastoid AHH-1 cells (Morris *et al.*, 1994). Minimal metabolic activation of 6-nitro- or 6-aminochrysene by AHH-1 cells may account for the lack of a positive mutagenic response.

6-Nitrosochrysene also induced mutations at the *Hprt* locus in Chinese hamster ovary cells, predominantly base-pair substitution at A:T base pairs (Manjanatha *et al.*, 1993). However, it mainly formed DNA adducts at deoxyguanosine (80%) while only 20% were probably formed through binding at deoxyadenosine, indicating that 6-nitrosochrysene-derived deoxyadenosine adducts are much more effective at producing mutations than their deoxyguanosine counterparts. In contrast, 1,2-DHD-6-AC-induced *Hprt* mutations in Chinese hamster ovary cells involved primarily G:C base-pair substitutions, which was consistent with the formation of a single major deoxyguanosine adduct formed by the microsome-catalysed reaction of 1,2-DHD-6-AC with DNA (Li *et al.*, 1993).

The mutational profiles of 6-nitrochrysene and its metabolites, *N*-hydroxy-6-aminochrysene, 6-aminochrysene, 1,2-DHD-6-NC, 1,2-DHD-6-AC and 1,2-DHD-6-NHOH-C, were compared in the *cII* gene of *lacI* rat mammary epithelial cells *in vitro* (Guttenplan *et al.*, 2007). 1,2-DHD-6-NHOH-C induced a mutational profile that was most similar to that of 6-nitrochrysene *in vivo* (Boyiri *et al.*, 2004). When the mutation profiles of (\pm)-1,2-DHD-6-NC were examined in these cells, the [*R,R*]-isomer was a more potent mutagen than the [*S,S*]-isomer (Sun *et al.*, 2009). The major types of mutation induced by the [*R,R*]-isomer were G:C to T:A, A:T to G:C and A:T to T:A mutations, which were similar to those obtained in the mammary gland of rats treated with 6-nitrochrysene *in vivo* (Boyiri *et al.*, 2004).

6-Nitrochrysene induced morphological transformation in cultured Syrian hamster embryo cells (DiPaolo *et al.*, 1983; Sala *et al.*, 1987), Syrian hamster kidney BHK-21/cl13 cells (Purchase *et al.*, 1978), rat tracheal epithelial cells (Mitchell & Thomassen, 1990; Gray *et al.*, 1994; West & Rowland, 1994) and human lung fibroblast WI38 or human liver cells (Purchase *et al.*, 1978) but not in murine BALB/c 3T3 or C3H 10T1/2 cells (Sala *et al.*, 1987; Sheu *et al.*, 1994).

(c) Other biological effects of 6-nitrochrysene

The effect of 6-nitrochrysene on the expression of p53 and p21^{Cip1} proteins and cell-cycle regulation was examined in MCF-7 and MCF-10A cells (Sun *et al.*, 2007). Although treatment with 6-nitrochrysene did not increase the level of total p53 protein in either cell line, p21^{Cip1} protein and a concomitant increase in cells in the G1 phase were observed in MCF-10A, but not MCF-7 cells. Treatment with 1,2-DHD-6-NHOH-C resulted in a significant induction of p53 protein in MCF-7 cells. The authors concluded that the lack of a p53 response to 6-nitrochrysene may imply a lack of the protective functions that are mediated by p53 (i.e. DNA repair mechanisms).

6-Nitrochrysene inhibited the growth of cultured rat but not human epidermal cells (Chun *et al.*, 2000).

4.3 Other relevant data

No data were available to the Working Group.

4.4 Mechanistic considerations

6-Nitrochrysene can be activated metabolically via ring oxidation, nitroreduction or a combination of both pathways to yield reactive electrophilic species that can react with DNA and result in the formation of DNA adducts (Boyiri *et al.*, 2002; El-Bayoumy *et al.*, 2004). 6-Nitrochrysene induces hepatic and pulmonary

CYP1A1 and can thereby enhance its own CYP-mediated bioactivation (Chen *et al.*, 1998). Several DNA adducts have been detected in rats and mice treated with 6-nitrochrysene (IPCS, 2003). Such DNA damage has been suggested to contribute to its potent mutagenic and carcinogenic activities, including the induction of rat mammary adenocarcinomas (Delclos *et al.*, 1987b; Li *et al.*, 1994; Chae *et al.*, 1996; Krzeminski *et al.*, 2000). These DNA lesions are liable to cause mutations if they are not removed by cellular defence mechanisms before DNA replication occurs. A recent study in human cell extracts demonstrated the inefficient nucleotide excision repair of two DNA adducts – *N*-(dG-8-yl)-6-AC and 5-(dG-*N*²-yl)-6-AC – derived from the nitroreduction pathway of 6-nitrochrysene (Krzeminski *et al.*, 2011). However, the efficiency of nucleotide excision repair of DNA adducts of 6-nitrochrysene derived from a combination of ring oxidation and nitroreduction pathways has not yet been determined.

Nitroreduction and ring oxidation leads to the formation of 1,2-DHD-6-NHOH-C. The mutation profile of 6-nitrochrysene in the rat mammary gland *in vivo* was compared with that of its known metabolites in the *cII* gene of *lacI* mammary epithelial cells *in vitro* and showed that the profile of 1,2-DHD-6-NHOH-C was the most similar (Guttenplan *et al.*, 2007). These results, in conjunction with the known structure of the major DNA adduct detected in the rat mammary gland (i.e. 5-(dG-*N*²-yl)-1,2-DHD-6-AC), support the hypothesis that 1,2-DHD-6-NHOH-C is the ultimate mutagen/carcinogen. The mutation profile and spectra of the proximate mutagens [*R,R*]- and [*S,S*]-1,2-DHD-6-NC were compared in the *cII* gene of *lacI* mammary epithelial cells *in vitro* and, although the [*R,R*]-enantiomer was a significantly more potent mutagen than the [*S,S*]-enantiomer, the mutation spectra were similar (Sun *et al.*, 2009). While the *in vitro* results suggest that the [*R,R*]-enantiomer is the proximate carcinogen in the rat mammary

gland, the comparative carcinogenic activity of both enantiomers in this organ *in vivo* has not yet been determined.

In contrast to numerous other DNA-damaging agents, such as benzo[*a*]pyrene, that are known to stimulate the expression of p53 protein, the lack of a p53 response to 6-nitrochrysene implies the lack of protective functions mediated by this protein (e.g. DNA repair mechanisms) after exposure to 6-nitrochrysene, which may, in part, account for the carcinogenicity of 6-nitrochrysene in rat mammary glands (Sun *et al.*, 2007).

5. Summary of Data Reported

5.1 Exposure data

6-Nitrochrysene was reported to be a constituent in the particulate phase of both diesel and gasoline engine exhaust emissions. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. No evidence was found of any source of exposure to 6-nitrochrysene other than traffic exhaust emissions. In ambient air, 6-nitrochrysene was found at concentrations in the subnanogram and low to subpicogram per cubic metre range in airborne particulate matter collected at urban, and rural and remote locations, respectively. Blood analysis confirmed exposure to 6-nitrochrysene of inhabitants in a rural area and several occupational groups.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Several studies have investigated tumour formation in rodents that were administered 6-nitrochrysene shortly after birth and then observed for up to 1 year after treatment. Eight studies of intraperitoneal injection were conducted in newborn mice and one in newborn rats.

In mice, increases in the incidence of malignant lymphoma in one study, and of benign or malignant tumours of the lung (adenoma or adenocarcinoma) and liver (hepatocellular adenoma or carcinoma) in both sexes were observed. The study in rats showed an increased incidence of colon adenocarcinoma in both sexes, but no lung or liver tumours. Thus, intraperitoneal injection caused a species-specific increase in the incidence of lung and liver tumours in mice and of colon tumours in rats. In two studies of oral administration and one of intramammary injection in rats, an increased incidence of mammary adenocarcinomas was observed. In addition, 6-nitrochrysene showed initiating activity in one skin tumour initiation-promotion study in mice.

5.4 Mechanistic and other relevant data

6-Nitrochrysene is metabolically activated via nitroreduction, ring-oxidation or a combination of both pathways, leading to the formation of DNA adducts. When administered orally or by intramammary injection to rats, 6-nitrochrysene induced mammary adenocarcinomas; its carcinogenic activity in the rat mammary gland exceeds that of benzo[*a*]pyrene – classified as a Group 1 human carcinogen by the IARC. Furthermore, 6-nitrochrysene was found to induce lung, liver and skin tumours in mice. Human hepatic and pulmonary microsomes and human mammary epithelial cells metabolized 6-nitrochrysene to reactive metabolites

that caused DNA damage. 6-Nitrochrysene induced the same DNA adducts in human mammary epithelial cells as those detected in the mammary gland – the target organ – of rats. Haemoglobin adducts derived from 6-nitrochrysene have been identified in humans exposed to diesel engine exhaust. The mutation spectrum induced by 6-nitrochrysene in mammary tissue in *lacZ*-transgenic mice was dominated by mutations at G and A residues and could be linked to the mutation profile of 1,2-dihydro-dihydroxy-6-hydroxyamino-chrysene, a metabolite that is formed by a combination of nitroreduction and ring-oxidation. The formation of its major adduct, 5-(deoxyguanosin-*N*²-yl)-1,2-dihydro-dihydroxy-6-amino-chrysene, in the rat mammary gland supports the hypothesis that 1,2-dihydro-dihydroxy-6-hydroxyamino-chrysene is the ultimate carcinogen. Mutations in the *H-Ras* and *K-Ras* oncogenes were observed in lung tumours from mice exposed to 6-nitrochrysene.

Overall, the Working Group considered that there is *strong mechanistic evidence* to support the carcinogenic properties of 6-nitrochrysene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 6-nitrochrysene.

6.3 Overall evaluation

6-Nitrochrysene is *probably carcinogenic to humans (Group 2A)*.

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