Environmental Health Criteria 229

SELECTED NITRO- AND NITRO-OXY-POLYCYCLIC AROMATIC HYDROCARBONS

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The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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ENVIRONMENTAL HEALTH CRITERIA FOR SELECTED NITRO- AND NITRO-OXY-POLYCYCLIC AROMATIC HYDROCARBONS

NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

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The WHO Environmental Health Criteria Programme is financially supported by the US Environmental Protection Agency, European Commission, German Federal Ministry of the Environment, Nature Conservation, and Nuclear Safety, and Japanese Ministry of Health, Labour and Welfare.

Environmental Health Criteria

Objectives

In 1973, the WHO Environmental Health Criteria Programme was initiated with the following objectives:

(i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;

(ii) to identify new or potential pollutants;

(iii) to identify gaps in knowledge concerning the health effects of pollutants;

(iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976, and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g. for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration, the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical

Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In this manner, with the strong support of the new partners, the importance of occupational health and environmental effects was fully recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effects on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for the evaluation. However, they do not describe *every* study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered, and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are used only when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and *in vitro* studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.

Content

The layout of EHC monographs for chemicals is outlined below.

- Summary — a review of the salient facts and the risk evaluation of the chemical
- Identity — physical and chemical properties, analytical methods
- Sources of exposure
- Environmental transport, distribution and transformation
- Environmental levels and human exposure
- Kinetics and metabolism in laboratory animals and humans
- Effects on laboratory mammals and *in vitro* test systems
- Effects on humans
- Effects on other organisms in the laboratory and field
- Evaluation of human health risks and effects on the environment
- Conclusions and recommendations for protection of human health and the environment
- Further research
- Previous evaluations by international bodies, e.g., IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the

substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for environment; international concern, i.e., the substance is of major interest to several countries; adequate data on the hazards are available.

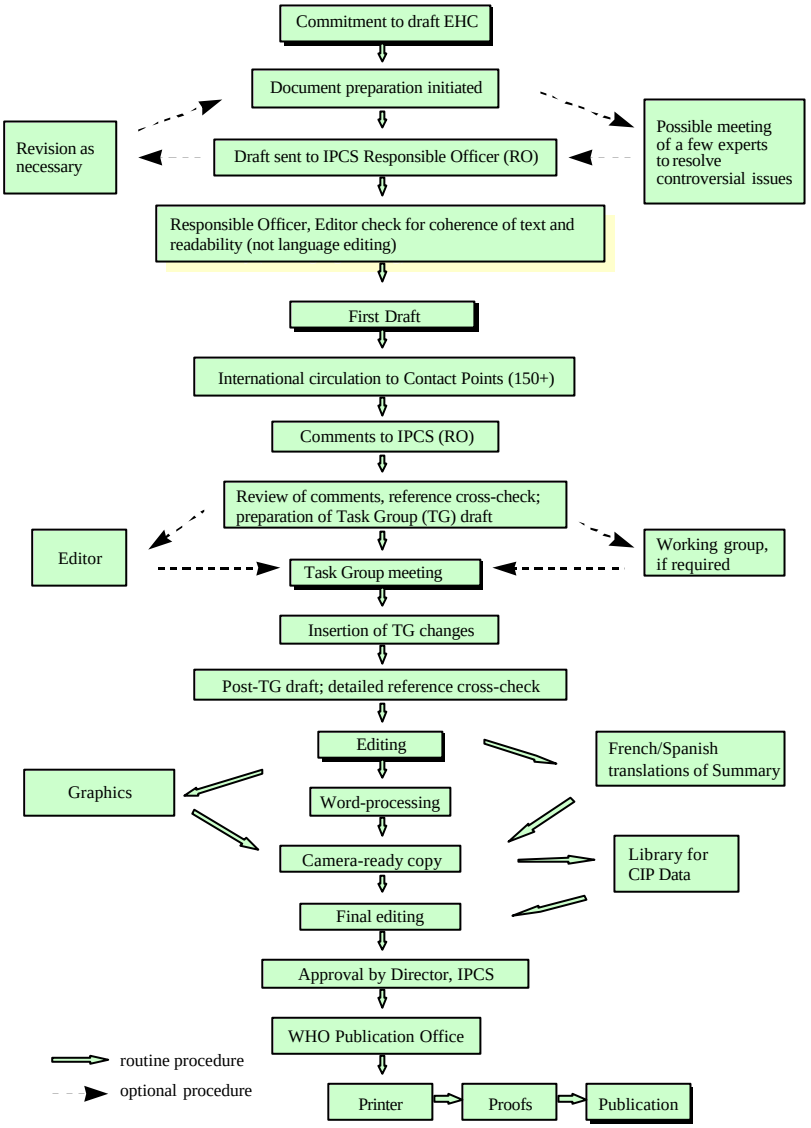
If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.

Procedures

The order of procedures that result in the publication of an EHC monograph is shown in the flow chart on p. xvii. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based on extensive literature searches from reference databases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

EHC PREPARATION FLOW CHART



The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. Although observers may provide a valuable contribution to the process, they can speak only at the invitation of the Chairperson. Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet *in camera*.

All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the document, it then goes for language editing, reference checking and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time, a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or

environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.

WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR SELECTED NITRO- AND NITRO-OXY-POLYCYCLIC AROMATIC HYDROCARBONS

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ENVIRONMENTAL HEALTH CRITERIA SELECTED NITRO- AND NITRO-OXY-POLYCYCLIC AROMATIC HYDROCARBONS

The first and second drafts of this monograph were prepared by the authors, Drs J. Kielhorn, U. Wahnschaffe and I. Mangelsdorf.

A WHO Task Group on Environmental Health Criteria for Selected Nitro- and Nitro-oxy-Polycyclic Aromatic Hydrocarbons met at the Fraunhofer Institute of Toxicology and Aerosol Research, in Hanover, Germany, on 26–30 November 2001. The group reviewed the draft and the peer review comments, revised the draft and made an evaluation of the risks for human health and environment from exposure to selected nitro- and nitro-oxy-polycyclic aromatic hydrocarbons.

Dr P. Jenkins and Mr T. Ehara of the IPCS central unit were responsible for the scientific aspects of the monograph, and Ms. Marla Sheffer was responsible for the technical editing.

The efforts of all, especially the Fraunhofer Institute of Toxicology and Aerosol Research, which helped in the preparation and finalization of the monograph, are gratefully acknowledged.

ACRONYMS AND ABBREVIATIONS

BaP	benzo[*a*]pyrene
bw	body weight
CAS	Chemical Abstracts Service
cDNA	complementary (or copy) DNA
CHO	Chinese hamster ovary
CYP	cytochrome P450
D2	no. 2 diesel fuel
dA	deoxyadenosine
dA-C8-2-AP	*N*-(deoxyadenosin-8-yl)-2-aminopyrene
DCM	dichloromethane
dG	deoxyguanosine
dG-C8-AAF	*N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene
dG-C8-AF	*N*-(deoxyguanosin-8-yl)-2-aminofluorene
dG-C8-1-amino-6-NP	*N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene
dG-C8-1-amino-8-NP	*N*-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene
dG-C8-AP	*N*-(deoxyguanosin-8-yl)-1-aminopyrene
dG-C8-2-AP	*N*-(deoxyguanosin-8-yl)-2-aminopyrene
dG-C8-4-AP	*N*-(deoxyguanosin-8-yl)-4-aminopyrene
dG-N^2-AAF	C3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene
dG-1-nitroBaP-DE	10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene
dG-3-nitroBaP-DE	10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydro-3-nitro-benzo[*a*]pyrene
6-dG-N^2-1-aminoBaP	6-(deoxyguanosin-N^2-yl)-1-aminobenzo[*a*]pyrene
6-dG-N^2-3-aminoBaP	6-(deoxyguanosin-N^2-yl)-3-aminobenzo[*a*]pyrene
dI	deoxyinosine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNP	dinitropyrene

EC	electron capture
EC_{50}	median effective concentration
ECD	electron capture detector
ED_{50}	median effective dose
EHC	Environmental Health Criteria monograph
EI	electron impact
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency (USA)
FAO	Food and Agriculture Organization of the United Nations
FTP	Federal Test Procedure (USA)
GC	gas chromatography
GPC	semipreparative gel permeation chromatography
GST	glutathione *S*-transferase
Hb	haemoglobin
HCFC-22	chlorodifluoromethane
HDD	heavy-duty diesel
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
ILO	International Labour Organization
i.m.	intramuscular
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_{oc}	organic carbon/water partition coefficient
K_{ow}	*n*-octanol/water partition coefficient
LC_{50}	median lethal concentration
LC_{100}	lethal concentration for 100% of test organisms
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level

LOEL	lowest-observed-effect level
LPG	liquefied petroleum gas
MA	metabolic activation
MN	micronucleus induction
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTD	maximum tolerated dose
MW	molecular weight (relative molecular mass)
NADH	nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide
NAT	*N*-acetyltransferase
NB	nitrobenzanthrone
nd	not detected
NICI	negative ion chemical ionization
NIST	National Institute of Standards and Technology (USA)
nitroPAH	nitro-polycyclic aromatic hydrocarbon
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
NPD	nitrogen–phosphorus detector
NP-LC	normal-phase high-performance liquid chromatography
NPR	NADPH-cytochrome P450 reductase
OCC	oxidation catalytic converter
OECD	Organisation for Economic Co-operation and Development
OH-AAF	*N*-acetyl-2-aminofluoren-x-ol
OH-2-NF	2-nitrofluorenol
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PEF	potency equivalency factor
PICI	positive ion chemical ionization
$PM_{2.5}$	particulate matter =2.5 μm in diameter
PM_{10}	particulate matter =10 μm in diameter
p.o.	per os
ppb	part per billion

ppm	part per million
ppt	part per trillion
PUF	polyurethane foam
RO	Responsible Officer
RP-LC	reversed-phase high-performance liquid chromatography
s.c.	subcutaneous
SCE	sister chromatid exchange
SEOM	solvent extractable organic matter
SOF	soluble organic fraction
SPE	solid-phase extraction
SRM	Standard Reference Material
TA98AT+	*N*-hydroxyarylamine *O*-acetyltransferase-overproducing *S. typhimurium* strain
TA98NR+	nitroreductase-overproducing *S. typhimurium* strain
TA98AT–	*N*-hydroxyarylamine *O*-acetyltransferase-deficient *S. typhimurium* strain
TA98NR–	nitroreductase-deficient *S. typhimurium* strain
TEA	thermal energy analyser
TID	thermionic detector
TLC	thin-layer chromatography
UDS	unscheduled DNA synthesis
UN	United Nations
UNEP	United Nations Environment Programme
UV	ultraviolet
WHO	World Health Organization
XOC	extractable organic component

1. SUMMARY

1.1 Identity, physical and chemical properties, and analytical methods

Nitro-polycyclic aromatic hydrocarbons (nitroPAHs) are derivatives of polycyclic aromatic hydrocarbons (PAHs), which contain two or more fused aromatic rings made of carbon and hydrogen atoms. NitroPAHs occur in the environment as a mixture together with parent PAHs and hundreds of other organic compounds. NitroPAHs are usually present in much smaller quantities than PAHs.

NitroPAHs in the environment occur either in the vapour phase or adsorbed to particulate matter. NitroPAHs are insoluble or sparingly soluble in water but mostly soluble in organic solvents.

The sampling of nitroPAHs is similar to that of PAHs. Ambient air is sampled by collecting particulate matter on special filters by means of high-volume samplers. Vapour-phase nitroPAHs are commonly collected on solid sorbents such as polyurethane foam.

Solvent extraction is followed by cleanup using liquid chromatography with silica gel or alumina, high-performance liquid chromatography (HPLC) or solid-phase extraction. The nitroPAH fraction must be separated from the PAH fraction and oxygenated PAH fraction by HPLC on silica. Methods used for the separation and detection of nitroPAHs include gas chromatography with a variety of detectors, HPLC with fluorescence, chemiluminescence or electrochemical detector, and mass spectrometric techniques. Analysis is dependent on the standards available.

Another approach to analysis of complex mixtures is bioassay-directed chemical analysis, where mutagenically active fractions are bioassayed and characterized until the major class or specific compounds potentially responsible for the mutagenicity are identified. The use of bacterial tester strains selectively sensitive to nitroarenes has

led to the identification of nitroPAHs as potent mutagens in complex mixtures from diverse sources. Synthetic standards are required for this type of analysis.

The nitroketone 3-nitrobenzanthrone and nitrolactones, such as 2- and 4-nitrodibenzopyranone, are nitro-oxy compounds, which have been detected together with nitroPAHs and are analysed by similar methods.

1.2 Sources of human and environmental exposure

NitroPAHs originate primarily as direct or indirect products of incomplete combustion. Only a few nitroPAHs are produced industrially; commercially produced nitronaphthalenes and 5-nitroacenaphthene, for example, are used primarily as chemical intermediates.

NitroPAHs originate from PAHs (generally adsorbed on particulate matter and themselves products of incomplete combustion) by at least two distinct processes: (1) through nitration during combustion processes (e.g., in vehicle exhaust, particularly diesel, but also gasoline and aircraft emissions; industrial emissions; domestic residential heating/cooking; wood burning) and (2) through atmospheric formation from PAHs by either gas-phase reactions — daytime hydroxyl radical addition to the PAH followed by reaction with nitrogen dioxide and loss of a water molecule and nighttime nitrate radical addition to the PAH followed by reaction with nitrogen dioxide and loss of nitric acid — or heterogeneous gas–particle interaction of parent PAHs adsorbed onto particles with nitrating agents.

The distribution of nitroPAH isomers in samples of ambient air has been found to be significantly different from that in direct emissions from combustion. 2-Nitrofluoranthene and 2-nitropyrene are ubiquitous components of particulate matter, although they are not directly emitted from most combustion sources. The nitroPAH profile, or the relative quantities of certain “marker” PAHs, is a pointer to the source of formation of the nitroPAH. The most abundant nitro isomers of pyrene, fluorene and fluoranthene observed in diesel exhaust are 1-nitropyrene, 2-nitrofluorene and 3-nitrofluoranthene, whereas the isomers formed

from the hydroxyl radical reactions of these PAHs are 2-nitropyrene, 3-nitrofluorene and 2-nitrofluoranthene.

The majority of ambient nitroPAHs are now thought to be formed in the atmosphere from the gas-phase reactions of PAHs with four rings or less.

Many mono- and some di- and trinitroPAH isomers have been identified and quantified in various samples of diesel exhaust, 1-nitropyrene usually being the most abundant. 1-Nitropyrene is the "marker" nitroPAH for diesel exhaust, and its presence in ambient air samples is a sign of pollution by diesel vehicle traffic. Diesel fuel, engine types and catalytic traps are continually being modified, so the various studies of nitroPAHs in diesel exhaust cannot be directly compared. In general, the mass emission of particles, emissions of particle-bound PAHs and nitroPAHs, and mutagenic activity levels generally decreased with the use of either particulate traps or catalytic converters.

The concentration of 1-nitropyrene was much less in gasoline exhaust particles than in diesel exhaust particles, but the concentrations of 1,3-, 1,6- and 1,8-dinitropyrenes were found to be almost the same in gasoline and diesel exhaust particles.

There is evidence of the presence of nitroPAHs in jet aeroplane exhaust.

NitroPAHs have been detected in the emissions of kerosene heaters, fuel gas and liquefied petroleum gas (LPG) burners, which are used in many countries for heating and cooking at home.

3-Nitrobenzanthrone has been detected in diesel exhaust particulate and in urban air samples. 2-Nitrodibenzopyranone and 4-nitrodibenzopyranone as well as nitropyrene lactones have been observed in ambient particulate matter.

1.3 Environmental transport, distribution and transformation

1.3.1 Environmental transport and distribution

NitroPAHs can be transported in the vapour phase or adsorbed onto particulate matter. Those with liquid-phase vapour pressures greater than 10^{-4} Pa at ambient air temperature (i.e., two- to four-ring PAHs and two-ring nitroPAHs) will exist at least partially in the gas phase.

Owing to their low aqueous solubility or insolubility, nitroPAHs are not expected to be transported in water. Data available give high values for sorption coefficients (log K_{oc}), suggesting that nitroPAHs, similar to PAHs, adsorb onto soil and sediments. Leaching into groundwater is thought to be negligible. Some nitroPAHs may be slowly biodegradable under certain conditions.

The values for the *n*-octanol/water partition coefficient (log K_{ow}) range from 2.5 for 1-nitronaphthalene to 6.3 for 3-nitroperylene, suggesting a potential for bioaccumulation. There were no data available on biomagnification.

1.3.2 Biotransformation

Many anaerobic and aerobic bacteria reduce nitroPAHs to mutagenic aminoPAHs. Nitroreduction by intestinal microflora plays a major role in the metabolism of nitroPAHs in mammals. Although a wide variety of bacteria, fungi and algae have been shown to degrade the parent PAHs containing two to five rings, nitro-substituted PAHs are only slowly degraded by indigenous microorganisms and may persist in soils and sediments. The recalcitrance of high molecular weight nitroPAHs is due in part to the strong adsorption to soil organic matter, low solubility, large molecular size and the polar character of the nitro group.

Time course studies in microcosms showed that 1-nitropyrene was degraded slowly under aerobic and anaerobic conditions in estuarine sediments.

Sphingomonas paucimobilis strain EPA 505 (a soil bacterium capable of utilizing fluoranthene as the sole source of carbon and energy) biodegraded 1-nitropyrene to 48.6% after 6 h.

The filamentous fungus *Cunninghamella elegans* has been shown to oxidatively metabolize, via a cytochrome P450 monooxygenase, a number of nitroPAHs (1-nitropyrene, 2-nitrofluorene, 2- and 3-nitrofluoranthene, 6-nitrochrysene, 1-nitrobenzo[*e*]pyrene and 6-nitrobenzo[*a*]pyrene) to products that are less mutagenic than the nitroPAHs themselves.

A plant cell culture derived from alligator weed (*Alternanthera philoxeroides*) detoxified 1-nitropyrene and 1,3-, 1,6- and 1,8-dinitropyrene, all direct-acting mutagens, when incubated with them, as shown by mutagenicity response in the *Salmonella typhimurium* TA98 assay.

1.3.3 Abiotic degradation

The photolysis of nitroPAHs has been studied under varied conditions of irradiation. The rate of photolysis depends not only on the conditions of irradiation but also on whether the nitroPAH is in the gaseous phase (e.g., 1-and 2-nitronaphthalene), in solution (type of solvent) or bound to solids/particles. In the latter case, the type and age of the particle seem to influence the photochemistry of the respective nitroPAH. The rate of photodecomposition, identification of photolytic products and resulting loss or gain of metabolic activity as determined by the *S. typhimurium* assay have been the main end-points studied.

Calculated atmospheric lifetimes of nitroPAHs due to photolysis and gas-phase reactions with hydroxyl and nitrate radicals and with ozone under atmospheric conditions show that the dominant loss process for nitroPAHs (e.g., 1- and 2-nitronaphthalene) is photolysis.

Particle oxidation of nitroPAHs by ozone may be the main loss process at night.

1.4 Environmental levels and human exposure

NitroPAHs that have been detected in ambient air include 1- and 2-nitronaphthalene and methylnitronaphthalenes (predominantly in the vapour phase), 2-nitrofluorene, 9-nitroanthracene, 9-nitrophenanthrene, 2-, 3- and 8-nitrofluoranthene, 1- and 2-nitropyrene, 1,3-, 1,6- and 1,8-dinitropyrene and 6-nitrochrysene.

At remote and forest sites, nitroPAHs were either not detected or detected in the low picogram per cubic metre range (e.g., 17 pg/m^3 for 2-nitrofluoranthene; 4 pg/m^3 for 1-nitropyrene). The concentration of nitroPAHs in the atmosphere of urban regions depends on the season, the type of heating used and the number and regulation of traffic vehicles. Reported levels in air do not usually exceed 1 ng/m^3, although maxima of up to 13 ng/m^3 have been reported.

Various studies have been performed monitoring certain isomeric nitroPAHs. Investigators have concentrated on the nitroPAHs that seem to be of quantitative/environmental (e.g., nitroPAHs of relative molecular mass 247: 1-nitropyrene, 2-nitropyrene, 2-nitrofluoranthene) or carcinogenic (e.g., 1-nitropyrene, 2-nitrofluorene, dinitropyrenes) importance.

Studies of daytime/nighttime concentrations of specific isomeric nitroPAHs in certain regions (in particular California, USA) and parallel environmental chamber studies have led to an understanding of the atmospheric formation of certain nitroPAHs (2-nitrofluoranthene and 2-nitropyrene). Concurrent studies of certain nitroPAHs (1-nitropyrene, dinitropyrenes) and traffic volume have confirmed that traffic emission is a source of nitroPAHs.

Most seasonal studies show higher winter/spring concentrations of marker nitroPAHs, which parallels the use of domestic heating, although this is not always the case.

1.4.1 Indoor air

As nitroPAHs have been detected in the emissions of kerosene heaters, fuel gas and LPG burners used for heating and cooking at home, as well as in the fumes of cooking oils, there is therefore a potential indoor exposure to nitroPAHs in poorly ventilated conditions.

Concentrations of polyaromatic compounds, including nitroPAHs, were measured in a study of indoor and outdoor air levels associated with 33 homes located in two US cities: Columbus, Ohio, and Azusa, California. The overall levels were much higher in homes occupied by smokers, but the use of natural gas heating and cooking appliances also appeared to increase the nitroPAH levels slightly.

1-Nitropyrene (4.2–25 600 ng/litre) was detected in 36 of 55 samples of wastewater from oil–water separating tanks of gasoline stations and in used crankcase oil.

1- and 2-nitronaphthalene and 1,3- and 1,5-dinitronaphthalene were detected in river water in Japan at concentrations of 1.3, 11.7, 1.7 and 3.2 ng/litre, respectively. In another water sample, 1-nitropyrene was identified.

There are only limited data on the presence of nitroPAHs in samples of soil, sewage sludge, sediment and incinerator ash (e.g., for 1-nitropyrene, 0.03–0.8 µg/kg dry weight in soil, 0.68 µg/kg in sewage sludge, 25.2 µg/kg in sediment and <0.01–0.89 mg/kg in incinerator ash).

1.4.2 Food and beverages

With the exception of spices, smoked and grilled foods and peanuts, the concentrations of nitroPAHs in foods are below 5 µg/kg.

In a study in the United Kingdom, foodstuffs were monitored for the presence of 9-nitroanthracene and 1-nitropyrene. Twenty-five out of 28 foods contained no detectable levels of these nitroPAHs. 9-Nitroanthracene was tentatively identified in peated malt, at 0.9 µg/kg, and 1-nitropyrene in two samples of tea leaves, at 1.7 and 0.17 µg/kg.

Another survey of nitroPAH levels in various foods in Austria showed mostly detectable levels of 2-nitrofluorene, 1-nitropyrene and 2-nitronaphthalene. The highest concentrations were found in spices, smoked foods and teas, in particular Mate tea, which is roasted. Nitro-PAHs were also detected in vegetables and fruits, probably due to atmospheric pollution.

1-Nitropyrene was detected in grilled corn, mackerel and (in considerable amounts) pork and yakitori (grilled chicken) grilled with sauce (up to 43 ng/g).

1.4.3 Other products

In 1980, studies showed that extracts of selected xerographic toners and paper photocopies were mutagenic. The fraction of the carbon black B responsible for 80% of the mutagenicity contained 1-nitropyrene, 1,3-, 1,6- and 1,8-dinitropyrene, 1,3,6-trinitropyrene and 1,3,6,8-tetranitropyrene. As a result of this finding, the manufacturers modified the production of carbon black B, substantially reducing the levels of nitropyrenes.

1.4.4 Occupational exposure

Occupational exposure to nitroPAHs has been demonstrated in workplaces associated with the use of diesel engines. For example, concentrations of 1-nitropyrene in air were measured in various work-places associated with the use of diesel engines. The highest levels (42 ng/m^3) reported were determined in the breathing zones of the underground workers (drivers of diesel-powered excavators) at an oil shale mine in Estonia.

1.5 Kinetics and metabolism in laboratory animals and humans

1-Nitropyrene and 2-nitrofluorene administered by various routes are rapidly absorbed, and the resulting metabolites are conjugated and excreted. Radiolabelled 1-nitropyrene was found to be widely distributed in the body of rats and mice following administration by all routes. Other nitroPAHs have not been as well studied.

The metabolism of nitroPAHs is complex. It seems that there are at least five metabolic activation pathways through which mutations can be induced by nitroPAHs in bacterial and mammalian systems and/or through which DNA binding occurs. These are 1) nitroreduction; 2) nitroreduction followed by esterification (in particular acetylation); 3) ring oxidation; 4) ring oxidation and nitroreduction; and 5) ring oxidation and nitroreduction followed by esterification. In bacteria, nitroreduction seems to be the major metabolic pathway, whereas the fungus *Cunninghamella elegans* is an example of a species in which nitroPAHs are metabolized by ring oxidation.

Nitroreduction of nitroPAHs *in vivo* probably occurs mainly by bacteria in the intestinal tract. In oxidative metabolism, the first step is transformation to phase I primary metabolites such as epoxides, phenols and dihydrodiols, and then to secondary metabolites, such as diol epoxides, tetrahydrotetrols and phenol epoxides. In mammalian systems, the phase I metabolites are then conjugated with glutathione, sulfate or glucuronic acid to form phase II metabolites, which are more polar and water-soluble than the parent hydrocarbons. On reaching the intestine, the conjugated metabolites can be deconjugated by the intestinal microflora and absorbed, entering enterohepatic circulation. Nitroreduction and *N*-acetylation can occur, resulting in the excretion in urine and faeces of metabolites such as acetylaminopyrenols after 1-nitropyrene administration.

Different cytochrome P450 enzymes may be involved in the metabolism of a specific nitroPAH, and these may differ in the related isomers, resulting in possibly different kinetics and pathways. Cytochrome P450 enzymes responsible for the metabolism of nitroPAHs may

vary between species and in different target organs and in different cell types within target organs.

All nitroPAHs do not follow the same activation pathways. Some are mutagenic when reduced to an arylhydroxylamine (e.g., 1-nitropyrene is metabolized mainly by hydroxylation of the aromatic moiety, followed by nitroreduction and *N*-acetylation); others (e.g., 1,8- and 1,6-dinitropyrene) are reduced to the arylhydroxylamine and then require further *O*-esterification (in particular *O*-acetylation) to an acyloxy ester for mutagenicity. Some may be mutagenic only after activation by oxidation to reactive epoxides or dihydrodiol epoxides (as possibly in 6-nitrobenzo[*a*]pyrene, similar to benzo[*a*]pyrene, or BaP). The main DNA adducts detected with nitroPAHs *in vivo* and *in vitro* are C8-substituted deoxyguanosine adducts; however, N^2-substituted deoxyguanosine and C8-substituted deoxyadenosine derivatives have also been detected and may predominate in nitroPAHs with greater hydrocarbon character (e.g., 3-nitrobenzo[*a*]pyrene and 6-nitrochrysene). DNA adducts of dinitropyrenes are formed only via nitroreduction, presumably owing to the high electron deficiency in the aromatic rings caused by the presence of two nitro groups. The DNA adducts resulting from the nitroreduction of nitroPAHs are better characterized than those arising from oxidative metabolism, although the latter may be of more importance in mammalian metabolism.

1.6 Effects on laboratory mammals and *in vitro* test systems

Only six nitroPAHs have been tested for acute toxicity. In rats, an LD_{50} of 86 mg/kg of body weight (kg bw) after intraperitoneal (i.p.) application was reported for 1-nitronaphthalene; in mice, an oral LD_{50} of 1300 mg/kg bw was reported for 2-nitronaphthalene. In further studies on both substances, systemic effects on the target organs lung and liver were observed after single high doses; however, 2-nitronaphthalene seemed to be less toxic than 1-nitronaphthalene. 5-Nitroacenaphthene at an i.p. dose of 1700 mg/kg bw was lethal to all treated rats. For 2-nitrofluorene, an oral LD_{50} of 1600 mg/kg bw in mice was reported, whereas gavaging with up to 5000 mg 1-nitropyrene/kg bw resulted in no observable toxic effects. Local inflammation and

ulceration were seen in rats after subcutaneous (s.c.) injection of 8 mg 3-nitrofluoranthene/kg bw.

Data on systemic or local non-neoplastic effects caused by short-term or long-term treatment with nitroPAHs are limited, as the end-point of most studies has been carcinogenicity. In most cases, non-neoplastic toxic effects were observed at doses at which carcinogenic responses are also manifested. Systemic non-neoplastic toxic effects, such as reduced body weight or increased mortality, appeared presumably independently of carcinogenic effects in feeding studies with 5-nitroacenaphthene at a dose level of 500 mg/kg bw per day (rat) or 40 mg/kg bw per day (mice) and with 2-nitrofluorene at a dose of 25 mg/kg bw per day (rat). Medium-term exposure via inhalation to 1-nitropyrene resulted in metaplasia of the upper respiratory tract at concentrations of ≥0.5 mg/m^3.

No data are available on skin and eye irritation, sensitization or reproductive toxicity.

Data on genotoxicity *in vitro* are available on 95 nitroPAHs; for 74 nitroPAHs, however, only one or two end-points, mainly in bacterial test systems, were investigated. A sufficient database, including eukaryotic test systems, has been found only with 21 nitroPAHs. Most of these substances (67 out of 95) showed positive results, but the results were derived from a small database. Clearly positive results were obtained for 19 nitroPAHs, and questionable results for 8 nitroPAHs. With none of the nitroPAHs were clearly negative results obtained.

For 86 nitroPAHs, data on the *S. typhimurium* microsome test are available. In contrast to the parent PAHs, most nitroPAHs were clearly more effective in the *Salmonella* microsome test without metabolic activation. There are five nitroPAHs that showed exceptionally high mutagenic potency (≥100 000 revertants/nmol) in this test system: 3,7- and 3,9-dinitrofluoranthene, 1,6- and 1,8-dinitropyrene, and 3,6-dinitrobenzo[*a*]pyrene.

Bacterial nitroreductase and acetyltransferase are involved in the metabolic activation of nitroPAHs, but not all nitroPAHs follow the same metabolic activation pathways. Furthermore, there is no uniform mutagenic effect of the different nitroPAHs, as they produce both frameshift and base pair substitutions in the *S. typhimurium* microsome test. There is evidence that nitroPAHs with nitro groups perpendicular to the aromatic ring are not as mutagenic as isomers having parallel nitro orientation.

Data on the *in vivo* genotoxicity of nitroPAHs are available for 15 nitroPAHs. All nitroPAHs that gave positive results *in vivo* were also positive *in vitro*. Four nitroPAHs that were positive in *in vitro* genotoxicity tests revealed inconsistent or inconclusive genotoxicity (2-nitronaphthalene, 5-nitroacenaphthene and 3-nitrofluoranthene) or negative genotoxicity (2,7-dinitrofluorene; limited validity) results *in vivo*.

3-Nitrobenzanthrone, like 1,6- and 1,8-dinitropyrene, is highly mutagenic in bacteria through nitroreduction and *O*-esterification. 3-Nitrobenzanthrone is also an effective gene mutagen and causes micronuclei formation in human cells *in vitro* and in mice *in vivo*.

2-Nitrodibenzopyranone was reported to be highly mutagenic in the *S. typhimurium* microsome test in strain TA98 (–S9), being more mutagenic than 2-nitrofluorene and 1-nitropyrene. 1- and 3-nitro pyrene lactones have been found to be highly mutagenic in the *S. typhimurium* microsome test.

Studies on the *in vitro* genotoxicity of 2-nitrodibenzopyranone in forward mutation assays using two human B-lymphoblastoid cell lines are conflicting. Nitropyrene lactones were found to induce mutations at the *tk* and *hprt* loci in both cell lines. Further, they induced kinetochore-positive and -negative micronuclei in the CREST modified micronucleus assay, which detects chromosomal loss and breakage events.

Data on carcinogenic effects are available for 28 nitroPAHs. Although inhalation is the main exposure route in humans, no long-

term inhalation study on any nitroPAH is available. Most studies examined the carcinogenic effects of nitroPAHs by oral administration, topical application, pulmonary implantation or intratracheal administration.

Owing to the limitations in experimental design, none of the negative studies confirmed the absence of carcinogenic effects in animals. However, results showed carcinogenic effects in experimental animals for 5-nitroacenaphthene, 2-nitrofluorene, 3-nitrofluoranthene, 3,7- and 3,9-dinitrofluoranthene, 1- and 4-nitropyrene, 1,3-, 1,6- and 1,8-dinitropyrene and 6-nitrochrysene. Some carcinogenic effects in experimental animals were observed for 2-nitropyrene, 7-nitrobenz[*a*]-anthracene, 2- and 6-nitrobenzo[*a*]pyrene, 3,6-dinitrobenzo[*a*]pyrene, 7-nitrodibenz[*a*,*h*]anthracene and 3-nitroperylene. For the remaining 10 nitroPAHs tested, not enough data were available with which to evaluate their carcinogenicity in experimental animals.

Besides local effects at the site of injection, nitroPAHs induced mainly systemic tumours in mammary tissue, lung, liver and the haematopoietic system. 6-Nitrochrysene appears to be the most carcinogenic of the nitroPAHs considered here. With systemic effects after s.c. or i.p. injection, 1-nitropyrene was more carcinogenic than the dinitropyrenes. The carcinogenicity of 1-nitropyrene and dinitropyrenes varies, depending on the route of administration.

Nitrated benzo[*a*]pyrenes are generally less potent carcinogens than the parent compound BaP. However, the mono- or dinitrated pyrenes are more carcinogenic than pyrene. Similar results were presented for 3-nitroperylene compared with perylene and for 6-nitrochrysene compared with chrysene; with local effects after dermal exposure, however, 6-nitrochrysene was less active than chrysene.

Data were available on carcinogenic effects of some metabolites of 2-nitrofluorene, 1-nitropyrene and 6-nitrochrysene. Comparing 2-nitrofluorene with its metabolites in rats, the highest carcinogenic potency was shown by 2-acetylaminofluorene. 1-Nitropyrene was significantly more carcinogenic after oral application in rats than either 1-nitrosopyrene or 1-aminopyrene. In contrast, 1-nitrosopyrene

induced a higher incidence of liver tumours in mice after i.p. application than 1-nitropyrene; no effects were observed with ring hydroxylated metabolites. 6-Nitrosochrysene and 6-aminochrysene were inactive, in contrast to the ring hydroxylated metabolites, which showed carcinogenic activity in the liver similar to that of the parent compound 6-nitrochrysene; this indicates that the metabolic activation of 6-nitrochrysene occurs by ring oxidation and/or a combination of ring oxidation and nitroreduction.

1.7 Effects on humans

There are no reports on the effects of individual nitroPAHs on humans. As would be expected, since nitroPAHs occur in complex mixtures in the atmosphere and exhaust, the exact contribution of nitroPAHs to the adverse health consequences of exposure to polluted atmospheres and to exhaust cannot be elucidated.

At present, investigations on the effects of nitroPAHs on human health are being carried out using biomarkers of exposure. Several reports have described the development of methods for and provided data on the evaluation of 1-nitropyrene as a biomarker for occupational exposure to diesel exhaust. Urinary metabolites of PAHs and nitroPAHs were determined in the urine of diesel mechanics using the enzyme-linked immunosorbent assay (ELISA). In another study, metabolites of 1-nitropyrene (namely, *N*-acetyl-1-aminopyren-6-ol and *N*-acetyl-1-aminopyren-8-ol) were measured in the urine of workers in a shipping department. Several studies have focused on measuring the haemoglobin and plasma adducts of metabolites of 1-nitropyrene and other nitroPAHs and may provide appropriate biomarkers in future molecular epidemiological investigations.

1.8 Effects on other organisms in the laboratory and field

Data on the acute toxicity of nitroPAHs to aquatic organisms are available only for 1-nitronaphthalene. An LC_{50} (96 h) of 9.0 mg/litre was reported for the fathead minnow (*Pimephales promelas*). Furthermore,

this nitroPAH inhibited the growth of the ciliate *Tetrahymena pyriformis*, with an EC_{50} (60 h) of 17.3 mg/litre.

Some studies have been concerned with the effect of nitroPAHs on the metabolism of some aquatic species — for example, the subcellular and tissue distribution of two- and one-electron NAD(P)H-dependent nitroreductase activity in marine invertebrates from three phyla: mussel (*Mytilus edulis*), crab (*Carcinus maenas*) and starfish (*Asteria rubens*). NADPH-dependent two-electron nitroreductase activity, occurring only under anaerobic conditions, was detected in the microsomal and cytosolic fractions of the major digestive tissues of mussel (digestive gland) and crab, but not in the gills of either species. 1-Aminopyrene was the only metabolite identified. No activity was detectable in the pyloric caeca or stomach region of the starfish. NAD(P)H-dependent one-electron nitroreduction was present in all subcellular fractions of the major digestive tissues of the three species.

In the presence of calf thymus DNA, adducts derived from 1-nitropyrene were detected *in vitro* using hepatic S9 fractions prepared from fish. The ability of 1-nitropyrene to form DNA adducts was also established *in vivo* using brown trout (*Salmo trutta*) and turbot (*Scophthalmus maximus*). These DNA adducts were comparable to those obtained in Wistar rats treated with 1-nitropyrene.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, AND ANALYTICAL METHODS

2.1 Identity

Nitro-polycyclic aromatic hydrocarbons (nitroPAHs) are derivatives of polycyclic aromatic hydrocarbons (PAHs), which contain two or more fused aromatic rings made of carbon and hydrogen atoms, formed as a result of incomplete combustion (see IPCS, 1998). NitroPAHs occur in the environment as a mixture together with parent PAHs and hundreds of other organic compounds (see chapter 3). NitroPAHs are usually present in smaller quantities (by 2 orders of magnitude) than PAHs.

Interest was focused on nitroPAHs in the early 1980s as correlations were found between the presence of nitroPAHs in diesel exhaust and environmental extracts and mutagenic activity. A large number of groups of nitro, oxy and mixed nitro-oxy compounds eluted together in the mutagenic fractions. Analytical methods were developed to separate and identify these compounds and to specify their isometric composition, as the biological action of these compounds also depends on their stereospecificity (see chapters 6 and 7). As it would be impossible to evaluate all these compounds in one document, a decision was made to include mono- and dinitroPAHs (2–5 rings) but, in general, not methylated or hydroxylated nitroPAHs. Some nitro-oxyPAHs are also included: nitroketones (3-nitrobenzanthrone) and selected nitrolactones (e.g., the nitrophenanthrene lactones: 2- and 4-nitrodibenzopyranone [2- and 4-nitro-6H-dibenzo[*b*,*d*]pyran-6-one] and nitropyrene lactones), which have recently been shown to be present in the extracts of the polar fractions of diesel exhaust and airborne particulates.

The nomenclature, molecular formula, relative molecular mass and Chemical Abstracts Service (CAS) number of selected nitroPAHs and nitro-oxyPAHs are given in Table 1. The structural formulas of some selected nitroPAHs and nitro-oxyPAHs are shown in Figure 1.

Table 1. Nomenclature, molecular formulas, relative molecular mass and CAS numbers of selected nitroPAHs and their oxygen-containing derivatives

Parent PAHs	Nitro derivative	Molecular formula	Relative molecular mass	CAS number
Two-ring PAHs				
Naphthalene	1-Nitronaphthalene	$C_{10}H_7NO_2$	173.17	86-57-7
	2-Nitronaphthalene	"	"	581-89-5
	1,3-Dinitronaphthalene	$C_{10}H_6N_2O_4$	218.17	606-37-1
	1,5-Dinitronaphthalene	"	"	605-71-0
	1,8-Dinitronaphthalene	"	"	602-38-0
	2,7-Dinitronaphthalene	"	"	24824-27-9
	2,3,5-Trinitronaphthalene	$C_{10}H_5N_3O_6$	263.17	87185-24-8
	1,3,6,8-Tetranitronaphthalene	$C_{10}H_4N_4O_8$	308.16	28995-89-3
Three-ring PAHs				
Acenaphthene	3-Nitroacenaphthene	$C_{12}H_9NO_2$	199.21	3807-77-0
	5-Nitroacenaphthene	"	"	602-87-9
Fluorene	1-Nitrofluorene	$C_{13}H_9NO_2$	211.22	22250-99-3
	2-Nitrofluorene	"	"	607-57-8
	3-Nitrofluorene	"	"	5397-37-5
	4-Nitrofluorene	"	"	24237-68-1
	2,7-Dinitrofluorene	$C_{13}H_8N_2O_4$	256.22	5405-53-8
Anthracene	2-Nitroanthracene	$C_{14}H_9NO_2$	223.23	3586-69-4
	9-Nitroanthracene	"	"	602-60-8

Table 1 (Contd).

Parent PAHs	Nitro derivative	Molecular formula	Relative molecular mass	CAS number
Anthracene (contd)	9,10-Dinitroanthracene	$C_{14}H_8N_2O_4$	268.23	33685-60-8
Phenanthrene	2-Nitrophenanthrene	$C_{14}H_9NO_2$	223.23	17024-18-9
	9-Nitrophenanthrene	"	"	954-46-1
	2,6-Dinitrophenanthrene	$C_{14}H_8N_2O_4$	268.23	
Four-ring PAHs				
Fluoranthene	1-Nitrofluoranthene	$C_{16}H_9NO_2$	247.25	13177-28-1
	2-Nitrofluoranthene	"	"	13177-29-2
	3-Nitrofluoranthene	"	"	892-21-7
	7-Nitrofluoranthene	"	"	13177-31-6
	8-Nitrofluoranthene	"	"	13177-32-7
	1,2-Dinitrofluoranthene	$C_{16}H_8N_2O_4$	292.25	33611-88-0
	2,3-Dinitrofluoranthene	"	"	105735-66-8
	2,4-Dinitrofluoranthene	"	"	102493-19-6
	2,5-Dinitrofluoranthene	"	"	102493-21-0
	3,4-Dinitrofluoranthene	"	"	
	3,7-Dinitrofluoranthene	"	"	105735-71-5
	3,9-Dinitrofluoranthene	"	"	22506-53-2
	1,2,4-Trinitrofluoranthene	$C_{16}H_7N_3O_6$	337.25	102493-20-9
	1,2,5-Trinitrofluoranthene	"	"	102493-22-1
	2,3,5-Trinitrofluoranthene	"	"	116331-54-5

Table 1 (Contd).

Parent PAHs	Nitro derivative	Molecular formula	Relative molecular mass	CAS number
Pyrene	1-Nitropyrene	$C_{16}H_9NO_2$	247.25	5522-43-0
	2-Nitropyrene	"	"	789-07-1
	4-Nitropyrene	"	"	57835-92-4
	1,3-Dinitropyrene	$C_{16}H_8N_2O_4$	292.25	75321-20-9
	1,6-Dinitropyrene	"	"	42397-64-8
	1,8-Dinitropyrene	"	"	42397-65-9
	1,3,6-Trinitropyrene	$C_{16}H_7N_3O_6$	337.25	75321-19-6
	1,3,6,8-Tetranitropyrene	$C_{16}H_6N_4O_8$	382.24	28767-61-5
Benz[*a*]anthracene	7-Nitrobenz[*a*]anthracene	$C_{18}H_{11}NO_2$	273.29	20268-51-3
Chrysene	2-Nitrochrysene	$C_{18}H_{11}NO_2$	273.29	3989-90-0
	5-Nitrochrysene	"	"	89455-17-4
	6-Nitrochrysene	"	"	7496-02-8
Five-ring PAHs				
Benzo[*e*]fluoranthene	3-Nitrobenzo[*e*]fluoranthene	$C_{20}H_{11}NO_2$	297.31	
Benzo[*a*]pyrene	1-Nitrobenzo[*a*]pyrene	$C_{20}H_{11}NO_2$	297.31	70021-997
	3-Nitrobenzo[*a*]pyrene	"	"	70021-98-6
	6-Nitrobenzo[*a*]pyrene	"	"	63041-90-7
	3,6-Dinitrobenzo[*a*]pyrene	$C_{20}H_{10}N_2O_4$	342.31	128714-76-1
Benzo[*e*]pyrene	1-Nitrobenzo[*e*]pyrene	$C_{20}H_{11}NO_2$	297.31	91259-16-4
	3-Nitrobenzo[*e*]pyrene	"	"	81340-58-1

Table 1 (Contd).

Parent PAHs	Nitro derivative	Molecular formula	Relative molecular mass	CAS number
Perylene	3-Nitroperylene	$C_{20}H_{11}NO_2$	297.31	20589-63-3
Dibenz[*a*,*h*]anthracene	7-Nitrodibenz[*a*,*h*]anthracene	$C_{22}H_{13}NO_2$	323.4	
Six-ring PAHs				
Benzo[*ghi*]perylene	4-Nitrobenzo[*ghi*]perylene	$C_{22}H_{11}NO_2$	321.34	
	7-Nitrobenzo[*ghi*]perylene	"	"	
Coronene	1-Nitrocoronene	$C_{24}H_{11}NO_2$	345.36	
Nitro-oxyPAHs				
	3-Nitrobenzanthrone	$C_{17}H_9NO_3$	275.26	1711-34-9
	2-Nitrobenzopyranone	$C_{13}H_7NO_4$	241.20	6623-66-1
	3-Nitrobenzopyranone	$C_{13}H_7NO_4$	241.20	6638-64-8
	4-Nitrobenzopyranone	$C_{13}H_7NO_4$	241.20	51640-90-5
	Nitropyrene lactones	$C_{15}H_7NO_4$	265.22	

Fig. 1 Structural formulae of some nitro-PAHs

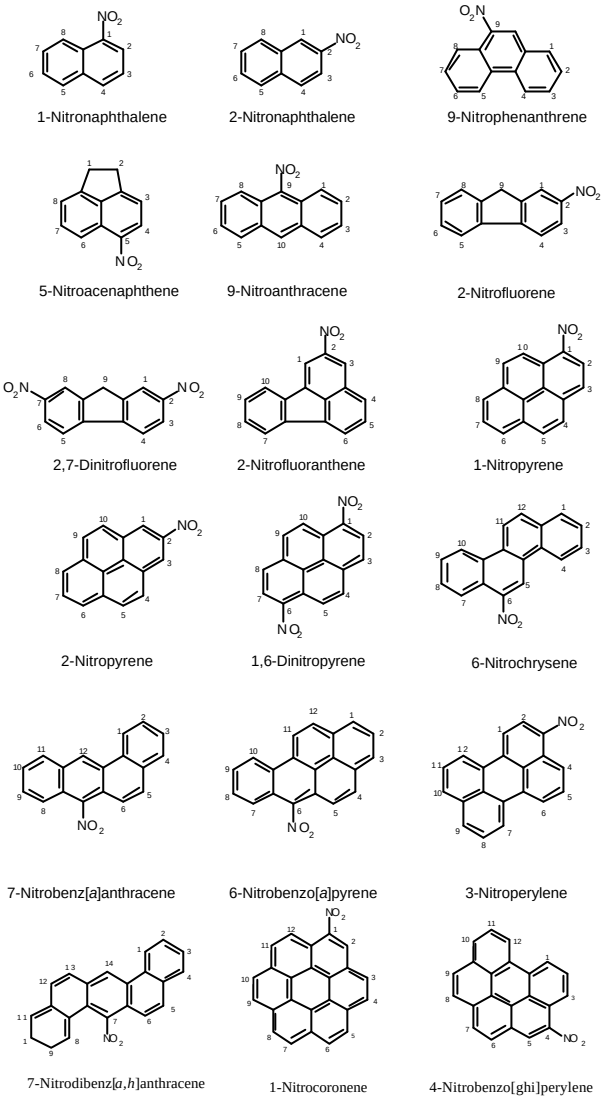
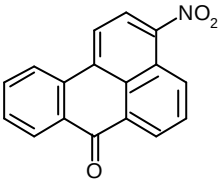
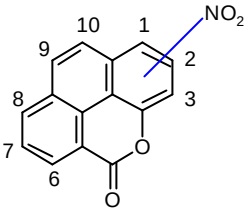


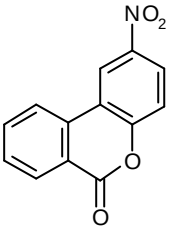
Fig. 1. Structural formulas of some nitroPAHs and some nitro-oxyPAHs.



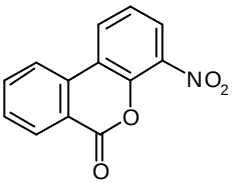
3-Nitrobenzanthrone



nitropyrene lactone



2-Nitrodibenzopyranone



4-Nitrodibenzopyranone

Fig. 1 (Contd).

2.2 Physical and chemical properties

At ambient temperatures, nitroPAHs are yellowish to orange solids that tend to sublime (White, 1985). NitroPAHs in the environment occur in the vapour phase or are absorbed and/or adsorbed to particulate matter, depending upon their vapour pressure and the ambient conditions. Two- to four-ring nitroPAHs are present partially in the vapour phase under certain conditions; for example, 1-nitronaphthalene (in certain climates) occurs mainly in the vapour phase (Arey et al., 1987), whereas 2-nitrofluorene occurs equally in both vapour and particulate phases, and 1-nitropyrene occurs in the particulate phase (Schuetzle & Frazier, 1986).

NitroPAHs are insoluble or sparingly soluble in water but are mostly soluble in organic solvents such as acetone, benzene, dimethyl sulfoxide (DMSO) and methylene chloride.

Table 2 gives details of some environmentally relevant physical and chemical properties of nitroPAHs together with those of the parent PAHs (see IPCS, 1998). Nitrodibenzopyranones have a lower vapour pressure than nitroPAHs and therefore are to be found predominantly in the particulate phase.

2.3 Conversion factors

Atmospheric concentrations of nitroPAHs are usually expressed as micrograms, nanograms or picograms per cubic metre. At 25 °C and 101.3 kPa, the conversion factors for a compound of given molecular mass are obtained as follows:

$$\text{ppb} = \mu g/m^3 \times 24.45/\text{relative molecular mass}$$
$$\mu g/m^3 = \text{ppb} \times \text{relative molecular mass}/24.45$$

where ppb is parts per billion, and one billion is 10^9.

For example, for 1-nitropyrene, 1 ppb = 10.1 $\mu g/m^3$, and 1 $\mu g/m^3$ = 0.099 ppb.

Table 2. Physical and chemical properties of nitroPAHs and their parent PAHs[a]

Parent PAHs; nitro derivatives	Melting point (°C)	Boiling point (°C) at 101.3 kPa	Vapour pressure (Pa at 25 °C)	Solubility in water at 25 °C (mg/litre)	Henry's law constant at 25 °C ($kPa \cdot m^3/mol$)	Log K_{ow}[b]	Log K_{oc}[b]
Two-ring PAHs							
Naphthalene	**81**	**218**	**10.4**	**31.7**	**4.9×10^{-2}**	**3.4**	
1-Nitro-	58–61.5 (56.5[c])	330; 314 sublimes[d] 312[e]	0.0154 (20 °C)[d] 3.2×10^{-2} [e]	34*f* 9.18[e]	6.1×10^{-1} [e]	2.50[c] 3.19[g,h]	3.02 2.98[e]
2-Nitro-	74–79 (76[c])	304[e]	3.2×10^{-2} [e]	26[f] 9.24[e]	6.1×10^{-1} [e]	2.78[c] 3.24[g,h]	3.09 3.03[e]
1,3-Dinitro-	144–149					2.83[g,h]	
1,5-Dinitro-	215–219					2.58[g,h]	
1,8-Dinitro-	171–172					2.52[g,h]	
2,7-Dinitro-	234						
1,3,6,8-Tetranitro-	195					2.29[g]	
Three-ring PAHs							
Acenaphthene	**95**	**279**	**2.9×10^{-1}**	**3.93**	**1.5×10^{-2}**	**3.92**	
3-Nitro-	151						
5-Nitro-	102 (101–102[c])					3.36[c] 3.85[g,h]	
Fluorene	**115**	**295**	**8×10^{-2}**	**1.98**	**1.0×10^{-2}**	**4.18**	
1-Nitro-		326	9.7×10^{-5}	0.28	7.2×10^{-2}		3.76[e]

Table 2 (Contd).

Parent PAHs; nitro derivatives	Melting point (°C)	Boiling point (°C) at 101.3 kPa	Vapour pressure (Pa at 25 °C)	Solubility in water at 25 °C (mg/litre)	Henry's law constant at 25 °C ($kPa \cdot m^3/mol$)	Log K_{ow}[b]	Log K_{oc}[b]
2-Nitro-	154–158 (158[c])	326	9.7×10^{-5}	0.216	9.5×10^{-2}	4.08[c] 3.37[g,h]	3.16[e]
3-Nitro-	105–106	326	9.7×10^{-5}	0.28	7.2×10^{-2}		3.76[e]
4-Nitro-	75–76	326	9.7×10^{-5}	0.28	7.2×10^{-2}		3.76[e]
2,7-Dinitro-	334					3.35[g,h]	
Anthracene	**216**	**342**	**8×10^{-4}**	**0.073**	**7.3×10^{-2}**	**4.5**	
2-Nitro-	172					4.23[g]	
9-Nitro-	141–146 (146[c])					4.16[c] 4.50[g] 4.78[i]	4.69[j]
9,10-Dinitro-	263, 310					4.10[c]	
Phenanthrene	**100.5**	**340**	**1.6×10^{-2}**	**1.29**			
2-Nitro-	119–120					4.23[g]	
9-Nitro-	116–117						
2,7-Dinitro-							
Four-ring PAHs							
Fluoranthene	**108.8**	**375**	**1.2×10^{-3}**	**0.26**	**6.5×10^{-4} (20 °C)**	**5.22**	
1-Nitro-						4.69[g]	
2-Nitro-		420[e]	9.9×10^{-7} [e]	0.019[e]	1.3×10^{-2} [e]		4.48[e]

Table 2 (Contd).

Parent PAHs; nitro derivatives	Melting point (°C)	Boiling point (°C) at 101.3 kPa	Vapour pressure (Pa at 25 °C)	Solubility in water at 25 °C (mg/litre)	Henry's law constant at 25 °C ($kPa \cdot m^3/mol$)	Log K_{ow} [b]	Log K_{oc} [b]
3-Nitro-	156–162 (166[c])					5.15 4.69[g]	
7-Nitro-	144–145	420[e]	9.9×10^{-7} [e]	0.017[e]	1.4×10^{-2} [e]	4.69[g]	4.48[e]
8-Nitro-	158–164	420[e]	9.9×10^{-7} [e]	0.017[e]	1.4×10^{-2} [e]	4.69[g]	4.48[e]
3,4-Dinitro-	279–280[k]						
3,7-Dinitro-	203–204[k]						
3,9-Dinitro-	275–276 222–224[k]						
Pyrene	**150.4**	**393**	**6.0×10^{-4}**	**0.135**	**1.1×10^{-3}**	**5.18**	
1-Nitro-	151–152 (153[c])	472[e]	4.4×10^{-6} [e]	0.017[e]	6.4×10^{-2} [e]	5.29[c] 4.69	4.48[e]
2-Nitro-	197–199	472[e]	4.4×10^{-6} [e]	0.021[e]	6.4×10^{-2} [e]		3.53[e]
4-Nitro-	190–192	472[e]	4.4×10^{-6} [e]	0.017[e]	6.4×10^{-2} [e]		4.48[e]
1,3-Dinitro-	295–297					4.44[g]	
1,6-Dinitro-	309–310					4.44[g]	
1,8-Dinitro-	299–300					4.44[g]	
1,3,6-Trinitro-						4.18[g]	
1,3,6,8-Tetranitro-	335					3.92[g]	

Table 2 (Contd).

Parent PAHs; nitro derivatives	Melting point (°C)	Boiling point (°C) at 101.3 kPa	Vapour pressure (Pa at 25 °C)	Solubility in water at 25 °C (mg/litre)	Henry's law constant at 25 °C ($kPa \cdot m^3/mol$)	Log K_{ow}[b]	Log K_{oc}[b]
Benz[*a*]anthracene	**160.7**	**400**	**2.8×10^{-5}**	**0.014**		**5.61**	
7-Nitro-	161–162[c]					5.34[c]	
Chrysene	**253.8**	**448**	**8.4×10^{-5}**	**0.002**		**5.91**	
2-Nitro-							
5-Nitro-							
6-Nitro-	208[c]					5.41[c] 5.41[g]	
Five-ring PAHs							
Benzo[*a*]pyrene	**178.1**	**496**	**7.3×10^{-7}**	**0.004**	**3.4×10^{-5} (20 °C)**	**6.50**	
1-Nitro-							
3-Nitro-							
6-Nitro-	260[c]	567[e]			0.012[e]	6.13[c] 5.87[e]	5.66[e]
3,6-Dinitro-							
Benzo[*e*]pyrene	**178.7**	**493**	**7.4×10^{-7}**	**0.005**		**6.44**	
1-Nitro-						5.87[g]	
Perylene	**277.5**	**503**		**0.0004**		**5.3**	
3-Nitro-	209[c]					6.34[c]	

Table 2 (Contd).

Parent PAHs; nitro derivatives	Melting point (°C)	Boiling point (°C) at 101.3 kPa	Vapour pressure (Pa at 25 °C)	Solubility in water at 25 °C (mg/litre)	Henry's law constant at 25 °C ($kPa \cdot m^3/mol$)	Log K_{ow} [b]	Log K_{oc} [b]
Six-ring PAHs							
Coronene	**439**	**525**	**2×10^{-10}**	**0.000 14**		**5.4**	
1-Nitro-							
Benzo[*ghi*]perylene	**278.3**	**545**	**1.4×10^{-8}**	**0.000 26**	**2.7×10^{-5} (20 °C)**	**7.10**	
4-Nitro-							
7-Nitro-							
Nitro-oxyPAHs							
3-Nitrobenzanthrone	256–257[d]						
2-Nitrodibenzo[*b*,*d*]-pyranone		368[e]	2.5×10^{-6} [e]	128[e,f]	2.9×10^{-6} [e]		2.04[e]
4-Nitrodibenzo[*b*,*d*]-pyranone		368[e]	2.5×10^{-6} [e]	128[e,f]	2.9×10^{-6} [e]		2.04[e]

[a] The data for parent PAHs are from IPCS (1998). The data for nitroPAHs are from White (1985), unless stated otherwise.
[b] Log K_{ow} = octanol/water partition coefficient; log K_{oc} = sorption coefficient.
[c] From Karcher et al. (1991).
[d] From IUCLID dataset on 1-nitronaphthalene, 2000.
[e] From Yaffe et al. (2001).

Table 2 (Contd).

[f] From Al-Bashir et al. (1994).
[g] From Compadre et al. (1990).
[h] Experimental values.
[i] From Sinks et al. (1997).
[j] From Nielsen et al. (1997).
[k] From Nakagawa et al. (1987).
[l] From Suzuki et al. (1997).

2.4 Analytical methods

A direct analysis of nitroPAHs from environmental sources is not possible, as all environmental matrices are very complex. The samples often contain thousands of combustion products, including parent PAHs and other closely related derivatives (in particular oxygenated PAHs such as aldehydes, ketones and carboxylic acids), which tend to co-elute with nitroPAHs under a variety of liquid and gas chromatographic conditions and are present at concentrations 1 or 2 orders of magnitude higher than those of the nitro-substituted compounds (Schuetzle, 1983; Vincenti et al., 1996; see Table 3). An extensive sample cleanup and prefractionation of the sample are tedious but necessary prerequisites for trace analysis of nitroPAHs.

Isomer-specific identification is necessary, as the biological activity depends on the position of the nitro substituent. The source of the nitroPAH (e.g., from combustion or from atmospheric reactions; see chapter 3) may determine the isomeric specificity of the nitroPAH.

Although 1- and 2-nitronaphthalene are expected to be found predominantly in the gas phase, other semivolatile nitroPAHs will be distributed between the particulate and gas phases, depending upon the ambient temperature. Thus, many ambient measurements will underestimate the total nitroPAHs present unless both gas- and particulate-associated species have been measured.

Analysis is hindered by a lack of adequate instrumental sensitivity or selectivity and limited availability of native and isotope-labelled standards (Chiu & Miles, 1996).

Bioassay-directed fractionation (usually using the Ames test or modifications of this with specific *Salmonella typhimurium* strains) and subsequent chemical characterization have been used for the identification of nitroPAHs in a number of complex mixtures (see section 2.4.5).

As most nitroPAHs are mutagenic, special precautions should be taken, even at ultratrace concentrations.

Table 3. Relative concentrations of various PAH compounds and PAH derivatives in the non-polar and moderately polar fractions of a diesel particle extract, showing only the nitroPAH fraction in detail[a]

Compound	Fraction concentration	
Non-polar fractions	**1000**	
Total PAHs, hydrocarbons, alkylbenzenes	1000	
Moderately polar fractions	**1000**	
PAH ketones	147	
PAH carboxyaldehydes	122.2	
PAH acid anhydrides	54.1	
HydroxyPAHs	113.1	
PAH quinones	71.3	
NitroPAHs	**2.9**	
Nitrofluorenes		0.34
Nitro(anthracenes and phenanthrenes)		0.71
Nitrofluoranthenes		0.05
Nitropyrenes		1.5
Methyl nitro(pyrenes and fluoranthenes)		0.25
Other oxygenated PAHs	83.4	
PAH carry-over phthalates, hydrocarbon contaminants	340	

[a] Adapted from Schuetzle (1983).

It should be noted that many nitroPAHs, in particular 9-nitro-anthracene, are unstable in the presence of light; therefore, reduced light conditions should be used (see chapter 4).

Details of methods used for the analysis of nitroPAHs in different matrices are given in Table 4. Reviews on the analysis of nitroPAHs are given by White (1985), Vincenti et al. (1996), CONCAWE (1998) and Hayakawa (2000).

2.4.1 Sampling

Methods of collecting air particulates include a) Mega sampler with 50% cut-off point of 20 µm and typically 6-h sampling periods

Table 4. Analysis of nitroPAHs[a]

Sample type	Extraction	Cleanup	Analysis	Detector	Detection limit[b]	References
Air						
Ambient air	Soxhlet (DCM)	NP-LC	GC	MS in SIM		Arey et al. (1987)
Reaction chamber study	Soxhlet (DCM)	NP-LC	GC RP-LC	MS in SIM UV		Atkinson et al. (1987a)
Ambient air	Ultrasonication (DCM)	Extensive, including NP-LC	GC	FPD; NPD; MS EI		Fernández & Bayona (1992)
Air particulate	Ultrasonication (DCM)	Silica gel	HPLC	Electrochemical		Galceran & Moyano (1993)
Air particulate	Ultrasonication (benzene/ethanol)	NP-LC	HPLC (with on-line reduction)	CL	0.3–5 fmol	Murahashi & Hayakawa (1997)
Air particulate	Ultrasonication (DCM)	Silica/cyclohexane	HPLC switching technique GC ELISA	FL HRMS	20 fmol (1-nitropyrene)	Zühlke et al. (1998)
Diesel exhaust						
Diesel	Soxhlet	NP-LC	GC	NPD (FL)		Schuetzle & Perez (1983)
Diesel particulate	Soxhlet (DCM)	NP-LC	GC GC	NPD MS	0.5 ppm 5 ppm	Paputa-Peck et al. (1983)
Diesel or air particulates	Soxhlet (DCM)	Extensive; also NP-LC	GC GC	FID TEA		Niles & Tan (1989)

Table 4 (Contd).

Sample type	Extraction	Cleanup	Analysis	Detector	Detection limit[b]	References
Diesel or air particulates	Ultrasonication (acetone)	SPE and on-line reduction	GC	MS EI	1 ng/g	Scheepers et al. (1994a)
Air and diesel particulates	Soxhlet (DCM)	NP-LC	LC On-line reduction	Electrochemical FL	60 pg 10 pg	MacCrehan et al. (1988)
Diesel or air particulates and gaseous	DCM	SPE	HRGC	HRMS	low ng/g (diesel particulate); pg/m^3 range	Chiu & Miles (1996)
Vehicle or air particulates	Ultrasonication (benzene/ethanol)	Precolumn reduction	HPLC (column switching)	CL with on-line reduction		Hayakawa et al. (1999a)
Water						
River water		DCM; florisil column	GC	MS		Takahashi et al. (1995)
River water	Concentration by blue chitin column; methanol		GC	MS		Nagai et al. (1999)
Wastewater	Fractionation into diethyl ether-soluble neutral, acidic and basic fractions		HPLC	UV and FL		Manabe et al. (1984)
Crankcase oil	Extraction with methanol, concentration and dissolution in water; then as above		HPLC	UV and FL		Manabe et al. (1984)

Table 4 (Contd).

Sample type	Extraction	Cleanup	Analysis	Detector	Detection limit[b]	References
Soil, sewage sludge, sediment						
Urban dust, residual of incinerator, soil	Soxhlet (acetonitrile)	SPE	GC	NPD		Librando et al. (1993)
Sewage sludge	Dimethyl formamide	LSE or SPE	GC	FID; GC-MS		Bodzek & Janoszka (1995)
River sediment	Ether; sodium hydroxide or benzene methanol; chloroform	Silica gel column; reduced/acylated	GC	ECD		Sato et al. (1985)
Soil	Methanol	Silica gel column; hexane/benzene/ chloroform; HPLC ODS columns	Analytical ODS column for HPLC	Reduction to amino derivatives; fluorescence	Dinitropyrenes 0.7–4 pg	Watanabe et al. (1999)
Soil	Methanol/acetone	SPE; HPLC	GC	MS EI or NICI	30 ng/kg dry weight	Niederer (1998)

Table 4 (Contd).

Sample type	Extraction	Cleanup	Analysis	Detector	Detection limit[b]	References
Biological samples						
Rat tissue	Homogenate treated with acetonitrile; evaporated under nitrogen; residue dissolved in methanol; extraction with Blue Rayon		HPLC; on-line reduction	Fluorescence		van Bekkum et al. (1999)
Human lung specimens	Ultrasonication (DCM)		Bioassay-directed chemical analysis, HPLC, GC	MS		Tokiwa et al. (1993a, 1998a)
Food and beverages						
Various food samples	Homogenization; acetonitrile; hexane	Silica gel column; DCM	GC	TEA	12 pg	Dennis et al. (1984)
Various food samples			GC GC	MS NPD	5 pg	Schlemitz & Pfannhauser (1996a)
Grilled and smoked food samples			HPLC; on-line reduction	Fluorescence		Schlemitz & Pfannhauser (1996b)
Fish, meat products and cheese			HPLC; on-line reduction	Fluorescence	50 ng/kg	Dafflon et al. (2000)

Table 4 (Contd).

[a] Abbreviations:

CL	chemiluminescence
DCM	dichloromethane
ECD	electron capture detector
ELISA	enzyme-linked immunosorbent assay
FID	flame ionization detector
FL	fluorescence detection
FPD	flame photometric detection
GC	gas chromatography
HPLC	high-performance liquid chromatography
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
LC	liquid chromatography
LSE	adsorption column chromatography on silica gel
MS	mass spectrometry
MS EI	mass spectrometer electron impact mode
NICI	negative-ion chemical ionization
NPD	nitrogen–phosphorus detection
NP-LC	normal-phase high-performance liquid chromatography
ODS	octadecylsilyl
ppm	parts per million
RP-LC	reversed-phase high-performance liquid chromatography
SIM	selective ion monitoring
SPE	solid-phase microextraction
TEA	thermal energy analyser
UV	ultraviolet

[b] The percent recovery was not given in most cases.

(used in Claremont, California, USA); b) an electrostatic precipitator with an impact stage designed for a 15-µm cut-off (used in Aurskog, Norway); and c) specially designed filter baghouses collected over a period of a year (National Institute of Standards and Technology [NIST] Standard Reference Materials [SRMs]) (Ramdahl et al., 1986). A 10-µm size-selective inlet (for particulate matter less than 10 µm in diameter, or PM_{10}) sampler is also reported (Nishioka & Lewtas, 1992).

For investigations into the formation and photochemistry of nitro-PAHs, three different collection media for ambient air sampling have been used: Tenax-GC solid adsorbent, polyurethane foam (PUF) and filters for high-volume sampling (Arey et al., 1991).

Three methods for the sampling of the semivolatile phase of diesel exhaust were compared: cryogenic sampling, adsorbent sampler with XAD-2 and PUF. The PUF technique gave the highest recovery of PAHs and mutagenic activity. The three sampling techniques for the semivolatile phase resulted in extracts with different chemical composition, different mutagenic potency and different mutagenicity profiles (Westerholm et al., 1991).

In general, vapour-phase constituents are collected on solid sorbents such as XAD and PUF, and particles are collected on Teflon-impregnated glass fibre filters (see Table 4).

For sampling of diesel exhaust particulates, a dilution tunnel method is mainly used (Hayakawa, 2000). The exhaust is diluted by the filtered air in the tunnel to simulate the real road conditions, and an aliquot is sampled on the filter. Gaseous substances are trapped in PUF. The sampling and analytical methods are reviewed by Levsen (1988).

Blue cotton bearing covalently linked copper phthalocyanine trisulfonates as a ligand adsorbs polyaromatic compounds and preconcentrates several nitroPAHs in water (Hayatsu, 1992).

2.4.2 Extraction

Extraction of filter and PUF samples can be carried out by dichloromethane (DCM) in a Soxhlet apparatus for 16 h, the Soxhlet body being loosely covered in aluminium foil to exclude light (Chiu & Miles, 1996). DCM was found to be the most efficient solvent for extraction of mutagenic compounds from diesel particles (Montreuil et al., 1992).

Toluene as solvent has also been reported (Spitzer, 1993; Vincenti et al., 1996). Supercritical fluid extraction of nitroPAHs from diesel exhaust particulate matter using carbon dioxide–chlorodifluoromethane (HCFC-22) or carbon dioxide–toluene has been demonstrated (Paschke et al., 1992).

Soil sample extraction has been described using the Soxhlet device using 1:1 (v/v) toluene:methanol (Vincenti et al., 1996) or 5% ethanol in toluene (Spitzer, 1993).

2.4.3 Cleanup

Various procedures for fractionation of particulate extracts have been described:

- open-column liquid chromatography with either silica gel or alumina (Yu et al., 1984; Lindskog et al., 1987; Lewtas, 1988; Kamiura et al., 1991; Galceran & Moyano, 1993; Spitzer, 1993);
- normal-phase high-performance liquid chromatography (HPLC), or NP-LC (Oehme et al., 1982; Nielsen, 1983; Paputa-Peck et al., 1983; Stray et al., 1984; Tomkins et al., 1984; Pitts et al., 1985a; Arey et al., 1986, 1987, 1988a; Ramdahl et al., 1986; Ciccioli et al., 1988; Niles & Tan, 1989; Veigl et al., 1994); or
- solid-phase microextraction (Xu et al., 1982; Jin & Rappaport, 1983; LaCourse & Jensen, 1986).

From open-column liquid chromatography, four main fractions are obtained by eluting with different solvents (in parentheses): aliphatics (hexane), aromatics (hexane/benzene), moderately polar (DCM) and highly polar (methanol) (Lewtas, 1988). The nitroPAHs are to be found in the moderately polar fraction, but together with a number of oxy derivatives (e.g., aldehydes, ketones, quinones), which may interfere with them. Therefore, a more effective fractionation is achieved by NP-LC, but here the nitroPAHs are not collected in a single fraction but are distributed in several fractions (Vincenti et al., 1996). Another approach to separate nitroPAHs from hydroxyPAHs uses a sequential cleanup with silica and alumina (Moyano & Galceran, 1997). Nitrated and hydroxylated PAHs extracted from air particulates could be fractionated from other micropollutants by semipreparative packed-column supercritical fluid chromatography on silica gel (Medvedovici et al., 1998).

Figure 2 shows a schematic diagram of an analytical method used for the cleanup and separation of particulate organic matter (Ciccioli et al., 1996).

2.4.4 Analytical separation and detection

The most frequently used techniques for the detection of nitro-PAHs are (Vincenti et al., 1996):

- gas chromatography **(**GC) combined with a thermionic or nitrogen–phosphorus selective detector (Nielsen, 1983; Paputa-Peck et al., 1983; Liberti et al., 1984; Nielsen et al., 1984; Matsushita & Iida, 1986; Warzecha, 1996), chemi-luminescence-based thermal energy analyser (Tomkins et al., 1984; Yu et al., 1984; Robbat et al., 1986; Niles & Tan, 1989) or electron capture detector (ECD) (Oehme et al., 1982; LaCourse & Jensen, 1986; Spitzer, 1993);
- HPLC with fluorescence (Tejada et al., 1986; MacCrehan et al., 1988; Kamiura et al., 1991; Hayakawa et al., 1993; Veigl et al., 1994), chemiluminescence (Sigvardson & Birks, 1984; Hayakawa et al., 1992, 1995a) or electrochemical (Jin & Rappaport, 1983; MacCrehan et al., 1988; Galceran &

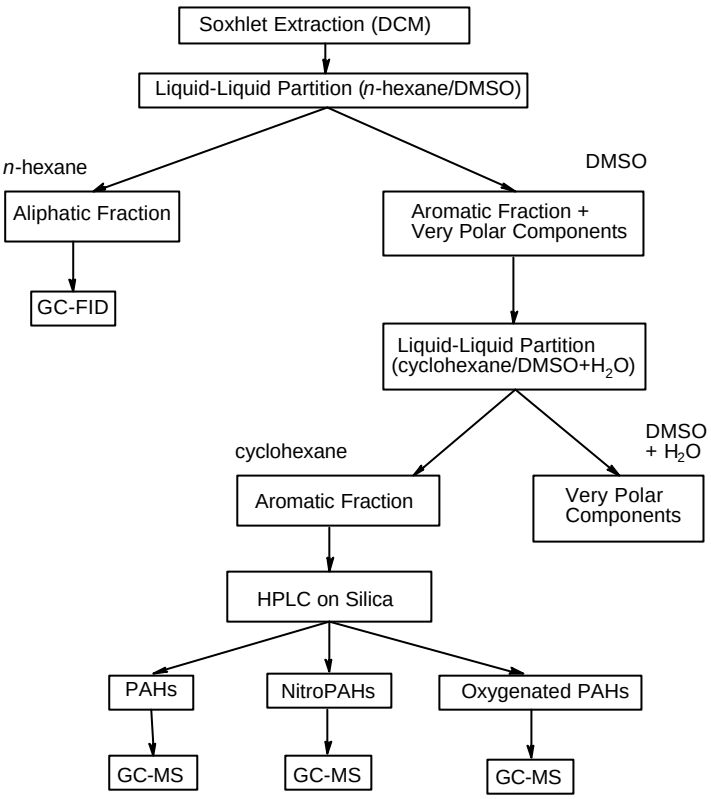


Fig. 2. Schematic diagram of the analytical method for the cleanup and separation of particulate organic matter (Ciccioli et al., 1996). DCM = dichloromethane; DMSO = dimethyl sulfoxide; GC-FID = gas chromatography with flame ionization detection; GC-MS = gas chromatography/mass spectrometry; HPLC = high-performance liquid chromatography.

Moyano, 1993) detector; for determination of 1,3-, 1,6- and 1,8-dinitropyrenes, HPLC using chemiluminescence after precolumn reduction has been developed, giving detection limits 2 orders lower than those with fluorescence (Hayakawa et al., 1992, 1995a; Maeda et al., 1994; Murahashi & Hayakawa, 1997);

- GC/mass spectrometry (GC-MS) in electron impact (EI) (Paputa-Peck et al., 1983; Liberti et al., 1984; Yu et al., 1984; Pitts et al., 1985a; Arey et al., 1986, 1987; Ramdahl et al., 1986; Niles & Tan, 1989; Paschke et al., 1992), positive ion chemical ionization (Schuetzle et al., 1982) or negative ion chemical ionization (NICI) electron capture (EC) (Oehme et al., 1982; Ramdahl & Urdal, 1982; Henderson et al., 1983; Liberti et al., 1984; Nielsen et al., 1984; Stray et al., 1984);
- EC-MS, which also provides adequate sensitivity and selectivity for the analysis of nitroPAHs in most matrices; however, there is still difficulty due to interference from oxy PAHs (Lewtas & Nishioka, 1990); and
- tandem mass spectrometry (MS/MS) (Schuetzle et al., 1982; Henderson et al., 1983). Combining this technique with GC-EC gives four "separation" stages: a chromatographic separation, a selective ionization method and two mass spectrometric analyses (Schilhabel & Levsen, 1989). The method has now been refined, and the high selectivity of the instrumentation techniques means that the preliminary sampling is no longer required on air particle samples (Vincenti et al., 1996).

A thin-layer chromatography method using plates coated with a silica gel layer has been developed for analysing nitroPAHs. DCM and DCM with *n*-hexane and methanol were used for the mobile phases. The chromatograms developed using a mixture of *n*-hexane–DCM (1:1 v/v) were observed under ultraviolet (UV) light before and after being sprayed with a reducing agent (sodium borohydride dissolved in methanol and copper chloride solution). Light at $\lambda = 254$ nm induced green fluorescence for 1-nitropyrene and 1,3-dinitropyrene only and a violet colour for the remaining compounds. Carbon disulfide quenched fluorescence (observed at $\lambda_{exc} = 365$ nm) for 1-nitronaphthalene, 9-nitroanthracene, 1-nitropyrene and 1,3-dinitropyrene only (Tyrpien, 1993; Tyrpien et al., 1997). Janoszka et al. (1997) used acetonitrile/water as the mobile phase. The method is suggested as a simple and quick method for identifying the above nitroPAHs in airborne particulate matter after separation in moderately polar fractions by column chromatography on silica gel. Isomeric nitroPAHs cannot be separated.

More recent developments in this field include a) selective detection of several nitroPAHs by using time-of-flight MS (Bentz et al., 1995; Dotter et al., 1996; Bezabeh et al., 1997); b) a method using supercritical fluid extraction and on-line multidimensional chromatographic methods (NP-LC coupled to a high-resolution GC) and ion trap detector MS (Lewis et al., 1995a,b; Feilberg et al., 2001); and c) particle beam liquid chromatography–MS with NICI mode (Bonfanti et al., 1996). High-resolution NICI is reported to be at least 20 times more sensitive than the low-resolution NICI or EI for determination of nitroPAHs in air samples (Chiu & Miles, 1996). A method has been developed involving the derivatization of nitroPAHs to their corresponding fluorinated derivatives, followed by GC-ECD analysis. The sensitivity of the method is an order of magnitude higher than that of direct GC-ECD analysis of nitroPAHs themselves. This method is suitable for routine monitoring of nitroPAHs in air samples (Jinhui & Lee, 2001).

2.4.4.1 *Difficulties in analysis*

Owing to small differences in GC retention times for 2- and 3-nitrofluoranthene, the 2-nitrofluoranthene present in some samples was incorrectly reported as 3-nitrofluoranthene in earlier reports. After reanalysis, the original reports were corrected (Ramdahl et al., 1986; Nishioka et al., 1988). Use of more selective stationary phases enabled better separation of isomeric pairs (Ciccioli et al., 1988). For the best separation of all of the nitrofluoranthene and nitropyrene isomers, the use of a DB13 (or SP30-type) column is recommended (A. Cecinato, personal communication, 2002).

Sampling gas-phase PAHs from environmental chambers onto Tenax adsorbent under conditions not typical of ambient atmospheres (e.g., 19 mg nitrogen dioxide/m^3, 44 mg nitrogen pentoxide/m^3) can lead to artificial formation of nitro derivatives via reactions with the Tenax adsorbent (Zielinska et al., 1986a).

2.4.4.2 Complex mixtures

The chemical analysis of trace levels of organic mutagens in ambient air is more complex than comparable analyses of emissions from specific sources. Source-emitted pollutants account for only part of the whole ambient air sample. Aging of air samples introduces unknown, highly variable chemical and meteorological factors. Further, the concentrations of pollutants are much lower in ambient air than at the sources (Greenberg et al., 1993).

2.4.4.3 Analysis of nitro-oxyPAHs

The procedures followed for investigating nitro-oxyPAHs in emissions and ambient air are, in general, the same as those adopted for nitroPAHs.

2.4.5 Use of bioassay (mutagenicity) fractionation and chemical analysis

Bioassay-directed fractionation closely coupled to chemical characterization has been developed as a method of determining nitroPAHs in complex mixtures (see Figure 3) (Schuetzle & Lewtas, 1986; Lewtas, 1988; Lewtas & Nishioka, 1990; Legzdins et al., 1995; Enya et al., 1997). In this approach, the complex mixture is fractionated, and each fraction is bioassayed; the mutagenic activity for each (HPLC) fraction is plotted in a manner analogous to a conventional chromatogram, and the plot is referred to as a mutagram (mutagenicity profile = mutagram).

Mutagenically active fractions are further fractionated, bioassayed and characterized until the major class of compounds or specific compounds potentially responsible for the mutagenicity are identified. Use of bacterial tester strains selectively sensitive to nitroarenes has led to the identification of nitroPAHs as potent mutagens in complex mixtures from diverse sources.

Non-polar fractions of an extract of diesel particulates (SRM 1650) accounted for less than 2–3% of the mutagenicity in the total

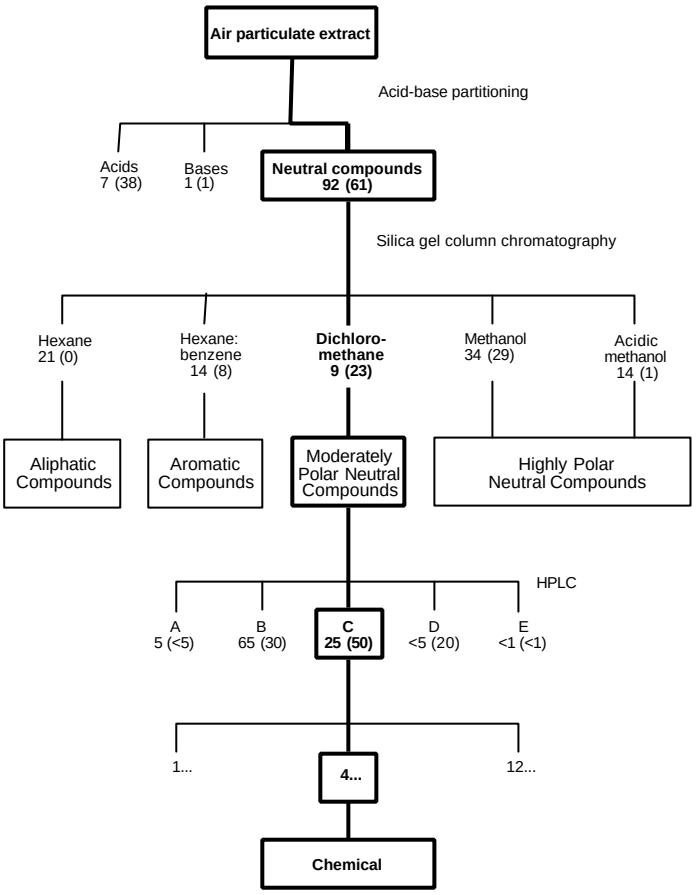


Fig. 3. Bioassay-directed chemical analysis scheme for the determination of air particulate matter. The numbers under each fraction represent the percent distribution of mass and mutagenicity in *Salmonella* tester strains (in parentheses); adapted from Schuetzle & Lewtas (1986) and Lewtas et al. (1990a).

extract. The distribution of mutagenicity in the moderately polar and polar fractions was dependent on the source sample (Schuetzle & Lewtas, 1986).

Studies using this bioassay-directed fractionation and chemical characterization (see also section 7.5.5) include, for example, studies of diesel exhaust extracts (Schuetzle et al., 1981; Nishioka et al., 1982; Claxton et al., 1992; Legzdins et al., 1994; Enya et al., 1997; Hayakawa et al., 1997), xerographic toners (Rosenkranz et al., 1980), cigarette smoke (Kier et al., 1974), ambient atmospheric particles (Nishioka et al., 1988; Lewtas et al., 1990a; Arey et al., 1992; Casellas et al., 1995; Hayakawa et al., 1995b) and the metabolites of 1-nitropyrene (Lewtas et al., 1990b).

For example, Casellas et al. (1995) made a detailed chemical analysis of mutagenic fractions using bioassay-directed chemical analysis in urban airborne particulate matter in Barcelona, Spain. The first fractionation of the solvent (DCM) extractable organic matter was achieved by semipreparative gel permeation chromatography (GPC). The second fractionation was achieved with NP-LC. The collected fractions were tested for mutagenicity using the *S. typhimurium* microsome assay with strains TA98, TA98NR– and TA98AT– (for more details, see chapter 7). The chemical characterization of mutagenic fractions was carried out by an extensive application of capillary GC-MS in the EI and NICI modes. Those fractions exhibiting the highest levels of mutagenicity were subjected to a third level of fractionation by reversed-phase HPLC (RP-LC) and analysed by GC-MS. Two sampling sites in Barcelona were monitored during 1990. Samples (24 h) of air particulate matter over periods of 1 week per season were processed.

The direct mutagenicity in the fractions NP-LC 3 (and 4) isolated from the GPC-2 fraction of airborne particulate matter collected in Barcelona (autumn 1990) seemed to be accounted for by nitrated arenes, 9-nitroanthracene, 2-nitrofluoranthene and 2-nitropyrene. 6-Nitrobenzo[*a*]pyrene in polar fraction 5 needed application of metabolic activation (+S9) for mutagenicity. In order to evaluate the contribution of nitro derivatives to the total mutagenicity, the NP-LC fractions were tested against TA98NR– and TA98AT–. Generally, a remarkable decrease in mutagenic activity was observed in all fractions; these decreases were more apparent in fractions NP-LC3 to NP-LC7, thus suggesting a significant contribution of nitroarenes to the mutagenicity of these fractions of medium and high polarity. Subfraction NP-LC2 was

fractionated further. The expected composition was PAHs, but two nitro derivatives, 2-nitrofluoranthene and 1-nitropyrene, were identified in subfraction RP-LC3, which may be responsible for the high direct-acting mutagenic activity observed in this fraction and in the NP-LC2 fraction (Casellas et al., 1995).

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

NitroPAHs originate primarily as direct or indirect products of incomplete combustion. Only a few nitroPAHs are produced industrially (e.g., nitronaphthalenes and 5-nitroacenaphthene).

3.1 Industrially produced nitroPAHS

3.1.1 Production levels and processes

Treatment of naphthalene with mixed sulfuric/nitric acids at 60 °C yields 95% 1-nitronaphthalene and 5% 2-nitronaphthalene, together with traces of dinitronaphthalene and dinaphthol. Higher temperatures (80–100 °C) result in a mixture of 1,5- and 1,8-dinitronaphthalene in about a 2:3 ratio (Booth, 1991). The dinitro isomers can then be further separated for specific uses.

Nitronaphthalenes are produced in Germany and Japan. The production capacity of dinitronaphthalenes in Japan was 1200 tonnes per year (no year given; Booth, 1991).

3.1.2 Uses of commercially produced nitroPAHs

1-Nitronaphthalene is used almost exclusively for catalytic reduction to 1-naphthylamine. Further uses, such as use as a deblooming agent for petroleum and oils and as a component in the formulation of explosives, are of historical interest only (Booth, 1991).

1,5-Dinitronaphthalene is an intermediate in the production of naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) and 1,5-naphthalenediamine, which is mainly converted to naphthalene 1,5-diisocyanate. It is further used as a sensitizing agent for ammonium nitrate explosives (Booth, 1991).

1,8-Dinitronaphthalene is catalytically hydrogenated to 1,8-naphthalenediamine for use mainly as a colorant intermediate for naphthperinones (Booth, 1991).

5-Nitroacenaphthene is reported to be an intermediate in the synthesis of naphthalimide dyes that are used as fluorescent whitening agents and photochemical agents (Yahagi et al., 1975; IARC, 1978).

3.2 Other sources of nitroPAHs

NitroPAHs in the environment originate from direct emissions from combustion sources and nitration of PAHs, primarily in the atmosphere.

The nitroPAHs emitted from combustion sources are nitroPAHs that would be formed through electrophilic nitration (e.g., 1-nitropyrene and 2-nitrofluorene; see section 3.2.1). NitroPAHs have been observed in vehicle exhaust (particularly diesel), industrial emissions and emissions from domestic residential heating/cooking and wood burning.

NitroPAHs are formed in the atmosphere from PAHs by the following reactions:

- daytime gas-phase reaction: through hydroxyl radical addition to the PAH followed by reaction with nitrogen dioxide and loss of a water molecule (formation of 2-nitrofluoranthene and 2-nitropyrene);
- nighttime gas-phase reaction: nitrate radical addition to the PAH followed by reaction with nitrogen dioxide and loss of nitric acid (formation of high yields of 2-nitrofluoranthene, nitronaphthalenes and methylnitronaphthalenes); and
- heterogeneous gas–particle interaction of parent PAHs adsorbed onto particles with nitrating agents (would be expected to produce electrophilic nitration products). This has been shown to occur in the laboratory under conditions, for example, of very high concentrations of nitrogen dioxide. Concerns that artefactual formation of nitroPAHs during ambient sampling could occur via heterogeneous reactions

with nitrogen oxides have been shown to be unfounded (Arey et al., 1988a; Dimashki et al., 2000). Recent ambient measurements suggest that some 9-nitroanthracene may be formed from heterogeneous reactions during transport (Feilberg et al., 2001).

These processes are described in more detail in section 3.2.2. Other less important pathways, which are briefly mentioned here, include:

- endogenous formation of nitroPAHs in the body due to reaction of PAHs ingested in food or inhaled in ambient air with nitrogen dioxide from, for example, cigarette smoke. This has been suggested in animal experiments where mutagens have been formed from intraperitoneal (i.p.) administration of PAHs during exposure to higher than ambient levels of nitrogen dioxide (1 ppm = 1.88 mg/m^3) — for example, 5–10 ppm (Kanoh et al., 1990), 10 ppm (Tokiwa et al., 1981a) and 20 ppm (Miyanishi et al., 1996); and
- formation in the water phase with nitrite as a donor of the nitro group, using mercury lamps as a UV radiation source (Ohe, 1984; Suzuki et al., 1987).

The distribution of nitroPAH isomers in samples of ambient air has been found to be significantly different from that in direct emissions from combustion (compare Table 5 and chapter 5). For example, 2-nitrofluoranthene and 2-nitropyrene are ubiquitous components of particulate matter that have been detected in urban, suburban, forest and remote areas located in Europe, America, Asia and Antarctica (Ciccioli et al., 1996), although they are not directly emitted from most combustion sources (see chapter 5). The nitroPAH profile, or the relative quantities of certain "marker" PAHs, is a pointer to the source of formation of nitroPAHs — for example, markers of direct emissions from combustion, in particular diesel exhaust, are 1-nitropyrene and 2-nitrofluorene, whereas the presence of 2-nitrofluoranthene and 2-nitropyrene points to atmospheric transformation.

Table 5. NitroPAHs detected in diesel emissions[a,b]

Parent PAH; nitro derivative	Concentration (ppm = µg/g, unless otherwise stated)														
	a) E	b) E	c) P	d) E	e) P	f) E	g) P	h) P	i) E	j) E	k) P	l) E	m) P µg/m^3	n) µg/km	o) P pmol/mg
Naphthalene															
1-Nitro-	X (nd)	X	X	0.7	0.88					0.013					
2-Nitro-	nd	X	X		0.02					0.039					
1,3-Dinitro-		X	X												
1,5-Dinitro-	X (nd)	X	X												
1,8-Dinitro-	X (nd)														
Acenaphthene															
3-Nitro-		X*													
Fluorene															
1-Nitro-							5.91*		0.08*						
2-Nitro-	X	X	X	nd	0.11	4.1	<0.01	0.27	0.001		X	27	8.77	0.99	nd
2,5-Dinitro-	X (nd)														
2,7-Dinitro-	X (nd)													0.13	
Anthracene															
1-Nitro-		X*		300*		4.6	X*								
2-Nitro-	X	X				10.1									
9-Nitro-	X	X	X			2.8	6.02	10	1.19	1.36	7	63		1.00	

Table 5 (Contd).

Parent PAH; nitro derivative	Concentration (ppm = µg/g, unless otherwise stated)														
	a) E	b) E	c) P	d) E	e) P	f) E	g) P	h) P	i) E	j) E	k) P	l) E	m) P µg/m^3	n) µg/km	o) P pmol/mg
Phenanthrene															
1-Nitro-						0.5									
2-Nitro-	X	X				X	16.5*		1.8*						
3-Nitro-						9.3									
4-Nitro-						0.7									
9-Nitro-						2.3	0.37		0.18	0.27					
Fluoranthene															
1-Nitro-	X (nd)	X				4.1	X*								
2-Nitro-	nd	X								0.06					
3-Nitro-	X (nd)	X		1		10	0.33		1.34	1.47	<1	10		0.06	
7-Nitro-	X (nd)	X				1.6									
8-Nitro-	X (nd)	X				2.0				0.25					
Pyrene															
1-Nitro-	75	X	X	590	2.84	43	18; 19.6*	20	16.6; 18.0*	16.4	19	450	0.2 (+1,3-DNP)	3.43	6.49
2-Nitro-	nd														
4-Nitro-	nd									0.07					0.04
1,3-Dinitro-	0.30	X		0.6			<0.1		0.58	0.6			with 7-NP	0.12 all DNP	0.17

Table 5 (Contd).

Parent PAH; nitro derivative	Concentration (ppm = µg/g, unless otherwise stated)														
	a) E	b) E	c) P	d) E	e) P	f) E	g) P	h) P	i) E	j) E	k) P	l) E	m) P µg/m^3	n) µg/km	o) P pmol/mg
Pyrene, continued															
1,6-Dinitro-	0.40			0.6			<0.1		0.64	1.39			0.37		0.04
1,8-Dinitro-	0.53			0.4			<0.1		0.49	1.55					0.07
Benz[*a*]anthracene															
7-Nitro-	nd	X		8*			0.45	2.8	1.99	1.62	<1	10	0.61		
Chrysene															
1-Nitro-	X (nd)			f			0.8*		2.9*						
5-Nitro-						4.6									
6-Nitro-		X					0.04		0.87	0.782	X	4	0.08		0.10
Benzo[*a*]pyrene															
1-Nitro-	1- + 3-			10*	1.2*		1.4*								
6-Nitro-	X		X			5.8	1.4	1.6		0.641	X			0.12	0.30
Benzo[*e*]pyrene															
1-Nitro-										0.83					
3-Nitro-										2.2					
Perylene															
3-Nitro-	X		X												0.64

Table 5 (Contd).

[a] E = extract; P = particulate; DNP = dinitropyrene; 7-NP = 7-nitropyrene; X = detected; * = mixture of isomers; X (nd) = detected by GC/nitrogen–phosphorus detector (NPD) (0.5 µg/g) but not by GC-MS (detection limit 5 µg/g); nd = not detected by either.

[b] Samples were as follows:

a) Light-duty diesel particulate extract (Paputa-Peck et al., 1983).
b) Levsen (1988); Schilhabel & Levsen (1989). Thirty-eight nitroPAHs were detected (including methyl-nitroPAHs and mixed isomers, not noted in the table). Identification by comparison with reference compounds or relative retention times reported by Paputa-Peck et al. (1983).
c) Hartung et al. (1984). Exhaust from light-duty diesel test engine.
d) Concentration in extract from VW Rabbit diesel (Nishioka et al., 1982, 1983).
e) Yu et al. (1984).
f) Campbell & Lee (1984); g/g light-duty diesel particulate extract (US Environmental Protection Agency [EPA] recalculated concentrations from mg/g extract to g/g particle using a value of 44% for extractable material).
g) Diesel particulate SRM 1650 (Chiu & Miles, 1996).
h) Diesel particulate SRM 1650 (MacCrehan et al., 1988).
i) Diesel extract SRM 1975 (Chiu & Miles, 1996).
j) SRM 1975 diesel particle extract (DCM extract of diesel particulate matter collected from an industrial diesel-powered forklift) (NIST, 2000) (SRMs are analytical reference samples for quality control, not implied as representative of environmental diesel samples).
k) Heavy-duty diesel particulate matter (SRM 1650) (Niles & Tan, 1989).
l) NitroPAHs extracted from bus soot (Paschke et al., 1992). Bus soot contained much higher concentrations of 1-nitropyrene than standard diesel sample SRM 1650 (450 compared with 20 µg/g); extraction by carbon dioxide–HCFC-22.
m) Particulate nitroPAH concentration; 1988 Cummins LTA engine operated under baseline conditions (no trap) at US EPA steady-state engine mode 9; mean of $n = 3$; vapour-phase exhaust was >0.025 µg/m^3. Details also given with mode 11; with and without ceramic particle trap and with copper fuel additive (Harvey et al., 1994).
n) µg/km US Federal Test Procedure 72 (FTP-72) cycle with hot start, four-cylinder engine, light diesel vehicle ($n = 3$–6) (Scheepers & Bos, 1992a).
o) pmol/mg; particulate emission from 1995 diesel engine vehicle, idling engine (Hayakawa et al., 1997).

The majority of ambient nitroPAHs are now thought to be formed in the atmosphere from the gas-phase reactions of PAHs with four rings or less (Atkinson & Arey, 1994).

3.2.1 Direct sources of nitroPAHs from combustion processes

3.2.1.1 Diesel exhaust

1) Qualitative and quantitative studies of nitroPAHs in diesel exhaust

NitroPAHs have been detected in particulate exhaust emissions of motor vehicles, in particular diesel exhaust emissions, together with hundreds of other organic compounds (see Table 3; Schuetzle, 1983). Interest was focused on nitroPAHs in the early 1980s because correlations were found between the presence of nitroPAHs in diesel exhaust (and environmental extracts) and mutagenic activity in *Salmonella typhimurium* (see chapter 7). A large number of groups of nitro, oxy and mixed nitro-oxy compounds eluted together in the mutagenic fractions. Analytical methods were developed to separate and identify as many mononitro- and dinitroPAHs in diesel exhaust as technically possible, first as isomer groups and then using isomer-specific identification (e.g., Schuetzle et al., 1981, 1982; Newton et al., 1982; Xu et al., 1982; Henderson et al., 1983; Paputa-Peck et al., 1983; Campbell & Lee, 1984; Levsen, 1988; Niles & Tan, 1989; Schilhabel & Levsen, 1989; Chiu & Miles, 1996; see also Table 5). The number of nitroPAHs quantified is generally limited due to lack of standards. Investigators have used different samples of diesel exhaust as well as different analytical methods. Further, the concentration of nitroPAHs adsorbed on diesel particulate varies substantially from sample to sample (Levsen, 1988). It is therefore difficult to compare the various nitroPAH profiles. Usually, 1-nitropyrene is the predominant component, and concentrations of 7–165 µg/g particulate have been reported (Levsen, 1988). However, 1-nitropyrene is not always the dominating substance. Especially in heavy-duty diesel, 2-nitrofluorene may exceed 1-nitropyrene by a factor of 1.8–15, with an average of 9.8. When 1-nitropyrene is the dominant substance, a 2-nitrofluorene concentration equal to 15% of that of 1-nitropyrene is common (Beije & Möller, 1988a).

Some studies have focused on measuring concentrations of specific nitroPAHs — for example, 1-nitropyrene as a marker of nitroPAH formation (see below), 2-nitrofluorene (Beije & Möller, 1988a; Möller et al., 1993a) or dinitroPAHs, in particular 1,3-, 1,6- and 1,8-dinitropyrenes, which have been found to be more mutagenic (although not necessarily more carcinogenic; see chapter 7) than mononitroPAHs (Pederson et al., 1984; Hayakawa et al., 1992, 1994) (see Table 6). Dinitropyrenes are formed from the further nitration of 1-nitropyrene and are present at only about 1% of its concentration (Schuetzle & Frazier, 1986). Mononitrofluoranthene and mononitropyrenes were separated and identified in diesel extracts (Ciccioli et al., 1988).

2) Formation of nitroPAHs and comparison of emissions

NitroPAHs are formed in vehicle engines from the reactions of PAHs with nitrating species that are provided by the conversion of nitrogen and oxygen at high temperatures in the combustion chamber (Scheepers & Bos, 1992a).

Studies compare the emissions of nitroPAHs from various engines (Henderson et al., 1983), under differing driving conditions (different speeds, loads, etc.) (Schuetzle & Perez, 1983; Draper, 1986; Lorber & Mollenhauer, 1989; Veigl et al., 1994), with and without an oxidation catalytic converter (Johnson et al., 1994; Mitchell et al., 1994; Pataky et al., 1994), with and without catalysed or non-catalysed particulate traps (Westerholm et al., 1986; Bagley et al., 1993; Harvey et al., 1994; Johnson et al., 1994) and using a variety of fuels and/or additives (Harvey et al., 1994; Johnson et al., 1994).

1-Nitropyrene concentrations in exhaust (given as µg/g dust) from heavy-duty trucks during simulated driving cycles are as follows: suburban, 1.94; urban, 3.39; and motorway, 1.94 and 2.73 (Scheepers et al., 1994a). 1-Nitropyrene emission rates in exhaust emissions from a heavy-duty diesel vehicle during transient driving conditions were 1.6 µg/km in the particulate and <0.05 µg/km in the semivolatile phase (Westerholm et al., 1991). Light-duty trucks with oxidation catalysts emitted 1-nitropyrene at 0.36 µg/km with the US Federal Test Procedure 75 (FTP-75) driving cycle and 0.32 and 0.22 µg/km with the

Table 6. Concentrations of 1-nitropyrene and 1,3-, 1,6- and 1,8-dinitropyrene in diesel particulate and gasoline exhaust[a]

NitroPAH	Concentration (µg/g)						
	a)	b)	c)	d)	e)	f)	g)
1-Nitropyrene	3.9	3.9–116	13	37	0.32	0.44	2.1 (<5)
1,3-Dinitropyrene	<0.005		0.07	0.08	0.05	0.06	
1,6-Dinitropyrene	0.033	0.04–4.47	0.07	0.15	0.06	0.12	
1,8-Dinitropyrene	0.013	0.04–6.24	0.06	0.23	0.08	0.10	

[a] Samples were as follows:
a) From 1978 Opel diesel (Gibson, 1982, 1983).
b) Range of nitropyrenes detected in diesel particulate from five different motors (1979–1983) under different driving speeds and cycles and collected by different methods (Pederson et al., 1984).
c) Mean from seven diesel vehicles (1983–1991) (Hayakawa et al., 1994).
d) Diesel exhaust from 1980 Isuzu truck (Sera et al., 1994).
e) Gasoline exhaust from 1985 Toyota automobile (Sera et al., 1994).
f) Exhaust from eight gasoline vehicles (year not given) (Hayakawa et al., 1994).
g) On-road emission factors for 1-nitropyrene of 0.49 versus <0.03 µg/km (2.1 versus <5 µg/g, by mass of the extracted particulate matter) were given for heavy-duty diesel trucks and spark ignition gasoline light-duty passenger cars, respectively (Gorse et al., 1983).

European driving cycle (cold and hot start, respectively; Scheepers et al., 1994a).

In a multivariate analysis of exhaust emissions, 10 different fuels were combusted using two different types of heavy-duty diesel engines. The levels of 1-nitropyrene in the exhaust from the different fuels ranged from 0.07 to 2.17 µg/km in one engine and from 0.32 to 7.19 µg/km in the other. It was not found that one fuel gave a high 1-nitropyrene emission in both engine types. There was a negative correlation between 1-nitropyrene and nitrogen oxides and pyrene content, indicating the formation of 1-nitropyrene from the reaction between nitrogen oxides and pyrene (Sjögren et al., 1996).

Studies using low-sulfur fuel showed that, independent of the type of engine and exhaust after-treatment device, the primary effect of reducing sulfur content in diesel fuel was to substantially decrease the sulfate emissions and the number of respirable particles (Bagley et al., 1996). The total particulate matter emissions were not significantly diminished. However, because sulfate particles are so small, decreases in the emissions of these particles do not significantly diminish the emissions of total particulate matter. Using low-sulfur fuel had no significant effect on emissions of semivolatile organic compounds in the vapour phase or of soluble organic compounds in the particulate phase. However, the emission of hydrocarbon gases, the emission of some PAHs in the particulate and vapour phases and the mutagenicity of particulate-phase fractions were significantly elevated under some operating conditions, which may have been due to differences in the hydrocarbon composition of the low-sulfur fuels compared with that of conventional, high-sulfur fuels. This study did not measure nitroPAHs but should be relevant for these compounds (Bagley et al., 1996).

Particulate-associated and vapour-phase emissions of 2-nitrofluorene, 1,3-, 1,6- and 1,8-dinitropyrene, 1-nitropyrene, 3-nitrofluoranthene, 7-nitrobenz[*a*]anthracene and 6-nitrochrysene were measured with a low-sulfur (0.01% by mass sulfur) fuel and a particulate trap at steady-state mode 9 (Johnson et al., 1994; Table 7). Relatively large amounts of nitroPAHs were found in the vapour phase. In contrast to

Table 7. Particulate-associated (SOF) and vapour-phase (XOC) nitroPAH emissions with a low-sulfur fuel and a particulate trap at steady-state mode 9[a]

	Phase[b]	Mean nitroPAH level (ng/m^3)						
		2-Nitro-fluorene	1,6/1,8-Dinitropyrene	1,3-Dinitropyrene	1-Nitropyrene	3-Nitro-fluoranthene	7-Nitro-benz[*a*]anthracene	6-Nitrochrysene
Baseline	SOF	420	550	420	120	1020	<24	<21
	XOC	<25	830	82	77	<38	10	140
Trap	SOF	920	1200	100	=16	25	=20	<12
	XOC	<16	1300	240	130	<24	170	38

[a] From Johnson et al. (1994).
[b] SOF = soluble organic fraction; XOC = extractable organic component.

other studies involving diesel exhausts, it is not clear why the dinitropyrene concentrations that were detected are so high relative to the 1-nitropyrene concentration (see also Harvey et al., 1994, in Table 5). The use of a low-sulfur fuel with the particulate trap is known to alter particle size distributions and partitioning of PAHs (see Table 8).

Table 8. Changes in PAH emissions with a non-catalysed particulate trap[a]

Fuel type	Particulate-associated PAH (%)	Vapour-phase PAH (%)	Overall PAH (%)
Commercial No. 2 (0.32% sulfur)	–25	+15	+2
"Low sulfur" (0.01% sulfur)	–65	+140	+18

[a] From CONCAWE (1998).

Low-sulfur no. 2 diesel fuel and 100% soy methyl ester biodiesel fuel were tested with and without an oxidation catalytic converter over a light-duty transient test cycle (Bagley et al., 1998). Of the nitroPAH compounds analysed (1-nitropyrene, 2-nitrofluorene, 6-nitrochrysene and 1,3- and 1,6-dinitropyrenes), only 1-nitropyrene was found in quantifiable levels in all particle-associated samples (although 1,3-dinitropyrene may also be present, as it co-elutes with 1-nitropyrene in the method used in this study). The use of the oxidation catalytic converter with low-sulfur no. 2 diesel fuel reduced 1-nitropyrene by 66%, but this was not significant.

From CONCAWE's review of studies on PAHs in automotive exhaust emissions, it can be said that, in general, after-treatment systems can substantially decrease PAH emissions. Diesel oxidation catalysts may be more effective in reducing the vapour-phase PAHs, whereas particulate traps seem to deal more effectively with PAHs condensed onto particulate matter. The few data on the effects of after-treatment (e.g., catalysts) on nitrated PAHs and the associated mutagenicity of the exhaust are variable, and the results of different studies can be contradictory (CONCAWE, 1998).

However, there does seem to be increasing evidence in recent studies that nitroPAHs, in particular in volatile and semivolatile fractions, are still emitted in diesel exhaust emissions. For example, in a

study by Sharp (2000), all of the PAH and nitroPAH compounds investigated were present in the exhaust of all three engines tested (Cummins N14, DDC Series 50 and Cummins B5.9) when operated on a special batch of diesel fuel, blended to meet the stringent specifications required by the US *Clean Air Act*, at levels well above the US Environmental Protection Agency (EPA) required threshold of 0.5 ng/hp-h (Table 9). Further, Sharp (2000) showed that emissions of PAH and nitroPAH compounds were substantially lower with biodiesel than with conventional diesel fuel. This is not unexpected, when considering that the biodiesel contains no aromatics and no PAH compounds. The catalyst-out nitroPAH data presented an unexpected trend, in that catalyst-out nitroPAH levels were significantly higher than engine-out levels. This trend was most evident with the lighter nitroPAH compounds.

Diesel fuel, engine types and catalytic traps/converters are continually being modified, so the various studies of nitroPAHs in diesel exhaust cannot be directly compared. A detailed survey of nitroPAHs in exhaust emissions under various conditions is beyond the scope of this document.

Although diesel use is likely to increase during the coming years (Lloyd & Cackette, 2001), a relatively small number of tests have been conducted to determine the gaseous, semivolatile and particulate organic matter in diesel fuel and exhaust. Very little is known about how this composition changes with different operating conditions and the introduction of new technologies. Further understanding will require significant improvements in the analytical methods and procedures used in emissions testing of diesel engines (Chow, 2001).

3) Diesel engine oil

New diesel engine oil did not contain 1-nitropyrene at a detection limit of 0.1 mg/litre; after a vehicle travel distance of 9000 km, however, 0.5 mg/litre was detected in the used oil. The presence of 1-nitropyrene in diesel engine oil points to another source of environmental contamination (e.g., used engine oil; Jensen et al., 1986).

Table 9. PAHs or nitroPAHs measured in exhaust from different types of engine with diesel fuel with and without an oxidation catalyst[a]

PAH or nitroPAH	Mass in exhaust (ng/hp-h)				
	Cummins N14	DDC 50		Cummins B5.9	
	No catalyst	No catalyst	Catalyst	No catalyst	Catalyst
Benzo[*a*]pyrene	1435	1614	226	723	241
2-Nitrofluorene	123	88	90	257	478
1-Nitropyrene	82	83	76	210	2171
7-Nitrobenz[*a*]anthracene	1.9	1.4	1.4	34	60
6-Nitrochrysene	0.8	0.8	5.8	11	56
6-Nitrobenz[*a*]pyrene	2.5	11.1	4.2	6.1	6.8

[a] From Sharp (2000).

3.2.1.2 *Diesel compared with gasoline exhaust*

Concentrations of some nitroPAHs detected in the exhaust particulate from mufflers of gasoline engines were much lower than those from diesel engines — for example, for 1-nitropyrene (0.16 versus 27.7 µg/g tar, respectively) and 2-nitrofluorene (0.16 versus 5.52 µg/g tar, respectively) (Handa et al., 1983). These differences were not as pronounced in a comparative study of 1-nitropyrene in the soluble organic fraction (SOF) of particles from exhaust emissions of in-use gasoline- and diesel-powered passenger cars under simulated driving conditions (mean 27.4 versus 53 µg/g, respectively) (Tejada et al., 1986).

Particulate-associated 1-nitropyrene was emitted at a rate of 0.03–0.05 µg/km driving distance from two three-way catalyst-equipped light-duty gasoline-fuelled vehicles using the US FTP-75 driving cycle under three different driving conditions — cold transient, stabilized and hot transient (Westerholm et al., 1996). (Three-way catalysts for gasoline exhaust seem to be effective in reducing both vapour- and particulate-phase PAHs [CONCAWE, 1998].)

Although the concentration of 1-nitropyrene, for example, was less in gasoline particles than in diesel particles, the concentrations of 1,3-, 1,6- and 1,8-dinitropyrene were found to be almost the same (Hayakawa et al., 1994; Sera et al., 1994; see Table 6).

3.2.1.3 *Aeroplane emissions*

Studies on the mutagenicity of aircraft (jet aeroplane) particulate extracts suggested the presence of nitroarenes, but no analytical determination was made (McCartney et al., 1986). The mutagenicity derived from idling aircraft was greater than that collected across an active runway. This is in agreement with the finding that particulates from idling aircraft are much richer in PAHs than those collected during simulated landings and takeoffs (Robertson et al., 1980). PAHs emitted during simulated idling are greatly enriched with respect to three- and four-ring structures. The majority of organic pollutants in airports come from idling aircraft. Takeoffs contribute only 1–2% to the total burden (Gelinas & Fan, 1979). In simulation experiments, the particulate-adsorbed PAHs constitute less than 1% of the total PAHs emitted by aircraft, the remainder residing in the gaseous phase (Robertson et al., 1980).

3.2.1.4 Emissions from combustion of heating oils

NitroPAHs have been detected in the emissions of kerosene heaters, fuel gas and liquefied petroleum gas (LPG) burners, which are used in many countries (e.g., Japan, China, Taiwan; also mobile homes in USA) for heating and cooking at home (Tokiwa et al., 1985, 1990a; Kinouchi et al., 1988).

In a study on indoor air concentrations in mobile homes with kerosene heaters, 1-nitronaphthalene, 2- or 3-nitrofluoranthene and 1-nitropyrene were found in the particulate phase (dinitropyrenes were below the detection limit). In the semivolatile organic fraction, from these nitroPAHs, only 1-nitronaphthalene was detected (naphthalene itself was present at a concentration more than 1000-fold higher than the 1-nitronapthalene concentration) (Mumford et al., 1991).

3.2.1.5 Fumes from cooking oils

Fume samples from three different commercial cooking oils frequently used in Taiwan were collected and analysed. As well as several PAHs (benzo[*a*]pyrene [BaP], benz[*a*]anthracene and dibenz[*a*,*h*]-anthracene), two nitroPAHs were also identified. Concentrations of 1-nitropyrene and 1,3-dinitropyrene were, respectively, 1.1 and 0.9 µg/m^3 in fumes from lard oil, 2.9 and 3.4 µg/m^3 from soybean oil, and 1.5 and 0.4 µg/m^3 from peanut oil (Wu et al., 1998). (The incidence of lung cancer in Chinese women is relatively high and is thought to be associated with cooking practices [Ko et al., 1997].)

3.2.1.6 Other combustion sources

Williams et al. (1986) reported significant levels of 18 different species of nitroPAHs in diesel extract but did not find any in coke oven mains, roofing tar vapour or cigarette smoke condensate at a detection level of <50 pg. The lack of 1-nitropyrene, 1-nitronaphthalene and 6-nitrochrysene in mainstream cigarette smoke was also shown by El-Bayoumy et al. (1985).

In another study, coke oven emission extractable organic matter was found to contain 3-nitrophenanthrene and 1-nitropyrene (66 and 27 ng/mg, respectively) in the slightly polar fraction and 9-nitroanthracene and 3-nitrophenanthrene (78 and 23 ng/mg, respectively) in the acidic fraction. However, 9-nitrophenanthrene, 3-nitrofluoranthene, 6-nitrochrysene and 6-nitrobenzopyrene were not detectable. *In vitro* mammalian cell cultures were used to determine whether DNA adducts derived from individual nitroPAHs or from organic extracts of coke oven emissions can be detected. Using the ^{32}P-postlabelling method, 4-nitropyrene, 6-nitrochrysene and 3-nitrofluoranthene were reported to cause 10–100 DNA adducts per 10^8 nucleotides. When the extractable organic matterwas used, the results suggest that nitroPAH adducts (detected by ^{32}P-postlabelling) were present and may contribute to the genotoxicity of coke oven emissions (Topinka et al., 1998).

Nitroarenes were found to be an important contributor to the mutagenic activity of the emissions from municipal waste incinerators (DeMarini et al., 1996; see also section 7.5.5.7). 1-Nitropyrene was identified in coal fly ash (Harris et al., 1984). NitroPAHs, in particular 2-nitrofluoranthene and 2-nitropyrene, have been detected in the stack emissions of a plant manufacturing carbon electrodes (Ciccioli et al., 1988, 1989).

3.2.2 Atmospheric formation of nitroPAHs

The gas-phase formation of nitroPAHs in the atmosphere was first proposed by Pitts et al. (1985a) to explain the unexpected presence of 2-nitrofluoranthene and 2-nitropyrene in particulate organic matter sampled in the Los Angeles basin in California, USA (see chapter 5). These two nitroPAHs have not been identified in diesel exhaust or other combustion products. The occurrence of these nitroPAHs in different locations of southern California (Arey et al., 1987; Atkinson et al., 1987a; Zielinska et al., 1989a) and Europe (Nielsen & Ramdahl, 1986; Ramdahl et al., 1986; Ciccioli et al., 1989; Feilberg et al., 2001) as well as in forest and remote areas of America and Asia (Ciccioli et al., 1996; see chapter 5 and Table 19) showed their ubiquitous presence. Further studies showing differences in daytime/nighttime concentrations of certain nitroPAHs under various climatic conditions (see

Figure 6 in chapter 5) provided further support for the gas-phase formation of nitroPAHs.

A further confirmation of the atmospheric origin of 2-nitrofluoranthene is the finding of its absence in tunnel air (Queensway, Birmingham, United Kingdom) and its abundant presence in the ambient atmosphere of this city (Dimashki et al., 2000; see also Table 10).

Table 10. Measurements of nitroPAHs in the atmosphere showing distribution in particulate and vapour phases[a]

Compound	Particulate (ng/m^3)		Vapour (ng/m^3)	
	Mean (*n* = 25)	Range	Mean (*n* = 5)	Range
1-Nitronaphthalene	$<0.61 \times 10^{-4}$		0.089	0.033–0.207
2-Nitronaphthalene	$<1.4 \times 10^{-4}$		0.067	0.027–0.176
9-Nitroanthracene	0.130	0.034–0.520	0.057	0.014–0.177
1-Nitropyrene	0.090	0.019–0.204	$<0.22 \times 10^{-4}$	
2-Nitrofluoranthene	0.221	0.046–0.586	$<0.21 \times 10^{-4}$	
7-Nitrobenz[*a*]anthracene	0.033	0.011–0.059	$<0.74 \times 10^{-4}$	

[a] From Dimashki et al. (2000); Birmingham, United Kingdom (November 1995 – February 1996).

Two-ring nitroPAHs are present mainly in the vapour phase; under some climatic conditions, other nitroPAHs are also present in the vapour phase. For example, according to Arey et al. (1987) in a study in California, the four-ring PAHs fluoranthene and pyrene were mainly (≥90%) present in the gas phase; even at 0 °C, 30% and 70%, respectively, were measured in the gas phase and were therefore available for reaction. However, the nitrofluoranthenes and nitropyrenes exist almost exclusively in the particulate phase. 2-Nitrofluoranthene and 2-nitropyrene formed in the gas phase by hydroxyl-initiated reactions were observed to condense on airborne particles immediately, so that these particles were not detected in the gas phase (Fan et al., 1995). Ambient data from California suggest that the nitrofluorenes are distributed between the gas and particulate phases (Helmig et al., 1992c). Table 10 shows the concentration of selected nitroPAHs measured in vapour and particulate phases in ambient air.

Laboratory studies were carried out to understand the formation of nitroPAHs in ambient air (e.g., Arey et al., 1986, 1989a, 1990; Zielinska et al., 1989b; Atkinson et al., 1990a,b). From these studies, it was concluded that gas-phase daytime hydroxyl radical- and nighttime nitrate radical-initiated reactions of simple volatile and semivolatile PAHs do form nitroPAH derivatives (Atkinson, 1990).

Both daytime hydroxyl radical-initiated reactions and nighttime nitrate radical-initiated reactions have been shown to produce ambient nitroPAHs. For references summarizing the atmospheric formation of nitroPAHs, see Atkinson & Arey (1994) and Arey (1998).

3.2.2.1 Reactions of gas-phase PAHs (and nitroPAHs) with the hydroxyl radical (daytime reactions)

The photolysis of ozone in the troposphere results in the formation of the hydroxyl radical. The hydroxyl radical-initiated reactions of PAHs lead to the formation of nitroPAHs in low yields (5% or less; see Table 11) — for example, 2-nitrofluoranthene (Arey et al., 1986; Zielinska et al., 1986b; Atkinson et al., 1990a), 2-nitropyrene (Arey et al., 1986; Atkinson et al., 1990a) and 3-nitrofluorene (Helmig et al., 1992c). The proposed mechanism involves hydroxyl radical reaction with the gaseous PAH, followed by nitrogen dioxide addition at the free radical site. Although this reaction occurs in competition with the reaction with oxygen, nitroPAH formation is preferred in the presence of sufficient nitrogen dioxide. The resulting nitroPAH products having a relatively low vapour pressure may then condense out on the surface of ambient particles (Atkinson & Arey, 1994).

This hydroxyl radical-initiated mechanism could also explain the formation of volatile nitroarenes such as 1- and 2-nitronaphthalene from gaseous naphthalene (Atkinson et al., 1987b, 1990a). Ambient measurements indicate that the atmospheric reaction products of the two-ring PAHs, such as nitronaphthalenes, remain predominantly in the gas phase. Phenanthrene is more abundant in ambient air than anthracene, fluoranthene and pyrene. Helmig et al. (1992a,b)

Table 11. NitroPAHs formed from the gas-phase reactions of PAHs known to be present in ambient air with hydroxyl radicals and nitrate radicals (both in the presence of nitrogen dioxide) and their yields[a]

PAH	Daytime reactions: Initiation by hydroxyl radical followed by reaction with nitrogen dioxide	Nighttime reactions: Reaction with nitrate radical
Naphthalene	1-Nitronaphthalene (0.3%) 2-Nitronaphthalene (0.3%)	1-Nitronaphthalene (17%) 2-Nitronaphthalene (7%)
1-Methyl-naphthalene	All 1-methylnitronaphthalene isomers except 1-methyl-2-nitronaphthalene (~0.4%)	All 1-methylnitronaphthalene isomers (~30%)
2-Methyl-naphthalene	All 2-methylnitronaphthalene isomers except 2-methyl-1- and 2-methyl-3-nitro-naphthalene (~0.2%)	All 2-methylnitronaphthalene isomers (~30%)
Acenaphthene	5-Nitroacenaphthene 3-Nitroacenaphthene 4-Nitroacenaphthene (S ~0.2%)	4-Nitroacenaphthene (40%)[b] 3-Nitroacenaphthene (~2%)[b] 5-Nitroacenaphthene (~1.5%)[b]
Acenaphthylene	4-Nitroacenaphthylene (2%)	No nitro isomers formed
Fluorene	3-Nitrofluorene (~1.4%) 1-Nitrofluorene (~0.6%) 4-Nitrofluorene (~0.3%) 2-Nitrofluorene (~0.1%)	
Phenanthrene	Two nitro isomers (not 9-nitrophenanthrene) in trace yields	Four nitro isomers (including 9-nitrophenanthrene) in trace yields
Anthracene[c]	1-Nitroanthracene, low yield 2-Nitroanthracene, low yield	1-Nitroanthracene, low yield 2-Nitroanthracene, low yield
Pyrene	2-Nitropyrene (~0.5%) 4-Nitropyrene (~0.06%)	4-Nitropyrene (~0.06%)
Fluoranthene	2-Nitrofluoranthene (~3%) 7-Nitrofluoranthene (~1%) 8-Nitrofluoranthene (~0.3%)	2-Nitrofluoranthene (~24%)

[a] From Arey (1998); Atkinson & Arey (1994).

[b] Yields for the nitrate radical addition pathway to the fused aromatic rings (Arey et al., 1989a).

[c] 9-Nitroanthracene was observed in both the hydroxyl and nitrate radical reactions, but may not be a product of these reactions, because it is also formed from exposure to nitrogen dioxide/nitric acid.

observed nitrophenanthrenes at only very low yields (#1%), although other authors (Wilson et al., 1995) found that 9-nitrophenanthrene was the second most abundant nitroPAH after 1-nitronaphthalene (see Figure 6 in chapter 5).

3.2.2.2 *Reactions of gas-phase PAHs (and nitroPAHs) with the nitrate radical (nighttime reactions)*

In ambient air, in contrast to environmental chambers, the nitrate radical is formed from the reaction of nitrogen dioxide with ozone. Concentrations of nitrate radical are low during daylight hours because of the rapid photolysis of the nitrate radical (with a photolysis lifetime at solar noon of approximately 5 s) and the rapid reactions of nitric oxide with ozone and nitrate with nitric oxide (Atkinson et al., 1992). At night, however, in the absence of nitric oxide, the concentrations of the nitrate radical and nitrogen pentoxide increase (Atkinson et al., 1986). Average nitrate radical concentrations in the lower troposphere over continental areas during nighttime hours have been estimated as 5×10^8 molecules/cm^3 (~20 ppt), but are lower over marine areas (Atkinson & Arey, 1994). In the dark, nitrate radicals react in the gas phase with PAHs to form nitro derivates in significant yield (see Table 11) (Pitts et al., 1985b,c,d; Sweetman et al., 1986; Zielinska et al., 1986b; Arey et al., 1989b; Atkinson et al., 1990a,b; Inazu et al., 1996, 1997).

The proposed mechanism of reaction of naphthalene in nitrogen pentoxide–nitrate–nitrogen dioxide–air mixtures occurs by the initial addition of the nitrate radical to the aromatic rings to form a nitrato-cyclohexadienyl-type radical, which then either decomposes to reactants or reacts exclusively with nitrogen dioxide (Atkinson & Arey, 1994).

3.3 Oxygen-containing nitroPAHs

3-Nitrobenzanthrone was found by bioassay-directed fractionation of diesel particulates (0.6–6.6 µg/g, depending on load; Enya et al., 1997). 3-Nitrobenzanthrone was also detected in airborne particle extracts from urban samples taken in autumn/winter during the day (nd–5.2 pg/m^3) and night (7.7–11.5 pg/m^3) (Enya et al., 1997). The

day/night differences could be the result of different emissions or different meteorological conditions.

Mutagenic nitropyrene lactones (El-Bayoumy & Hecht, 1986) were identified in environmental chamber simulations of atmospheric reactions of pyrene (Sasaki et al., 1995).

2- and 4-nitrodibenzopyranone were identified in the products of the gas-phase hydroxyl radical-initiated reaction of phenanthrene (Helmig et al., 1992a,b).

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION AND TRANSFORMATION

4.1 Transport and distribution between media

The transport and distribution of nitroPAHs depend on their physicochemical characteristics (see chapter 2), but data for nitroPAHs are scarce. Their behaviour in the environment is expected to be similar to that of the parent PAHs (see IPCS, 1998), for which there is more information.

4.1.1 Distribution and transport in the atmosphere

NitroPAHs are either formed in the atmosphere from PAHs or emitted directly into the atmosphere during combustion processes (see chapter 3). They can be transported in the vapour phase or adsorbed onto particulate matter. Those with liquid-phase vapour pressures greater than approximately 10^{-4} Pa at ambient air temperature (i.e., two- to four-ring PAHs and two-ring nitroPAHs) will exist at least partially in the gas phase (Atkinson & Arey, 1994). NitroPAHs having a relatively low vapour pressure will condense out on the surface of ambient particles. Based on ambient measurements, 1- and 2-nitronaphthalene are expected to be found predominantly in the gas phase (Arey et al., 1987). This has also been shown for 1-nitronaphthalene in diesel exhaust samples (Feilberg et al., 1999). Concentrations of a number of PAHs and nitroPAHs were measured (Arey et al., 1987; Atkinson & Arey, 1994), and it was shown that at daytime temperatures in, for example, California, USA, the four-ring PAHs fluoranthene and pyrene are mainly (≥90%) present in the gas phase; even at 0 °C, 30% and 70%, respectively, were measured in the gas phase and were therefore available for radical-initiated reactions. There is currently no clear understanding of the partitioning of PAHs between the gas and particulate phases or of the size distribution and mass of the particulates in exhaust gases (CONCAWE, 1998). This is equally true for ambient air, in particular for nitroPAHs.

Just as PAHs are ubiquitous in the environment, so are nitroPAHs. This has been shown in particular for nitroPAHs that are formed not by combustion but by atmospheric transformation (e.g., 2-nitrofluoranthene and 2-nitropyrene), which have been found in different types of airsheds throughout the world (Ciccioli et al., 1995, 1996; see also chapters 3 and 5).

4.1.1.1 Distribution of nitroPAHs between fine and coarse fractions of inhalable atmospheric particulates

In recent monitoring investigations in downtown Rome, Italy, 1-nitropyrene, a marker indicative of direct emissions, was found not only in the coarse fraction (2.5–10 µm) of atmospheric particulates, but also in the fine fraction (0.01–2.5 µm [$PM_{2.5}$]), whereas 2-nitrofluoranthene of photochemical origin was mostly found in the fine particulate fraction (Cecinato et al., 1999). 1-Nitropyrene and 1,3-, 1,6- and 1,8-dinitropyrene monitored in Kanazawa, Japan, were found almost exclusively in the particulate fraction =1.1 µm (Hayakawa et al., 1999b). It should be noted that particles with diameters below 2.1 µm can reach terminal bronchi and alveoli (Cecinato et al., 1999).

4.1.2 Distribution and transport in the hydrosphere

Owing to their low aqueous solubility or insolubility, nitroPAHs are not expected to accumulate in the hydrosphere. However, considering the low Henry's law constants (see Table 2 in chapter 2), nitro PAHs present in the hydrosphere are not expected to be transferred significantly to the gas phase.

4.1.3 Adsorption onto soils and sediments

Although data are scarce, the sorption coefficients (log K_{oc}) for nitroPAHs are high, indicating that nitroPAHs adsorb strongly to the organic fraction of soils and sediments. Leaching into groundwater is therefore thought to be negligible.

4.1.4 Bioaccumulation

The affinity of nitroPAHs for organic phases is much higher than that for water. The *n*-octanol/water partition coefficients ($\log K_{ow}$) range from 2.5 for 1-nitronaphthalene to 6.3 for 3-nitroperylene (see Table 2), indicating a potential for bioaccumulation. Bioaccumulation of 2-nitrofluorene by *Daphnia magna* was reported to follow first-order kinetics (Gang & Xiaobai, 1994). A bioconcentration factor of 170 was reported for daphnia exposed to a 2-nitrofluorene concentration of 0.124 mg/litre for up to 8 h.

4.1.5 Biomagnification

There were no data on biomagnification.

4.2 Transformation

4.2.1 Biotransformation

The diverse metabolic pathways for the microbial metabolism of nitroPAHs are summarized in Figure 4.

Less is known about the metabolism of nitroPAHs by aquatic and terrestrial microorganisms than for the parent PAHs. Although a wide variety of bacteria, fungi and algae have been shown to degrade the parent PAHs containing two to five rings, nitro-substituted PAHs are only slowly degraded by indigenous microorganisms and may persist in soils and sediments. The recalcitrance of high molecular weight nitroPAHs is due in part to the strong adsorption to soil organic matter, low solubility, large molecular size and the hydrophilic character of the nitro group (Cerniglia & Somerville, 1995).

4.2.1.1 Bacteria

The stability of 1-nitropyrene and 1,6-dinitropyrene was studied in four samples of water (sea, unpolluted river, polluted river and pond water) and filtrates of various soil suspensions with and without 0.1% peptone (Tahara et al., 1995). The mutagenicity decreased rapidly

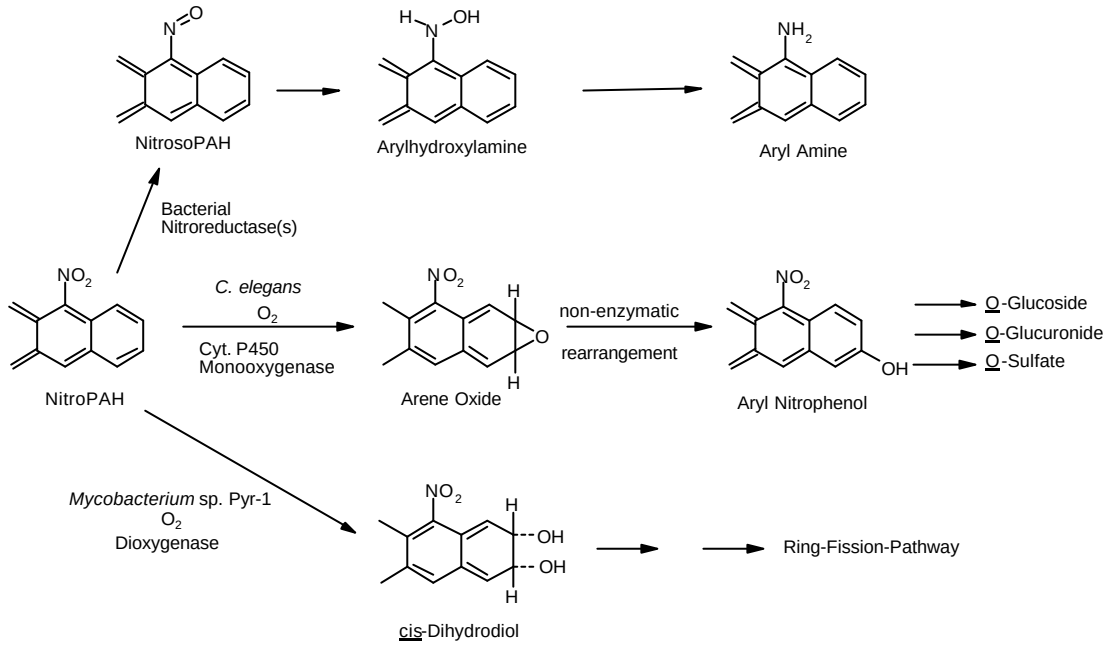


Fig. 4. Diverse metabolic pathways for the microbial metabolism of nitroPAHs (from Cerniglia & Somerville, 1995).

when 1-nitropyrene and 1,6-dinitropyrene were incubated at 30 °C, but not when the test solutions had been autoclaved. Mutagenicity attributed to 1-nitropyrene (3 µg/ml) decreased by 50% in 1.95–3.55 days for water samples and in 0.56–2.37 days for soil filtrate depending on the content of microflora in the test solutions. 1-Aminopyrene was detected as a degradation product of 1-nitropyrene. Mutagenicity attributed to 1,6-dinitropyrene (10 µg/ml) decreased by 50% in 0.53–2.15 days for water samples and in 0.50–0.61 days for soil filtrate.

Time course studies in microcosms showed that 1-nitropyrene was degraded slowly under aerobic and anaerobic conditions in estuarine sediments. Less than 1% had been converted to $^{14}CO_2$ after 8 weeks of aerobic incubation. Addition of 1-nitropyrene to anaerobic sediments resulted in no $^{14}CO_2$ evolution, but the 1-nitropyrene was reduced to 1-aminopyrene. The low mineralization of 1-nitropyrene compared with that of the parent compound pyrene could be due to the nitro substituent in the C1 position decreasing the enzymatic oxidation (Cerniglia & Somerville, 1995).

A bacterium isolated from sediments chronically exposed to petrogenic hydrocarbons mineralized 1-nitropyrene and 6-nitrochrysene only to a small extent (12.3% and 2%) compared with non-nitrated PAHs after 10 days of incubation (Heitkamp & Cerniglia, 1988). In pure culture, this bacterium, *Mycobacterium* sp. strain Pyr-1, was found to metabolize nitroPAHs by both oxidative and reductive pathways. In media with pyrene, the cells oxidized up to 20% of the added 1-nitropyrene to 1-nitro-*cis*-9,10- and 1-nitro-*cis*-4,5-dihydrodiols (Heitkamp et al., 1991). However, cells that had been grown in media without pyrene did not produce dihydrodiols, but reduced up to 70% of the 1-nitropyrene to aminopyrene. Further, extracts from cells that had been grown without pyrene activated 1-nitropyrene, 1,3- and 1,6-dinitropyrene and 6-nitrochrysene to DNA-damaging products, as shown in *Salmonella typhimurium* tester strains and by the *umu* test (Rafii et al., 1994).

Sphingomonas paucimobilis strain EPA 505 (a soil bacterium capable of utilizing fluoranthene as the sole source of carbon and energy) biodegraded 1-nitropyrene to 48.6% after 6 h (Ye et al., 1996).

4.2.1.2 Fungi

The filamentous fungus *Cunninghamella elegans* has been shown to metabolize a number of nitroPAHs via oxidation pathways to products that are, in general, less mutagenic than the nitroPAHs themselves. The nitroPAH is initially oxidized via a cytochrome P450 monooxygenase to arene oxides, which isomerize to form phenols (hydroxyl derivatives) or are enzymatically hydrated to form *trans*-dihydrodiols. The phenols can be subsequently conjugated with sulfate, glucose, xylose or glucuronic acid to form detoxified products (Cerniglia & Somerville, 1995) (Table 12 and Figure 4). The metabolites formed are similar to those oxidative metabolites found in rat microsomes and *in vivo* studies (see chapter 6); however, whereas the *trans*-dihydrodiol metabolites in rat liver microsomes are predominantly in the R,R configuration, metabolism by *C. elegans* produces *trans*-dihydrodiol metabolites in the S,S absolute configuration.

These studies with the fungus *Cunninghamella elegans* have been extended to include comparison of the biotransformation of nitroPAHs with that of their parent PAHs. For example, comparison of the metabolism pattern between 1-nitrobenzo[*e*]pyrene and its parent PAH, benzo[*e*]pyrene, indicates that the nitro group at the C1 position of benzo[*e*]pyrene drastically altered the regioselectivity of the metabolism (Pothuluri et al., 1999a).

The fungal biotransformation of a mixture of 2- and 3-nitrofluoranthenes was similar to that of the individual nitrofluoranthenes; however, the mammalian system (rat liver microsomes) showed differences in the regioselectivity of nitrofluoranthene at positions C4, C5, C8 and C9 (Pothuluri et al., 1998a).

4.2.1.3 Plants

A plant cell culture (100 mg/ml wet weight) derived from alligator weed (*Alternanthera philoxeroides*) detoxified 1-nitropyrene and 1,3-, 1,6- and 1,8-dinitropyrene, all direct-acting mutagens, when incubated with them, as shown by mutagenicity response in the *Salmonella typhimurium* TA98 assay (Shane et al., 1993).

Table 12. Biotransformation of nitroPAHs by the fungus *Cunninghamella elegans* (48-h incubation)

NitroPAH	Metabolites (major metabolites are in bold)	Reference
1-Nitropyrene	Glucoside conjugates of **1-nitropyren-6-ol** and **1-nitropyren-8-ol**	Cerniglia et al. (1985)
2-Nitrofluoranthene	Sulfate conjugates of **2-nitrofluoranthen-8-ol** and **2-nitrofluoranthen-9-ol**	Pothuluri et al. (1998a)
3-Nitrofluoranthene	Sulfate conjugates of **3-nitrofluoranthen-8-ol** and **3-nitrofluoranthen-9-ol**	Pothuluri et al. (1994)
6-Nitrochrysene	6-Nitrochrysene-1-sulfate 6-Nitrochrysene-2-sulfate	Pothuluri et al. (1998b)
1-Nitrobenzo[*e*]pyrene	Sulfate and glucoside conjugates of **1-nitrobenzo[*e*]pyren-6-ol**	Pothuluri et al. (1999a)
6-Nitrobenzo[*a*]pyrene	Glucoside conjugates of **6-nitrobenzo[*a*]pyren-1-ol** and **6-nitrobenzo[*a*]pyren-3-ol** Sulfate conjugate of 6-nitrobenzo[*a*]pyren-1-ol	Millner et al. (1986); Cerniglia & Somerville (1995)
2-Nitrofluorene	**2-nitrofluoren-9-ol**, 2-nitro-9-fluorenone, 2-nitro-fluoren-6-ol Sulfate conjugates of 2-nitro-9-fluorenon-7-ol, 2-nitrofluoren-7-ol	Pothuluri et al. (1996)
9-Nitroanthracene	Phenol and dihydrodiol derivatives	Pothuluri et al. (1999b)

4.2.1.4 *Aquatic animals*

The biotransformation of aquatic species is discussed in chapter 9.

4.2.2 Abiotic degradation

4.2.2.1 *Direct photolysis*

The photolysis of nitroPAHs has been studied under varied conditions of irradiation (see Table 13). The rate of photolysis depends not only on the conditions of irradiation but also on whether the nitroPAH is in the gaseous stage (e.g., 1-and 2-nitronaphthalene), in solution (type of solvent) or bound to solids or particles. In the latter case, the type and age of the particle seem to influence the photochemistry of

Table 13. Rates of photolysis of nitroPAHs in different media and comparison of the mutagenic activity of the products

NitroPAH	Media	Source of light	Major product	Mutagenic activity[a]	Rate of photolysis	Reference
1-Nitronaphthalene	Gaseous	Sunlight	1,4-Naphthoquinone		Atmospheric lifetime = 1.7 h	Atkinson et al. (1989)
1- and 2-nitro-naphthalenes	Gaseous	Sunlight			0.5 h and 11 h, respectively	Feilberg et al. (1999)
1- and 2-nitro-naphthalenes	2-Propanol	320–418 nm for 2 h			No degradation	Stärk et al. (1985)
3-Nitrofluoranthene	DMSO			Less	12.5 days	Holloway et al. (1987)
2-Nitrofluorene	DMSO	Cool-white light or artificial sunlight; up to 5 h		More		White et al. (1985)
9-Nitroanthracene	Acetone and silica gel		Mainly 9,10-anthraquinone (via iminoxyl radical)			Chapman et al. (1966); Pitts et al. (1978)
9-Nitroanthracene	Hexane		Anthraquinone		Transformed to 40% after a 1-day exposure to light	Schlemitz & Pfannhauser (1997)
1-Nitropyrene	Solid	Sunlight	Pyrenol, pyrene quinone, pyrenediol	Less	Biphasic; half-times = 14 h and 533 h	Benson et al. (1985)
1-Nitropyrene	Methanol	>300 nm in presence of oxygen	Pyren-1-ol (88%) and 2-nitropyren-1-ol (7%)		95% transformation after 2.5 h	van den Braken-van Leersum et al. (1987)
1-Nitropyrene	Benzene	Sunlight	Nitropyren-9-ol	Less	Rapid, 10% after 1 h; 53% after 10 h	Koizumi et al. (1994)
1-Nitropyrene	Soot	Sunlight	Nitropyren-9-ol	Less	Slow, 42% in 40 days	Koizumi et al. (1994)
1-Nitropyrene	DMSO	Light (>310 nm)		Less	1.2 days	Holloway et al. (1987)

Table 13 (Contd).

NitroPAH	Media	Source of light	Major product	Mutagenic activity[a]	Rate of photolysis	Reference
1-Nitropyrene	Silica	Light (>310 nm)		Less	6 days	Holloway et al. (1987)
1-Nitropyrene	2-Propanol	320–418 nm for 90 min		Almost total loss	Rapid	Stärk et al. (1985)
1-Nitropyrene	DMSO	Fluorescent sun-lamps; 4 and 24 h	Pyrene quinone, pyrenol, 1-nitropyrenol	Less		Yang et al. (1994)
2-Nitropyrene	Methanol	>300 nm in presence of oxygen	Very stable		Only 19% conversion after 7.5 h	van den Braken-van Leersum et al. (1987)
4-Nitropyrene	Methanol	>300 nm in presence of oxygen	Pyrene (9%) and traces of other products			van den Braken-van Leersum et al. (1987)
1,8-Dinitropyrene	DMSO	Light (>310 nm)	1-Nitropyren-8-ol		0.7 days	Holloway et al. (1987)
1,8-Dinitropyrene	Silica	Light (>310 nm)	1-Nitropyren-8-ol		5.7 days	Holloway et al. (1987)
1,8-Dinitropyrene	Silica			Less	>20 days	Holloway et al. (1987)
7-Nitro-benz[*a*]-anthracene	DMSO	Cool-white light or artificial sunlight; up to 5 h		More		White et al. (1985)
7-Nitrodibenzo[*a*,*h*]-anthracene	DMSO	Fluorescent sun-lamps; 4, 24 and 48 h		More at 4 h, then decreasing		Yang et al. (1994)
9-Nitrodibenzo[*a*,*c*]-anthracene	DMSO	Fluorescent sun-lamps; 4, 24 and 48 h		More at 4 h, then decreasing[b]		Yang et al. (1994)
1-Nitrobenzo[*a*]-pyrene	DMSO	Fluorescent sun-lamps; 4, 24 and 48 h		Less		Yang et al. (1994)
6-Nitrobenzo[*a*]-pyrene	Silica gel		Benzo[*a*]pyrene quinones: 1,6-, 3,6- and 6,12- isomers			Pitts (1983)

Table 13 (Contd).

NitroPAH	Media	Source of light	Major product	Mutagenic activity[a]	Rate of photolysis	Reference
6-Nitrobenzo[*a*]-pyrene	DMSO	Cool-white light or artificial sunlight; up to 5 h		More		White et al. (1985)
6-Nitrobenzo[*a*]-pyrene	In solution	UV light	Benzo[*a*]pyrene-3,6-quinone via benzo[*a*]pyren-6-oxyl radical			Ioki (1977)
3,6-Dinitrobenzo[*a*]-pyrene		UV light at 312 nm	3-Nitrobenzo[*a*]pyrene-6-quinone			Sera et al. (1991)
3-Nitrobenzo[*e*]-pyrene	DMSO	Cool-white light or artificial sunlight; up to 5 h		More		White et al. (1985)

[a] Compared with original nitroPAH.
[b] Also increasing cytotoxicity with length of exposure time.

the respective nitroPAH. The rate of photodecomposition, identification of photolytic products and the resulting loss or gain of mutagenic activity as determined by the *Salmonella typhimurium* assay have been the main end-points studied. Decomposition products include quinones, hydroxy-nitroPAHs and hydroxy PAHs (see Table 13). Although most studies show that the mutagenic activity of decomposition products was less than that of the original nitroPAH, some results show an increase.

1) Photolysis of gaseous nitroPAHs

Feilberg et al. (1999) showed that 1-nitronaphthalene photolyses faster than 2-nitronaphthalene. Using a large outdoor smog chamber facility, the gas-phase photolysis rates were determined to be $0.07 \times k_{NO2}$ and $0.005 \times k_{NO2}$ for 1- and 2-nitronaphthalene, respectively (Feilberg et al., 1999). Using an average k_{NO2} of 5.2×10^{-3}/s, the life times of these two nitroPAHs with respect to photolysis were calculated to be 0.5 and 11 h, respectively. Therefore, gas-phase photolysis is the major degradation pathway for 1-nitronaphthalene; for 2-nitronaphthalene, other pathways (such as reaction with hydroxyl radicals; see Tables 14 and 15) may be important (Feilberg et al., 1999).

Table 14. Room temperature rate constants, *k*, for the gas-phase reactions of hydroxyl radicals, nitrate radicalsand ozone with nitronaphthalenes[a]

Nitronaphthalene	*k* (cm^3/molecule per second) for reaction with		
	Hydroxyl (OH)[b]	Nitrate (NO_3)	Ozone (O_3)[b]
1-Nitronaphthalene	5.4×10^{-12}	3.0×10^{-29} $[NO_2]$[b]	$<6 \times 10^{-19}$
2-Nitronaphthalene	5.6×10^{-12}	2.7×10^{-29} $[NO_2]$[b]	$<6 \times 10^{-19}$

[a] Adapted from Atkinson & Arey (1994).
[b] Atkinson (1991).

2) Photolysis of PAHs in solution or on solids

Studies comparing the photodecomposition of nitroPAHs on solids or in solution show that decomposition rates or times are much longer on solids (see Table 13; e.g., with 1-nitropyrene [Koizumi et al., 1994]; 1-nitropyrene, 1,8-dinitropyrene, 3-nitrofluoranthene

Table 15. Calculated atmospheric lifetimes of nitronaphthalenes due to photolysis and gas-phase reactions with hydroxyl and nitrate radicals and with ozone

Nitronaphthalenes	Atmospheric lifetimes due to reaction with			
	Hydroxyl (OH)	Nitrate (NO_3)	Ozone (O_3)	Photolysis (solar radiation)
1-Nitronaphthalene	2.7 days[a]	18 years[b]	>28 days[c]	0.5 h[d]
2-Nitronaphthalene	2.6 days[a]	20 years[b]	>28 days[c]	11 h[d]

[a] From Atkinson & Arey (1994) for a 12-h daytime average hydroxyl radical concentration of 1.6×10^6 molecules/cm^3 (Prinn et al., 1992).

[b] From Atkinson & Arey (1994) for a 12-h average nighttime nitrate radical concentration of 5×10^8 molecules/cm^3 (Atkinson, 1991) and nitrogen dioxide concentration of 2.4×10^{11} molecules/cm^3.

[c] From Atkinson & Arey (1994) for a 24-h average ozone concentration of 7×10^{11} molecules/cm^3 (Logan, 1985).

[d] From Feilberg et al. (1999) using an average 12-h daytime nitrogen dioxide photolysis rate $k_{NO_2} = 5.2 \times 10^{-3}$/s.

[Holloway et al., 1987]; see also section 3) below on photostability of nitroPAHs on particles).

Several authors have studied the photolysis of isomeric or other groups of nitroPAHs and compared their rates of decomposition and their mutagenicities with their differences in chemical structure (see Table 13).

Some nitroPAHs can be readily decomposed when exposed to light both in solution or on particles to form quinones and possibly phenolic derivatives (Pitts, 1983). The reactions are complex and depend on the presence or absence of air, the type of solvent and the wavelength of light used (Chapman et al., 1966). A mechanistic theory was suggested using the example of 9-nitroanthracene. Illumination in acetone resulted in rearrangement into a nitrite, followed by dissociation into nitric oxide and a phenoxy-type radical and ultimately anthraquinone (Chapman et al., 1966). 9-Nitroanthracene forms 9,10-anthraquinone on irradiation both in solution and on silica gel (Pitts et al., 1978).

UV light-induced oxidation of 6-nitrobenzo[*a*]pyrene resulted in the formation of benzo[*a*]pyrene-3,6-quinone (Ioki, 1977). 3,6-Dinitrobenzo[*a*]pyrene was readily decomposed by UV radiation at 312 nm to 3-nitrobenzo[*a*]pyrene-6-quinone (Sera et al., 1991).

6-Nitrobenzo[*a*]pyrene on silica gel photolyses rapidly to BaP quinones (1,6-, 3,6- and 6,12- isomers); the 1- and 3- isomers are more stable (Pitts, 1983). (According to Chapman's hypothesis, the 6-nitro-isomer with two *peri*-hydrogens should be less stable than the 1- and 3- isomers with only one *peri*-hydrogen.)

A 1-day exposure of 9-nitroanthracene to light resulted in 40% conversion to anthraquinone (Schlemitz & Pfannhauser, 1997).

The influence of the nitro group on the aromatic B-system of pyrene has been studied by comparing the spectroscopic and photo-chemical properties of 1-, 2- and 4-nitropyrene (van den Braken-van Leersum et al., 1987). Whereas the UV and mass spectra of 1- and 4-nitropyrene show an interaction normal for nitro-aromatic compounds, 2-nitropyrene shows a lack of interaction, reflected in a UV spectrum very similar to that of pyrene and a mass spectrum with a low abundance of [M–NO]. The photochemical behaviour of the three compounds is governed by the degree of interaction. In the presence of oxygen, 1-nitropyrene shows the nitro-nitrite rearrangement, leading to 1-hydroxypyrene (pyren-1-ol) (88%) and 1-hydroxy-2-nitropyrene (2-nitropyren-1-ol) (7%); under anaerobic conditions, equimolar amounts of 1-nitrosopyrene and 2-nitropyren-1-ol are formed. The photo-chemical products of 4-nitropyrene in air are pyrene (9%) and unstable products that react with the solvent. 1-Nitropyrene was more reactive than 4-nitropyrene. 2-Nitropyrene is very stable under photochemical conditions due to lack of interaction. After irradiation of a solution of 2-nitropyrene 3 times longer than needed for 95% conversion of 1-nitropyrene, 81% of the starting material was recovered.

Yang et al. (1994) studied the photodecomposition of four sets of isomeric nitroPAHs (total of 10 nitroPAHs) in DMSO for 4, 24 and 48 h under fluorescent sunlamps. Decomposition products were multiple — e.g., quinones and/or hydroxy-nitroPAHs. The order of ease of

photodecomposition for the different isomeric groups was as follows (see also Table 16):

6-nitro-BaP > 1-nitro-BaP $ 3-nitro-BaP
1-nitropyrene > 4-nitropyrene > 2-nitropyrene
9-nitroanthracene > 2-nitroanthracene
7-nitrodibenzo[*a*,*h*]anthracene = 9-nitrodibenzo[*a*,*c*]anthracene

Table 16. Comparison of the decomposition rates of the isomeric nitroPAHs after exposure in DMSO to fluorescent sunlamps[a]

Compound	Nitro orientation	Degree of decomposition
6-Nitrobenzo[*a*]pyrene	Perpendicular	+ + + + + +
1-Nitrobenzo[*a*]pyrene	Parallel	+ + +
3-Nitrobenzo[*a*]pyrene	Parallel	+ + +
1-Nitropyrene	Parallel	+ + +
4-Nitropyrene	Parallel	+ +
2-Nitropyrene	Parallel	+
9-Nitroanthracene	Perpendicular	+ + + +
2-Nitroanthracene	Parallel	+ +
7-Nitrodibenzo[*a*,*h*]anthracene	Perpendicular	+ + + +
9-Nitrodibenzo[*a*,*c*]anthracene	Perpendicular	+ + + +

[a] From Yang et al. (1994).

It appears that those nitroPAHs having a perpendicular orientation decompose faster than those with a parallel orientation (see Table 16).

1-Nitropyrene, 2-nitropyrene, 2-nitrofluoranthene, 3-nitrofluoranthene, 9-nitroanthracene and 6-nitrobenzo[*a*]pyrene were irradiated in cyclohexane solutions (Feilberg & Nielsen, 2000). In the absence of co-solutes, the photodegradation of nitroPAHs is strongly dependent on the orientation of the nitro group. In 9-nitroanthracene and 6-nitrobenzo[*a*]pyrene, the nitro group adapts an approximately perpendicular orientation relative to the aromatic plane, and, as expected, both compounds decayed very quickly, with less than 1% remaining after 15 min. 1-Nitropyrene decayed moderately, whereas 2-nitrofluorene, 2-nitrofluoranthene and 3-nitrofluoranthene were stable towards photolysis.

The mutagenicity of the photodecomposition products of 1-nitropyrene (a moderate direct-acting mutagen), 1-nitrobenzo[*a*]pyrene (a potent direct-acting mutagen), 7-nitrodibenzo[*a*,*h*]anthracene and 9-nitrodibenzo[*a*,*c*]anthracene (both non-direct-acting mutagens) were tested using the *Salmonella typhimurium* microsome assay with TA98 without metabolic activation (Yang et al., 1994). Most studies show that the mutagenic activity of decomposition products was less than that of the original nitroPAHs (Benson et al., 1985; Koizumi et al., 1994; Yang et al., 1994) (see Table 13). In contrast, following 3- or 5-h exposures in DMSO, 6-nitrobenzo[*a*]pyrene, 7-nitrobenz[*a*]anthracene, 3-nitrobenzo[*e*]pyrene and 2-nitrofluorene were found to have elevated mutagenicities in the *S. typhimurium* microsome assay when exposed to sunlight (White et al., 1985). Irradiated 6-nitrobenzo[*a*]pyrene was about 15 times more mutagenic than 7-nitrobenz[*a*]anthracene.

3) Photostability of nitroPAHs on particles

NitroPAHs that are particle-associated under atmospheric conditions may be fully or partially protected from photolysis (Atkinson & Arey, 1994).

In a chamber study, the stability of nitroPAHs (1-nitropyrene, 7-nitrobenz[*a*]anthracene and 6-nitrobenzo[*a*]pyrene) on airborne, fresh diesel soot particles was assessed (Kamens et al., 1994). They degraded very rapidly in the sunlight; the half-lives for the nitropyrenes were around 2.5 h. 6-Nitrobenzo[*a*]pyrene degraded more rapidly, with a half-life of 30 min. However, nitroPAHs associated with aerosolized diesel soot (SRM 1650) did not decay when exposed to sunlight (no further details given). 1-Nitropyrene adsorbed to coal fly ash was resistant to photodecomposition (Holder et al., 1994).

NitroPAHs, in spite of structural differences, all showed similar decay rates on diesel soot particles or wood smoke, indicating that other chemicals associated with the diesel particles somehow affect the photooxidation of nitroPAHs in sunlight and that the effect of the structure is not a dominant factor, as it is with individual nitroPAHs studied (Fan et al., 1996a; Feilberg & Nielsen, 2000, 2001).

4.2.2.2 *Other atmospheric transformations*

1) Gaseous nitroPAHs

Transformations of 1- and 2-nitronaphthalene with hydroxyl and nitrate radicals and ozone were studied, and the rate constants for the gas-phase reactions are given in Table 14.

The reactions of nitrate radicals and ozone with gaseous nitro-PAHs (see Table 15) appear to be of negligible importance as atmospheric loss processes (Atkinson et al., 1989). Reactions of gaseous nitroPAHs with hydroxyl radicals are probably of secondary importance to photolysis (e.g., for 1-nitronaphthalene), although recent studies with 2-nitronaphthalene suggest that this loss process may be as important as photolysis (Feilberg et al., 1999).

2) NitroPAHs on particles

No physical or chemical loss processes were found when 1-nitropyrene was coated on glass and Teflon filters exposed to 100 ppb of nitric acid-free nitrogen dioxide, ozone and sulfur dioxide (Grosjean et al., 1983). In contrast, 1,3-, 1,6- and 1,8-dinitropyrene were detected when 1-nitropyrene-coated particles on a filter surface were exposed to 260 mg/m^3 of nitrogen dioxide (Lee et al., 1989).

Oxidation of 1-nitropyrene adsorbed on silica gel with dimethyldioxirane led to the formation of 1-nitropyrene-4,5-oxide and 1-nitropyrene-9,10-oxide, in a ratio 74:26. When the adsorbed 1-nitropyrene was exposed to the gas-phase ozonolysis of tetramethylethylene, the same oxides were produced in the ratio 72:28. Reaction of 1-nitropyrene with ozone alone did not lead to oxide formation (Murray & Singh, 1998).

Degradation of nitroPAHs was observed in the presence of both ozone and nitrogen dioxide plus ozone on real soot particles studied in an outdoor smog chamber in both cool and warm temperatures (between 2 and 20 °C) (Fan et al., 1996b) (see Table 17). The degradation rate constants of 1- and 2-nitropyrene and 2-, 3- and 8-nitrofluoranthene with ozone ranged from 0.0015 to 0.0025/ppm per minute.

Table 17. Particle nitroPAH atmospheric half-lives of 2-nitrofluoranthene and 1-nitropyrene from photolysis and dark heterogeneous reactions with ozone[a,b]

Compound	Photolysis rate constant (min^{-1})	O_3 rate constant ($ppm^{-1}\,min^{-1}$)	Half-life in sunlight (h)	Half-life with 0.2 ppm O_3 (h)
2-Nitrofluoranthene	0.013	0.002	1	29
1-Nitropyrene	0.020	0.002	0.6	29

[a] Data from Fan et al. (1995, 1996b).

[b] NitroPAH photolysis rate constants are derived from an average nitrogen dioxide photolysis rate of k_{NO_2} = 0.5/min; an ozone concentration of 0.2 ppm (1 ppm = 1.96 mg/m^3) is assumed, which represents a moderately to highly polluted atmosphere.

The rate constant of particle nitroPAHs with nitrogen dioxide–nitrate–nitrogen pentoxide was less than 0.001/ppm per minute. Particle oxidation of nitroPAHs by ozone does not appear to be as important as photodegradation of nitroPAHs in daytime, but it may be the main loss process at night.

LINKS TO OTHER SECTIONS OF THE DOCUMENT