Liver Cancer

ATTACHED ARE THE IARC MONOGRAPHS TIED TO THE STATED OCCUPATIONAL FIREFIGHTING CANCER

IARC SUPPLEMENT 7

PAGES 100 & 101 (ARSENIC*KNOWN CARCINOGEN FOUND IN SMOKE), P. 343 (SOOT) KNOWN CARCINOGEN (refer to IARC 105 Composition of Smoke) P. 355 TETRACHLOROETHYLENE

IARC 98

PAGES 182, 399 (Additional Composition of Smoke), 400, 401, 534 (1,3 butadiene)536 (PCB's)

IARC 100F

PAGES 130, 131, & 137 (benzo{a}pyrene), 262(benzene), 349, 365, 366 & 369 (2,3,7,8-Tetrachlorodibenzo-paradioxin), 389, 390, 391 & 396 (ethylene oxide), 455, 456, 457 & 473 (Vinyl Chloride), 531 (trichloroethylene)

IARC 105

PAGES 525, 533, & 541 (1,6 Dinitropyrene), 580, 584 & 595 (6-Nitrochrysene), 607, 616 (2-Nitroflourene)

International Agency for Research on Cancer – *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; Volume 98 – Painting, Firefighting, and Shiftwork

Composition of Fire Smoke:

Smoke from fires comprises suspended liquid and solid particulate matter, gases, and vapors that result from the combustion or pyrolysis of material.

- **ALL** types of fire release toxic and carcinogenic substances.

Overall Evaluation: The agent is described according to the wording of one of the following categories, and the designated group is given. This categorization of an agent is a matter of scientific judgment that reflects the strength of evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable as to its carcinogenicity to humans
Group 4	Probably not carcinogenic to humans

Carcinogens Found in Smoke at Fires	
Chemicals measured in fires	Classification
1,3-Butadiene	1
* 2,3,7,8-tetrachloro dibenzo-*para*-dioxin	1
Arsenic	1
Asbestos	1
* Benzene	1
* Benzo[*a*]pyrene	1
Cadmium	1
Formaldehyde	1
* Polychlorinated biphenyls	1
Radioactivity (γ activity)	1
Radionuclides (α-particle-emitting)	1
Radionuclides (β-particle-emitting)	1
Silica (crystalline)	1
* Trichloroethylene	1
Dibenz[*a*,*h*]anthracene	2A
Dichloromethane (methylene chloride)	2A
Lead compounds, inorganic	2A
Tetrachloroethylene (perchloroethylene)	2A
Acetaldehyde	2B

Carcinogens Found in Smoke at Fires	
Chemicals measured in fires	Classification
2-Nitroanisole	2B
Benz[*a*]anthracene	2B
Benzo[*b*]fluoranthene	2B
Benzo[*k*]fluoranthene	2B
Benzofuran	2B
Carbon black	2B
Chrysene	2B
Ethylbenzene	2B
Furan	2B
Indeno-1,2,3-[*cd*]pyrene	2B
Isoprene	2B
Lead	2B
Naphthalene	2B
Polychlorophenols	2B
Styrene	2B
Toluene diisocyanates	2B
Trichloromethane (chloroform)	2B
Lead compounds, organic	3
Silica (amorphous)	3
Triphenylene	3

* KNOWN Products of Combustion FOR Liver Cancer

Several studies have been conducted with the purpose of identifying the chemicals and known carcinogens found **during the overhaul phase of a structure fire.**

- *Characterization of Firefighter Exposures During Fire Overhaul* (Phoenix FD and the University of Arizona Prevention Center and Arizona State University).
- *A Study on Chemicals found in the Overhaul Phase of Structure Fires using Advanced Portable Air Monitoring available for Chemical Speciation* (Tualatin Valley Fire & Rescue – Oregon)

Chemicals measured in overhaul environment	IARC Classification
1,3 Butadiene	1
Arsenic	1
Asbestos	1
* Benzene	1
Benzo(a)pyrene	1
Coal Tar Pitch	1
Diesel Exhaust	1
Formaldehyde	1
* Vinyl Chloride	1
Dibenz(a,h)anthracene	2A
N-Nitrodimethylamine	2A
1,2 Dichloroethane	2B
Acetaldehyde	2B
Benz(a) anthracene	2B
Benzo(b)fluoranthene	2B
Benzo(k)fluoranthene	2B
Benzofuran	2B
Ethyl benzene	2B
Furan	2B
Indeno(1,2,3-cd)pyrene	2B
Lead	2B
Napthalene	2B
Styrene	2B
Mercury	3
Hydrochloric Acid	3
Toluene	3
Acrolein	3
Furfural	3
Acenaphthene	3
Anthracene	3
Benzo(ghi)perylene	3
Fluoranthene	3
Fluorene	3
Phenanthrene	3
Pyrene	3

Diesel Engine Exhaust:

On June 12, 2012, the International Agency for Research on Cancer (IARC), part of the World Health Organization and the authority on cancer, classified diesel engine exhaust as a Group 1 Carcinogen, meaning that it causes cancer in humans.

Diesel engine exhaust in fire stations has been and continues to be a serious health problem for firefighters. This exhaust is generated whenever a fire apparatus leaves or returns to the station. If not properly captured and removed, it will remain in the apparatus bay as well as enter the firefighters' living quarters. As a result, firefighters can be exposed to diesel engine exhaust for a considerable portion of their shift.

Diesel exhaust contains multiple cancer-causing chemicals such as (Source IARC Monograph 105):

Metals	IARC Classification
Antimony Compounds	2B
Arsenic and inorganic arsenic compounds	1
Beryllium and beryllium compounds	1
Cadmium and cadmium compounds	1
Chromium (VI)	1
Cobalt and cobalt compounds	2B
Lead compounds (inorganic/organic0	2A/3
Nickel (metallic/compounds)	2B/1
Organic Chemicals	IARC Classification
1,3-Butadiene	1
Acetaldehyde	2B
Benzene	1
Bis(ethylhexyl)phthalate	2B
Ethylbenzene	2B
Formaldehyde	1
Propylene oxide	2B
Halogenated and other chemicals	IARC Classification
Dioxin/dibenzofurans	1
Polycyclic aromatic hydrocarbons	IARC Classification
Benz(a) anthracene	2B
Benzo(b)fluoranthene	2B
Benzo(k)fluoranthene	2B
Benzo(a)pyrene	1
Chrysene	2B
Dibenz(a,h)anthracene	2A
3,7-Dinitrofluoranthene	2B
3,9-Dinitrofluoranthene	2B
1,3-Dinitropyrene	2B
1,6-Dinitropyrene	2B
1,8-Dinitropyrene	2B
Indeno(1,2,3-*cd*)pyrene	2B
Napthalene	2B
3-Nitrobenzanthrone	2B
6-Nitrochrysene	2A
2-Nitrofluorene	2B
1-Nitropyrene	2A
4-Nitropyrene	2B
Styrene	2B

Soot:

Soot is a byproduct of the incomplete burning of organic (carbon-containing) materials, such as wood, fuel oil, plastics, and household refuse.

Soot particles absorb many hazardous chemical vapors that are released during fires, holding them in place on surfaces including firefighter's personal protective equipment (PPE), clothing and skin.

As firefighters work, their body temperature rises and they begin to sweat. Skin becomes more permeable and soot particles are more easily absorbed into the body.

- For every 5°increase in skin temperature, absorption increases by 400%.

The International Agency for Research on Cancer, part of the World Health Organization, lists soot in the Group 1 category meaning that the agent is ***"Carcinogenic in Humans."***

In their *13th Report on Carcinogens* which was released on October 2, 2014, the U.S. Department of Health and Human Services continues to list **soots** as a substance under the category of ***"Known To Be Human Carcinogens."***

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS ON THE EVALUATION OF THE CARCINOGENIC RISKS TO HUMANS

Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs* Volumes 1 to 42

SUPPLEMENT 7

LYON, FRANCE

1987

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. In 1980 and 1986, the programme was expanded to include the evaluation of carcinogenic risks associated with exposure to complex mixtures and other agents.

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed, and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in carcinogenesis and related fields; and to indicate where additional research efforts are needed.

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produced fibrosarcomas, sarcomas and haemangiosarcomas of the spleen and peritoneal cavity[1]. In several limited studies, largely negative results were obtained following oral administration to rats[1], subcutaneous injection of mice[1] and hamsters[3], and after single intraperitoneal injection of mice[4].

C. Other relevant data

No data were available on the genetic and related effects of aniline in humans.

Aniline induced sister chromatid exchanges, but not micronuclei, in bone-marrow cells of mice treated *in vivo*, and DNA strand breakage was induced in liver and kidney of rats *in vivo*. Sister chromatid exchange assays in human cells *in vitro* gave negative results. Syrian hamster embryo cells and virus-infected Fischer rat embryo cells were not transformed by aniline, but BALB/c 3T3 cells were. It induced sister chromatid exchanges and chromosomal aberrations but not DNA strand breaks or unscheduled DNA synthesis in mammalian cells *in vitro*. Aniline did not induce sex-linked recessive lethal mutations in *Drosophila* and did not induce mutation or mitotic recombination in fungi. It was not mutagenic to bacteria and did not cause DNA damage. Urine from rats treated with aniline was reported to be mutagenic to bacteria[5].

References

[1]*IARC Monographs*, *27*, 39-61, 1982

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[3]Hecht, S.S., El-Bayoumy, K., Rivenson, A. & Fiala, E.S. (1983) Bioassay for carcinogenicity of 3,2′-dimethyl-4-nitrosobiphenyl, *o*-nitrosotoluene, nitrosobenzene and the corresponding amines in Syrian golden hamsters. *Cancer Lett.*, *20*, 349-354

[4]Delclos, K.B., Tarpley, W.G., Miller, E.C. & Miller, J.A. (1984) 4-Aminoazobenzene and *N*,*N*-dimethyl-4-aminoazobenzene as equipotent hepatic carcinogens in male C57BL/6 × C3H/He F_1 mice and characterization of *N*-(deoxyguanosin-8-yl)-4-aminoazobenzene as the major persistent hepatic DNA-bound dye in these mice. *Cancer Res.*, *44*, 2540-2550

[5]*IARC Monographs*, *Suppl. 6*, 68-70, 1987

ARSENIC AND ARSENIC COMPOUNDS (Group 1*)

A. Evidence for carcinogenicity to humans (*sufficient*)

Many cases of skin cancer have been reported among people exposed to arsenic through medical treatment with inorganic trivalent arsenic compounds, particularly Fowler's

*This evaluation applies to the group of chemicals as a whole and not necessarily to all individual chemicals within the group (see also Methods, p. 38).

solution[1], and further reports have confirmed these findings[2-9]. In some instances, skin cancers have occurred in combination with other cancers, such as liver angiosarcoma (after six months' treatment with Fowler's solution giving a total intake of 0.24 g arsenic)[6], intestinal and bladder cancers[7] and meningioma[9]. Liver angiosarcomas have also been associated with medicinal exposure to arsenic[1,6,10].

Epidemiological studies of cancer following medical treatment with arsenic have shown an excess of skin cancers, but no clear association with other cancers has been obtained[1], as confirmed by a recent cohort study on individuals treated with Fowler's solution[11]. No relation was found between prostatic cancer and treatment of syphilis with arsenicals[12].

An association between environmental exposure to arsenic through drinking-water and skin cancer has been observed[1] and confirmed[13,14]; two cases of bladder cancer were also described, with latent periods of eight to 20 years[15]. The latent periods for two cases of skin cancer related to arsenic in drinking-water were 20 and 23 years, and the concentrations or uptake of arsenic were reported to be 1.2 and 1 mg per day, respectively, with an estimated total ingested dose of about 8 g in one study[14].

Epidemiological studies in areas with different frequencies of black-foot disease and where drinking-water contained 0.35-1.14 mg/l arsenic revealed elevated risks for cancers of the bladder, kidney, skin, lung, liver and colon in both men and women[16,17].

A case of liver angiosarcoma was reported in the 20-month-old child of an exposed worker living in the vicinity of a copper mine and smelter[18]. Four rather inconsistent studies describing the effect of air pollutants containing arsenic[1,19,20] were followed by further reports that indicated an effect on lung cancer incidence of arsenic in polluted air from smelters and pesticide production, with risk ratios of 2.0-2.5 near smelters[21,22]. Two further studies near smelters showed no clear effect[23,24].

Occupational exposure to inorganic arsenic, especially in mining and copper smelting, has quite consistently been associated with an increased risk of cancer[1]. A number of studies of smelter workers relate to populations that have been reported previously[1] and represent both partial[25-27] and total[28,29] updates. An almost ten-fold increase in the incidence of lung cancer was found in workers most heavily exposed to arsenic, and relatively clear dose-response relationships have been obtained with regard to cumulative exposure[29] and especially with 30-day ceiling levels[27]. Sulphur dioxide in the smelter environment appeared to play a minor role, if any, in the development of lung cancer[27]. Other forms of cancer were considered, but their incidences were not found to be consistently increased[28]. Other US smelter worker populations have been shown to have consistent increases in lung cancer incidence, as well as increases of about 20% in the incidence of gastrointestinal cancer and of 30% for renal cancer and haematolymphatic malignancies[30,31]. The observation in an earlier study of an increase in lung cancer risk among a population of Swedish smelter workers[1] has been confirmed, with a risk of six to eight fold among roasters[32].

A decrease in lung cancer risk after cessation of exposure to arsenic has been observed in some studies[30,33], possibly indicating a late-stage effect of arsenic[34,35].

With regard to histological type of lung cancer, a significant, relative excess of adeno-carcinomas and a slight excess of oat-cell cancers were seen among smelter workers[36].

References

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[5]Thomas, T.L. & Stewart, P.A. (1987) Mortality from lung cancer and respiratory disease among pottery workers exposed to silica and talc. *Am. J. Epidemiol., 125*, 35-43

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[7]*IARC Monographs, 34*, 133-190, 1984

[8]*IARC Monographs, Suppl. 6*, 494-496, 1987

SOOTS (Group 1) Product of Combustion

A. Evidence for carcinogenicity to humans (*sufficient*)

The carcinogenicity of soot is demonstrated by numerous case reports, dating back over 200 years, of skin cancer, particularly of the scrotum, among chimney-sweeps. More recent cohort studies of mortality among chimney-sweeps in Sweden and Denmark have shown a significantly increased risk of lung cancer. Supporting evidence for an association with lung cancer was provided by two earlier epidemiological studies in the German Democratic Republic and the UK. The potentially confounding and interactive effects of smoking could not be evaluated; however, cigarette smoking is not believed to have seriously biased these estimates. In addition to lung cancer, statistically significant excess mortality from oesophageal cancer, primary liver cancer and leukaemia was found among chimney-sweeps in one study[1].

B. Evidence for carcinogenicity to animals (*inadequate* for soots; *sufficient* for soot extracts)

Coal soot was tested in two experiments in mice by whole-body exposure, but the studies were inadequate for evaluation. Coal-soot extracts applied to the skin of mice produced skin tumours in two studies. A wood-soot extract applied to the skin of mice was inadequately tested. In limited studies, subcutaneous implants of wood soot in female rats produced a few local sarcomas; similar implants in the scrotal sac of rats did not. An extract of fuel-oil soot was inadequately tested by application to the skin of mice. Extracts of soot from the combustion of oil shale produced skin tumours in mice after dermal application and lung

C. Other relevant data

No data were available on the genetic and related effects of 1,1,2,2-tetrachloroethane in humans.

1,1,2,2-Tetrachloroethane did not transform BALB/c 3T3 cells and did not induce sex-linked recessive lethal mutations in *Drosophila*. It induced recombination, gene conversion and mutation in *Saccharomyces cerevisiae* under conditions in which endogenous levels of cytochrome P450 were enhanced. It was not mutagenic to bacteria but caused DNA damage[3].

References

[1]Norman, J.E., Jr, Robinette, C.D. & Fraumeni, J.F., Jr (1981) The mortality experience of army World War II chemical processing companies. *J. occup. Med.*, *23*, 818-822

[2]*IARC Monographs*, *20*, 477-489, 1979

[3]*IARC Monographs*, *Suppl. 6*, 511-513, 1987

TETRACHLOROETHYLENE (Group 2B) Product of Combustion

A. Evidence for carcinogenicity to humans (*inadequate*)

Tetrachloroethylene has been studied by observing laundry and dry-cleaning workers, who may also have been exposed to other solvents, especially trichloroethylene (see p. 364), but also petroleum solvents. In several cohort and proportionate mortality studies, excesses have been reported of lymphosarcomas[1], leukaemias[2] and cancers of the skin[1,2], colon[3], lung[2,4] and urogenital tract[1-5], although in one study no excess of urogenital cancer was seen among persons exposed mainly to tetrachloroethylene[5]. Some excess of lymphomas and of cancers of the larynx and bladder was seen in a large cohort of dry cleaners[6]. A familial cluster of chronic lymphocytic leukaemia has also been related to dry-cleaning[7]. A large case-control study of bladder cancer did not show any clear association with dry-cleaning[8]. In other case-control studies, dry-cleaning appeared to be a risk factor for pancreatic cancer[9] and for liver cancer[10]. Some excess of liver cancer was also seen in one of the proportionate mortality studies[2]. In two case-control studies of liver cancer[11,12], an increased risk with occupational exposure to organic solvents (in one of the studies in women only[12]) was observed; in the first study, one case and no control had had exposure to tetrachloroethylene; in the second, one of six female cases was in dry-cleaning workers. Even if there is some consistency in several studies with regard to an association between lymphatic malignancies and urogenital cancers, taken together, and exposure to tetrachloroethylene, this broad grouping and the small numbers involved do not permit any definite conclusion to be drawn about any causal connection.

Other cancer categories had very few deaths and provided little information. More detail can be found in Table 2.1.

Steenland & Palu (1999) updated a previous large cohort study of US painters by Matanoski *et al.* (1986): 42 170 painters and 14 316 non-painters were assembled from union records and followed for mortality through local and national registries from 1975–1994. The update added 15 years of follow-up during which time the number of deaths increased from 5313 to 23 458. When painters were compared to the general US population, the updated data showed significant but modest excesses for all cancers (SMR, 1.12; 95% CI: 1.09–1.15; 4674 deaths), cancers of the lung (SMR, 1.23; 95% CI: 1.17–1.29; 1746 deaths), of the bladder (SMR, 1.23; 95% CI: 1.05–1.43; 166 deaths), of the stomach (SMR, 1.39; 95% CI: 1.20–1.59; 197 deaths), and of the liver (SMR, 1.25; 95% CI: 1.03–1.50; 119 deaths). In an additional analysis comparing painters and non-painters directly at other anatomical sites, the standardized rate ratios (SRRs) were 1.23 (95% CI: 1.11–1.35) for cancer of the lung, 1.77 (95% CI: 1.13–2.77) for cancer of the bladder, 0.92 (95% CI: 0.68–1.25) for cancer of the stomach, and 1.36 (95% CI: 0.87–2.11) for cancer of the liver. Further analyses restricted to painters with at least 20 years of membership in the union, showed reductions in the SRRs for cancers of the bladder, stomach, and liver while the SRR for cancer of the lung increased slightly (to 1.32). Both painters and non-painters showed significant excesses of cirrhosis compared to the US population (SMRs, 1.21; 95% CI: 1.07–1.35, and 1.26; 95% CI: 1.03–1.51, respectively), suggesting an excess of alcohol consumption compared to the US population; nonetheless, as noted above, the excess of liver cancer persisted in a direct comparison of painters to non-painters.

Paint burn's and off-gas produced during fire's

The data were also adjusted indirectly for smoking using detailed information on smoking in the general population from two large US surveys (see Axelson & Steenland (1988) for the description of methods). The authors found that confounding by smoking when comparing painters to the US population would have resulted in a rate ratio of 1.14 for lung cancer and 1.05 for bladder cancer, compared to the observed SMRs of 1.23 and 1.23, respectively. While this suggested that confounding by smoking may have accounted for some of the lung cancer excess, the case for an occupational etiology was strengthened by the finding of an SRR of 1.23 (95% CI: 1.11–1.35) through a direct comparison painters to non-painters in the same union as both these groups were expected to have similar smoking habits.

The same Dutch cohort described by van Loon *et al.* (1997) was studied for incident cancers of the bladder (532 cases, 1630 subcohort members) and of the prostate (830 cases, 1525 subcohort members), using the same case–cohort design (Zeegers *et al.*, 2001, 2004). Using a case by case expert assessment, and adjustment for age, other occupational exposures as well as the amount and duration of cigarettes consumed, a positive trend for exposure to paint components was observed, with incident rate ratios of 1.00, 0.75 (95% CI: 0.33–1.72), 1.78 (95% CI: 0.94–3.37), and 1.31 (95% CI: 0.72–2.40) for increasing levels of estimated exposure (none, low, medium and high, respectively; *P*-value for trend, 0.09), based on 483, 8, 20, and 19 bladder cancer cases, respectively (Zeegers *et al.*, 2001). For the 765 prostate cancer cases that reported occupational history, job titles were coded using the

1.2 Composition of fire smoke

1.2.1 *Fire chemistry*

Smoke from fires comprises suspended liquid and solid particulate matter, gases and vapours that result from the combustion or pyrolysis of material. There is a very large number of toxic components in smoke (for reviews, see Tuve, 1985; Meyer, 1989; DiNenno *et al.*, 2002; Côté, 2003). The basic form of the overall combustion reaction of organic (carbon-containing) compounds is illustrated by the burning of methane:

$$CH_4 + 2O_2 + \text{Energy of activation} \rightarrow CO_2 + 2H_2O + \text{Heat of combustion} + \text{Light}$$

Given the appropriate ratio of fuel (wood, solvent, plastic, rubber), oxygen, and combustion temperature, the products of combustion should be only water and carbon dioxide (CO_2).

Complete combustion is approached only under carefully controlled conditions. Uncontrolled or unintentional combustion tends to be "fuel rich" and therefore incomplete. The combustion of methane (CH_4) illustrates the formation of free radicals in an 11-step chain reaction, the first two of which are:

$CH_4 \rightarrow CH_3^{\bullet} + H^{\bullet}$

$H^{\bullet} + O_2 \rightarrow OH^{\bullet} + O^{\bullet}$

The free radicals formed during combustion are very reactive and side reactions are propagated to yield hundreds of chemical products, and smoke.

Most polymers found in buildings will burn or thermally degrade to simpler monomers. Thermal degradation products include methane, ethane, ethylene, benzene, toluene, and ethylbenzene in addition to the following monomers: ethylene, vinyl chloride, acrylonitrile, tetrafluoroethylene, styrene, methyl methacrylate, ethylene glycol, terephthalic acid, phenol, formaldehyde, hexamethylenediamine, adipic acid, propene, vinyl chloride, vinyl acetate, vinylidene chloride, chloroprene, 1,3-butadiene, ethyl acrylate, ethylene oxide, methylacrylate, urea, phenol, and isoprene.

The burning of plastics typically produces voluminous amounts of soot, together with higher levels of hydrogen cyanide (HCN), hydrochloric acid (HCl) and acrolein (CH_2=CHCHO) than the burning of materials such as wood, and fossil fuels. More smoke evolves from fires involving aromatic polymers, such as polystyrene, compared to aliphatic polymers, such as polyethylene.

In addition to the chemical agents described above, particulate matter is produced under conditions of incomplete combustion. The particulate matter is an aerosol consisting of condensed phase components of the products of combustion and finely divided carbon particulates that have not undergone combustion but remain suspended in the air. Although the particles themselves are microscopic in size (0.3–1.6 μm), they

rapidly coalesce and thereby become visible. These particles are also adsorbents (similar to activated charcoal) and are an additional vehicle for the transport and inhalation of toxic combustion products. Smouldering yields a substantially higher conversion of fuel to toxic compounds than does flaming, although it occurs more slowly (Ohlemiller, 2002).

1.2.2 *Modern versus pre-modern fires*

All types of fire release toxic and carcinogenic substances, including benzene, 1,3-butadiene, and formaldehyde. The focus has generally been on substances having short-term acute effects: carbon monoxide (CO), carbon dioxide, hydrogen cyanide, nitrogen oxides (NO_x), sulfur dioxide (SO_2) and hydrogen chloride. With the increasing use of polymers in building construction and furnishings, there is concern that the burning of these new materials might release large quantities of other highly toxic substances (Austin *et al.*, 2001b).

Combustion and pyrolysis products from newer building materials and furnishings were believed to be more toxic than smoke from fires in buildings built before these materials became commonplace, and more toxic than smoke from wildland fires (Betol *et al.*, 1983; Alarie, 1985). However, many of the carcinogenic products of combustion identified are volatile organic compounds and are common to most burning materials. In a more recent study, no new or unusual non-polar volatile organic compounds (VOCs) were observed in current structural fires compared to the combustion of wood (Austin *et al.*, 2001b, 2001c). Adding polyvinyl chloride (PVC) to the fire load at simulated apartment fires was observed to significantly increase levels of polychlorinated phenols (IARC Group 2B), while polycyclic aromatic hydrocarbon (PAH) levels remained essentially unchanged (Ruokojärvi *et al.*, 2000). The increases in levels of polychlorinated biphenyls (PCBs, 0.021 to 0.031 mg/m^3), polychlorinated benzenes (0.002 to 0.010 mg/m^3) and I-TEQs [or PCDD/F] (3.5 to 5.4 ng/m^3) as products of combustion were not significant [possibly due to the small sample size]. In another study, proportionately higher levels of ethyl benzene (IARC Group 2B) were found at an electronics factory fire when compared to levels at residential and mixed occupancy fires (Austin *et al.*, 2001b).

The emission of combustion products (in mg per kg of material burned) for the same material varies greatly depending on combustion conditions such as ventilation (oxygen supply), temperature, and heating rate. Nonetheless, the relative amounts of the various non-polar VOCs found in smoke at municipal structural fires have been found to be remarkably similar from fire to fire, namely with the same 14 of 144 target compounds, dominated by benzene (IARC Group 1), toluene and naphthalene (IARC Group 2B) (Austin *et al.*, 2001b, 2001c).

1.2.3 *Carcinogens found in smoke at fires*

Table 1.1 lists the agents in Groups 1, 2A, and 2B that have been detected at fires in one or more studies, together with corresponding IARC evaluations, human and animal evidence of carcinogenicity, and for the agents in Group 1, the cancer sites in humans.

sister chromatid exchanges in bone-marrow cells of mice, chromosomal aberrations in bone-marrow cells of rats and Chinese hamsters and sperm-head anomalies in mice treated *in vivo*. It induced chromosomal aberrations and mutation in human cells *in vitro* (IARC, 1987). In-vitro studies strongly imply that the genotoxicity of benzene is derived primarily from its metabolites hydroquinone and 1,4-benzoquinone through their ability to inhibit topoisomerase II and microtubule function, induce oxidative stress, and damage DNA (ATSDR 2005).

4.1.4 *1,3-Butadiene*

Product of Combustion

Butadiene is absorbed through inhalation and is systemically distributed. It is metabolized primarily by CYP2E1 and CYP2A6 (Evelo *et al.*, 1993). The metabolic rate in lung is greater at lower doses, and in liver, at higher doses. The butadiene metabolite epoxy-1,2-butanediol is reportedly the major electrophile binding with DNA and haemoglobin (Swenberg *et al.*, 2001). Adducts formed by reaction of the metabolites 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol with haemoglobin and urinary mercapturic acids derived from 1,2-epoxy-3-butene have been detected in workers exposed to 1,3-butadiene. There is considerable interindividual variability in the ability of human liver microsomes to metabolize 1,3-butadiene and 1,2-epoxy-3-butene *in vitro* (Swenberg *et al.*, 2001).

There are conflicting results on whether 1,3-butadiene increases *HPRT* mutations in lymphocytes from humans exposed to 1,3-butadiene compared with unexposed controls (IARC, 1999). One study of workers exposed to 1,3-butadiene demonstrated an increase in *HPRT* variant frequency in lymphocytes with high (mean 1.48 ppm) as compared with low (mean 0.15 ppm) exposures (Ammenheuser *et al.*, 2001). However, sister chromatid exchanges, micronuclei, chromosomal aberrations and DNA-strand breaks were not significantly elevated above control levels in peripheral blood lymphocytes of occupationally exposed workers. 1,3-Butadiene induces DNA adducts and damage in both mice and rats *in vivo* and is mutagenic in virtually all in-vitro and in-vivo test systems. Activated K-*ras* oncogenes have been detected in lymphomas and in liver and lung tumours induced in mice by 1,3-butadiene. Mutations in the *p53* tumour suppressor gene have been detected in mouse lymphomas (IARC, 1999).

4.1.5 *Free radicals*

Smoke contains highly reactive oxygen- and carbon-centred radicals, which may initiate cancer through the oxidative activation of a procarcinogen and/or through binding of the radical to DNA. The major effect should therefore be on the epithelial layer of the respiratory tract. Cigarette smoke, which has been better studied than wood smoke, contains a quinone–hydroquinone–semiquinone system that can reduce oxygen to produce superoxide, and hence, hydrogen peroxide and the hydroxyl radical, as well as penetrate viable cells, bind to DNA, and cause nicks (Pryor, 1997; Church & Pryor, 1985).

epoxides. Both oxides and diol epoxides are ultimate DNA-reactive metabolites. PAH oxides and diol epoxides can form stable DNA adducts and induce mutations (e.g. in *ras* proto-oncogenes) that are strongly associated with the tumorigenic process (IARC, 2010a). Measured end-points in human populations include mutagenicity in urine and the presence of aromatic DNA adducts in the peripheral lymphocytes of exposed workers. Cytogenetic effects such as micronucleus formation have also been reported. Other mechanisms of carcinogenesis have been proposed for PAHs, but these are less well developed and include generation of reactive oxygen species, activation of the aryl hydrocarbon receptor with regulation of phase I and II metabolism, lipid peroxidation, production of arachidonic acid-reactive metabolites, decreased levels of serum thyroxine and vitamin A and persistent activation of the thyroid hormone receptor, as well as activation of mitogen-activated protein kinase pathways, suppression of immunity by p53-dependent and other pathways (IARC, 2010a).

4.1.8 *PCBs*

PCBs are absorbed by inhalation and dermal contact. Wester *et al.* (1990) found that PCBs penetrated skin in a time-dependent manner, but that 93±7% of PCBs were removed from the skin with five successive washes using soap and water, the removal efficiency decreasing with increasing time from initial contact. Washing 24 hours following skin contact removed only 25% of the initial PCBs. The highest concentrations are found in adipose tissue, with metabolism occurring in the liver. PCBs are metabolized by cytochrome P450 followed by conjugation with glutathione or glucuronic acid. The rate of metabolism depends on the extent of chlorination, the location of the chlorine atoms, and the levels of P450 isozymes and other enzymes. Metabolites of PCBs with low chlorine content are predominately eliminated in the urine. PCBs with high chlorine content and substitution patterns resistant to metabolism are either retained or excreted unchanged in the faeces. In one study, following exposure to an electrical transformer fire in New York, USA, serum PCBs were higher in firefighters initially when compared to levels 9 months later (Kelly *et al.*, 2002). Orris *et al.* (1986) reported two cases of lesions consistent with chloracne in firefighters, although in both cases the blood PCB level was less than 10 µg/L.

A wide variety of cancers have been reported in association with PCB exposure. Exposure to PCB mixtures predominantly causes liver tumours in rats; although tumours in mouse lung and mouse skin have also been observed (IARC, 1987). Cancer mechanisms that are both dependent on and independent of the aryl hydrocarbon receptor may be involved. PCBs may be involved in tumour initiation and promotion. Metabolism of less chlorinated PCBs in rat microsomes can lead to covalently modified macromolecules including proteins and DNA, although PCB mixtures generally are inactive as mutagens, and are not potent genotoxicants. PCB promotion of liver tumours may involve increased cell proliferation following cell or tissue injury caused by reactive oxygen species, resulting from induction of CYP oxygenases and GSTs, decreased activity of glutathione

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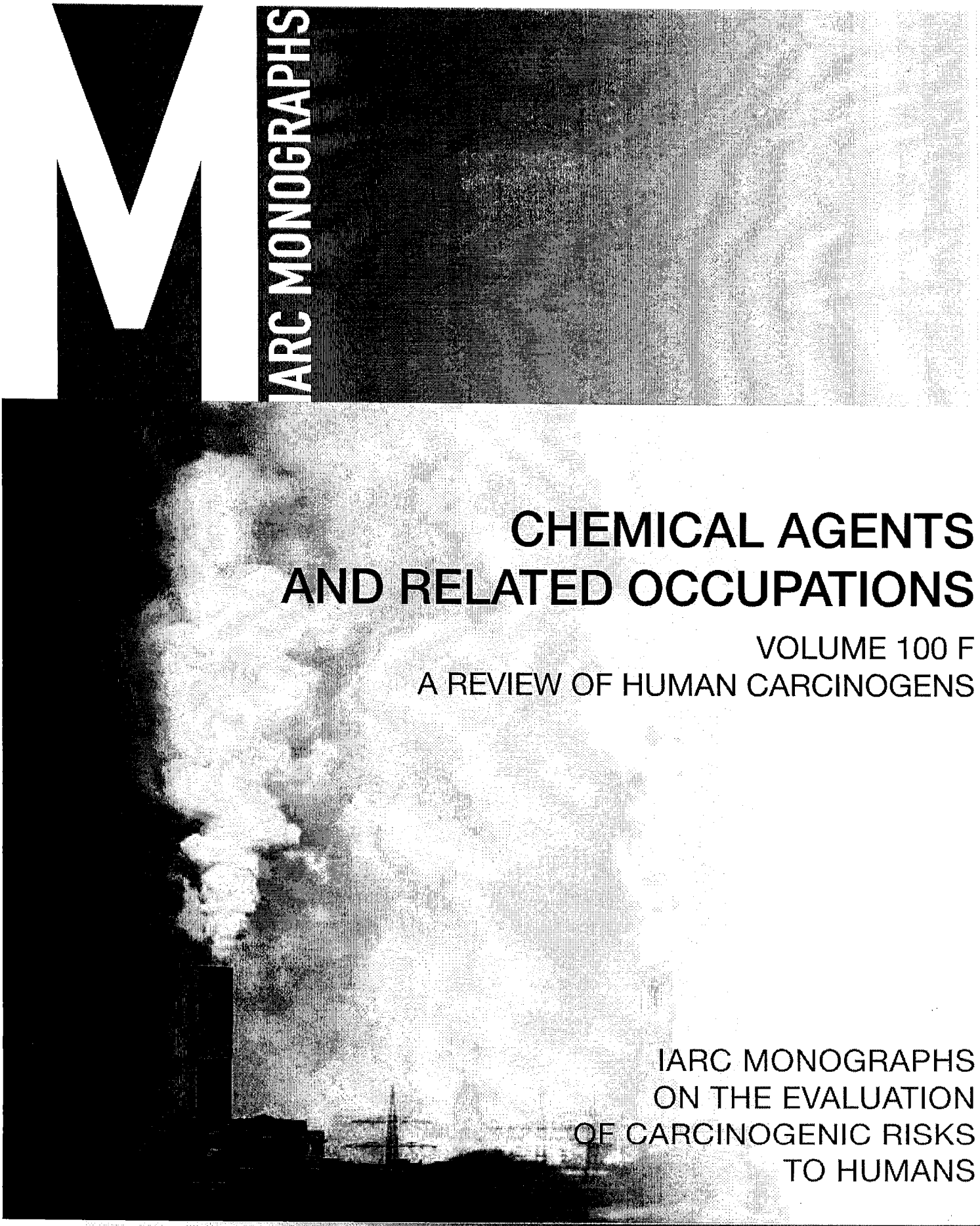

CHEMICAL AGENTS AND RELATED OCCUPATIONS

VOLUME 100 F
A REVIEW OF HUMAN CARCINOGENS

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

Reference IARC 105 - Composition of Fire Smoke, Overhaul & Diesel Exhaust

Known Prod. of combustion

Table 3.2 Summary of reports of malignant tumours clearly induced in experimental animals by benzo[*a*]pyrene

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

3.4 Intraperitoneal injection

In a series of studies in newborn and adult mice, intraperitoneal injection of benzo[*a*]pyrene increased the incidence of liver (adenomas and carcinomas) and lung (adenomas and adenocarcinomas) tumours and, occasionally, forestomach (squamous cell papillomas and carcinomas) and lymphoreticular tumours (Vesselinovitch *et al.*, 1975a, b; Wislocki *et al.*, 1986; Lavoie *et al.*, 1987; Busby *et al.*, 1989; Rippe & Pott, 1989; Mass *et al.*, 1993; Nesnow *et al.*, 1995; Ross *et al.*, 1995; Weyand *et al.*, 1995; Rodriguez *et al.*, 1997; Von Tungeln *et al.*, 1999).

In one study in rats with a single intraperitoneal injection of benzo[*a*]pyrene, a high incidence of abdominal mesotheliomas and sarcomas was observed (Roller *et al.*, 1992).

3.5 Inhalation

In a lifetime inhalation study (Thyssen *et al.*, 1981) in male hamsters, benzo[*a*]pyrene induced dose-related increases in the incidence of papillomas and squamous-cell carcinomas in both the upper respiratory tract (nose, larynx and trachea) and the upper digestive tract (pharynx, oesophagus and forestomach).

3.6 Intrapulmonary injection

Dose-related increases in the incidence of malignant lung tumours (mainly epidermoid and squamous-cell carcinomas and a few pleomorphic sarcomas) were found after injection of benzo[*a*]pyrene into the lung of rats (Deutsch-Wenzel *et al.*, 1983; Iwagawa *et al.*, 1989; Wenzel-Hartung *et al.*, 1990; Horikawa *et al.*, 1991).

3.7 Intratracheal administration

Intratracheal administration of benzo[*a*]pyrene alone or mixed with particulates and suspended in saline with or without suspendents resulted in benign and malignant respiratory tumours in mice (Heinrich *et al.*, 1986a), rats (Pott *et al.*, 1987; Steinhoff *et al.*, 1991) and in numerous studies in hamsters (IARC, 2010). This treatment also induced forestomach tumours in hamsters (Saffiotti *et al.*, 1972; Sellakumar *et al.*, 1973; Smith *et al.*, 1975a, b, Stenbäck & Rowland, 1979). Larger benzo[*a*]pyrene particles were generally more effective than smaller ones.

Mice that lack the nucleotide excision-repair gene *XPA* (*XPA*$^{-/-}$ mice) showed a stronger lung-tumour response after intratracheal instillation of benzo[*a*]pyrene than did their similarly treated *XPA*$^{+/+}$ and *XPA*$^{+/-}$ counterparts (Ide *et al.*, 2000).

3.8 Buccal pouch application

Repeated application of benzo[a]pyrene to the buccal pouch mucosa of male hamsters resulted in a high incidence of forestomach papillomas (Solt *et al.*, 1987).

3.9 Subcutaneous tracheal grafts transplantation

In one study conducted in rats transplanted with subcutaneous rat tracheal grafts exposed to beeswax pellets containing various amounts of benzo[a]pyrene, a high incidence of squamous-cell carcinomas was reported (Nettesheim *et al.*, 1977).

3.10 Intramammilary administration

In three studies in rats, benign and malignant mammary gland tumours developed after intrammilary injection of benzo[a]pyrene (Cavalieri *et al.*, 1988a, b, 1991).

3.11 Intracolonic instillation

In three experiments in mice, intracolonic instillation of benzo[a]pyrene induced lymphomas and a variety of benign and malignant tumours in various organs including the forestomach (Toth, 1980; Anderson *et al.*, 1983).

3.12 Intravaginal application

Intravaginal application of benzo[a]pyrene in mice produced invasive cervical carcinoma; no such tumours were seen in controls (Näslund *et al.*, 1987).

3.13 Intrafetal injection

In one study in male and female Swiss mice, intrafetal injection of benzo[a]pyrene produced lung adenomas (Rossi *et al.*, 1983).

4. Other Relevant Data

P.O.C.

Benzo[a]pyrene is a carcinogen that induces tumours in many animal species. Some of the examples relevant for this review are: lung tumours in mice, rats, and hamsters; skin tumours in mice; liver tumours in mice; forestomach tumours in mice and hamsters; and mammary gland tumours in rats (Osborne & Crosby, 1987; IARC, 2010). In humans, occupational exposures to benzo[a]pyrene-containing mixtures have been associated with a series of cancers: coke production: lung; coal gasification: lung, bladder; paving and roofing: lung; coal tar distillation: skin; soots: lung, oesophagus, haematolymphatic system, skin; aluminum smelting: lung, bladder; tobacco smoking: lung, lip, oral cavity, pharynx, oesophagus, larynx, bladder (IARC, 1984, 1985, 1986, 2010).

Studies on the mechanisms of action of benzo[a]pyrene have been reviewed (Xue & Warshawsky, 2005; IARC, 2010).

4.1 Metabolism

Benzo[a]pyrene is metabolized by both phase-I and phase-II enzymes to form a series of arene oxides, dihydrodiols, phenols, and quinones and their polar conjugates with glutathione, sulfate, and glucuronide (Osborne & Crosby, 1987). Benzo[a]pyrene-7,8-diol is a key metabolite that is formed by the action of epoxide hydrolase on benzo[a]pyrene-7,8-epoxide. This dihydrodiol can be further metabolized by cytochrome P450s (CYPs) to a series of benzo[a]pyrene-7,8-diol-9,10-epoxides, which form one class of ultimate carcinogenic metabolites of benzo[a]pyrene.

4.5.3 Relationship of biomarkers to human cancer

Mutations in *TP53* are common in lung cancers from smokers and less common in nonsmokers. These mutations are G→T transversions with hotspots in codons 157, 248 and 273 (Hainaut & Pfeifer, 2001; Pfeifer *et al.*, 2002) and they are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts. The active metabolite *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide causes a unique spectrum of *TP53* mutations distinct from those found in cancers that are not associated with smoking (Campling & el-Deiry, 2003). Similar G→T mutations have been reported in lung tumours from nonsmoking Chinese women whose tumours were associated with exposure to PAHs from smoke generated by burning smoky coal in unventilated homes. The mutations were clustered at the CpG rich codons 153–158 of the *TP53* gene, and at codons 249 and 273. The mutation spectrum was fully consistent with exposure to PAHs (DeMarini *et al.*, 2001).

4.6 Synthesis

Benzo[*a*]pyrene is metabolically activated to a series of reactive intermediates by CYP450 and related enzymes under control of the aryl-hydrocarbon receptor. There is strong evidence that the benzo[*a*]pyrene diolepoxide mechanism operates in mouse-lung tumorigenesis, while there is also strong evidence that both the radical-cation and the diolepoxide mechanisms are involved in mouse-skin carcinogenesis. The meso-region mechanism has been studied only in rat liver, while the mechanism that involves the formation of *ortho*-quinone/reactive oxygen species has only been studied *in vitro*, although reactive oxygen species can be formed *in vivo* by other benzo[*a*]pyrene-mediated mechanisms. All these pathways reflect genotoxic mechanisms, as they involve alterations to DNA. Benzo[*a*]pyrene is pleotropic and has the ability to affect many cell- and organ-based systems. Therefore, there are probably many modes of carcinogenic action operating to different extents *in vivo*. These include mechanisms that involve AhR, oxidative stress, immunotoxicity and epigenetic events.

Based on the best available, consistent and strong experimental and human mechanistic evidence it is concluded that benzo[*a*]pyrene contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAH mixtures that contain benzo[*a*]pyrene. The most commonly encountered – and most widely studied – mechanistically relevant DNA lesion is the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct. The formation of this adduct is consistent with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-associated genotoxic effects in surrogate tissues and with the mutation pattern in the *TP53* gene in lung tumours from humans exposed to PAH mixtures that contain benzo[*a*]pyrene. The fact that those PAH mixtures and benzo[*a*]pyrene itself induce genotoxic effects like sister chromatid exchange, chromosomal aberrations, micronuclei, DNA damage (comet assay) and 8-oxo-deoxyguanosine, supports the notion that benzo[*a*]pyrene contributes to human cancer.

5. Evaluation

There is *sufficient evidence* for the carcinogenicity of benzo[*a*]pyrene in experimental animals.

[No epidemiological data on benzo[*a*]pyrene alone were available to the Working Group.]

The genotoxic mechanism of action of benzo[*a*]pyrene involves metabolism to highly reactive species that form covalent adducts to DNA. These anti-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts induce mutations in the K-*RAS* oncogene and the *TP53* tumour-suppressor gene in human lung tumours, and

et al., 2003) and in two others statistically significant increases in risk were observed (Lynge *et al.*, 1997; Sorahan *et al.*, 2005). A case–control study from Canada showed no association of exposure to benzene with lung cancer overall or with the major histological subtypes (Gérin *et al.*, 1998; see Table 2.16 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.16.pdf).

2.3 Cancer of the kidney

Cohort studies with results on kidney cancer are shown in Table 2.17 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.17.pdf). Results generally do not show any association. In a case–control study among males in Germany an association was found between exposure to benzene and an increased risk for kidney cancer (Pesch *et al.*, 2000), but in a study in Montreal, Canada, there was little evidence of an association (Gérin *et al.*, 1998) (see Table 2.18 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.18.pdf).

2.4 Other cancers

In the evaluation of the cohort studies that provided data on the cancer sites considered above, it was apparent that associations have occasionally been found with other cancer sites including malignant melanoma (Schnatter *et al.*, 1996; Consonni *et al.*, 1999; Lewis *et al.*, 2003), nose and stomach cancer (Fu *et al.*, 1996) and prostate cancer (Collingwood *et al.*, 1996), but overall there was no consistency across the cohorts.

3. Cancer in Experimental Animals

Studies on the carcinogenesis of benzene in rats and mice after exposure by inhalation, intragastric gavage, skin application, and by intraperitoneal or subcutaneous injection have been reviewed in *IARC Monographs* Volume 29 and in Supplement 7 (IARC, 1982, 1987). In Supplement 7 it was concluded that there is *sufficient evidence* in experimental animals for the carcinogenicity of benzene. Results of adequately conducted carcinogenicity studies reported before and after 1987 are summarized in Tables 3.1, 3.2, 3.3, 3.4.

Exposure to benzene by inhalation increased the incidence of Zymbal gland carcinomas, liver adenomas, and forestomach and oral cavity carcinomas in female rats (Maltoni *et al.*, 1982a, c, 1983, 1985, 1989). It also increased the incidence of lymphohaematopoietic (lymphoma, myelogenous) neoplasms in male and female mice (Snyder *et al.*, 1980; Cronkite *et al.*, 1984, 1989; Farris *et al.*, 1993), and Zymbal gland carcinomas, squamous cell carcinomas of the preputial gland, and lung adenomas in male mice (Snyder *et al.*, 1988; Farris *et al.*, 1993).

Oral administration of benzene increased the incidence of Zymbal gland carcinomas and oral-cavity papillomas and carcinomas in rats of both sexes, of carcinomas of the tongue, papillomas and carcinomas of the skin and of the lip and papillomas of the palate in male rats, of forestomach acanthomas in both sexes of the rat, and of forestomach carcinomas in female rats (Maltoni & Scarnato, 1979; Maltoni *et al.*, 1982b, 1983, 1988, 1989; NTP, 1986; Maronpot, 1987; Huff *et al.*, 1989; Mehlman, 2002). Given by the oral route, benzene also increased the incidence of Zymbal gland carcinomas, forestomach papillomas, lymphomas, and lung adenomas and carcinomas in mice of both sexes, of liver carcinomas, adrenal gland pheochromocytomas, harderian gland adenomas and preputial gland squamous cell carcinomas in male mice,

cancers including major cancers are, overall, inconsistent between studies. It should be borne in mind that the general population is exposed to levels that are much lower than those experienced by the industrial populations.

The Working Group did not review the epidemiological evidence of other PCDDs, PCDFs or PCBs with a dioxin-like activity.

3. Cancer in Experimental Animals

3.1 *2,3,7,8-Tetrachlorodibenzo-para-dioxin*

Carcinogenicity studies with several strains of rats, mice and Syrian hamsters treated with 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) via the oral route (gavage or diet), by intra-peritoneal injection, or by skin application have been reviewed in *IARC Monograph* Volume 69 (IARC, 1997). At the time, the review of the available data led to the conclusion that there is *sufficient evidence* in experimental animals for the carcinogenicity of TCDD. The present *Monograph* also evaluates relevant carcinogenicity studies in TCDD-treated experimental animals that were published since 1997. The results of adequately conducted carcinogenicity studies are summarized below and in Table 3.1 and Table 3.2.

TCDD was tested for carcinogenicity by oral administration (gavage or dose feed) in four studies in mice and six studies in rats, by skin (topical) application in two studies in mice, by intraperitoneal injection in one study in mice, one study in rats and one study in hamsters and by subcutaneous injection in one study in hamsters. TCDD produced tumours in both sexes of mice and rats, and in multiple organs and tissues.

Oral administration of TCDD caused increased incidences of thyroid follicular adenomas and hepatocellular adenomas and carcinomas in male and female mice, of alveolar/bronchiolar adenomas and carcinomas in male mice, and of histiocytic lymphomas and subcutaneous fibrosarcomas in female mice. In rats, it caused increased incidences of hepatocellular adenomas in males and females, cholangiocarcinomas and hepatocellular carcinomas in females, lung cystic keratinizing epitheliomas and squamous-cell carcinomas in females, adrenal gland (cortex) adenomas and squamous-cell carcinomas of the hard palate/nasal turbinates in males and females, tongue squamous-cell carcinomas and thyroid follicular adenomas and carcinomas combined in males, subcutaneous fibromas in males and subcutaneous fibrosarcomas in females, and pituitary adenomas, uterine and oral mucosa (gingival) squamous-cell carcinomas and pancreatic adenomas and carcinomas combined in females (Van Miller *et al.*, 1977; Kociba *et al.*, 1978; Tóth *et al.*, 1979, NTP, 1982a, 2006a; Della Porta *et al.*, 1987; Goodman & Sauer, 1992; Hays *et al.*, 1997, Yoshizawa *et al.*, 2005). Skin application or gavage caused benign and malignant tumours of the skin in female mice including transgenic mice (NTP, 1982b; Wyde *et al.*, 2004). Hamsters that received TCDD by intraperitoneal or subcutaneous injection developed squamous-cell carcinomas of the facial skin (Rao *et al.*, 1988). Intraperitoneal injection caused increased incidence of hepatocellular adenomas and carcinomas in female mice and of lymphomas in male and female mice (Della Porta *et al.*, 1987).

Several studies in mice showed that administration of TCDD with known carcinogens enhanced the incidence of skin papillomas, lung adenomas, liver adenomas and hepatoblastomas. In female rats, TCDD co-administered with various nitrosamines enhanced the incidence of focal hepatic lesions. In one study, TCDD enhanced the incidence of lung carcinomas in ovariectomized female rats following administration of *N*-nitrosodiethylamine (NDEA) (IARC, 1997). In two more recent studies in female rats, TCDD given orally or subcutaneously enhanced

the carcinogenicity of previously administered NDEA (Davis *et al.*, 2000; Viluksela *et al.*, 2000). In another study, the oral administration of TCDD to pregnant rats increased 7,12-dimethylbenz[*a*] anthracene-induced mammary-gland tumours in offspring (Brown *et al.*, 1998; see Table 3.3).

3.2 Dioxin-like compounds

3.2.1 2,3,4,7,8-Pentachlorodibenzofuran

Oral administration of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) resulted in significant dose-dependent trends for increased incidence of cholangiocarcinomas and hepatocellular adenomas (Walker *et al.*, 2005; NTP, 2006b). (see Table 3.4)

Skin application of PeCDF after a single dose of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine resulted in an increased incidence of skin papillomas in mice (Hébert *et al.*, 1990). Subcutaneous injections of PeCDF after oral treatment with NDEA resulted in an increased multiplicity of hepatocellular carcinomas and liver hyperplastic nodules in male rats (Nishizumi & Masuda, 1986). Subcutaneous injections of PeCDF after a single intraperitoneal injection of NDEA increased the number of focal hepatic lesions in female rats (Waern *et al.*, 1991).

3.2.2 3,3',4,4',5-Pentachlorobiphenyl

Oral administration of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) resulted in significantly increased incidence of hepatocellular adenomas, cholangiocarcinomas, lung cystic keratinizing epitheliomas, and oral mucosa (gingiva) squamous-cell carcinomas in female rats (Walker *et al.*, 2005; NTP, 2006c).

4. Other Relevant Data

4.1 AhR activation

Most, if not all of the effects of TCDD are related to its binding to and activation of the aryl hydrocarbon receptor (AhR), a member of the basic helix–loop–helix/Per-Arnt-Sim family of transcription factors. This receptor was first identified in mouse liver (Poland *et al.*, 1976) where it showed high affinity towards TCDD. Further studies found that AhR is expressed in most mammalian tissues and that many other halogenated aromatic compounds can bind this receptor, including the coplanar polychlorinated biphenyls and the polychlorinated dibenzodioxins and dibenzofurans. It is generally proposed that the toxic and carcinogenic effects of dioxin and other halogenated compounds are due to their high affinity to AhR, and to the sustained pleiotropic response from a battery of genes – many of which encode drug-metabolizing enzymes – that follows the receptor-ligand complex formation (Mandal, 2005; Walker, 2007). Much of the research in the three decades since the discovery of the AhR has focused on dissecting this pleiotropic response to fully understand the mechanisms involved in dioxin-mediated toxicity.

The free AhR resides in the cytoplasm as an inactive complex containing a heat-shock protein dimer, Hsp90, XAP2 and p23 (Meyer *et al.*, 1998). When the AhR binds to a ligand, XAP2 is released and, through a conformational change, the complex is moved to the nucleus where the Hsp90 dimer dissociates and the AhR-nuclear-translocator (ARNT) binds to the PAS domains of the receptor. The activated AhR/ARNT complex forms a heterodimer that is then capable of binding to the 5'-regulatory region of dioxin-responsive genes (Mimura & Fujii-Kuriyama, 2003). The primary targets following activation of AhR include genes encoding many phase-I and phase-II metabolic enzymes (e.g. *CYP1A1*,

CYP1A2, *CYP1B1*, *NQO1*, *UGT1A2*, *GSTA1* and *ALDH3A1*) (Nebert *et al.*, 2000; Schwarz & Appel, 2005). However, through direct and indirect pathways, TCDD is able to alter the expressions of a much larger number of genes (Martinez *et al.*, 2002; Dere *et al.*, 2006; Pastorelli *et al.*, 2006; Schwanekamp *et al.*, 2006). In addition, there is cross-talk with several other receptor-mediated systems including the estrogen receptor (Safe & Wormke, 2003) and the retinoic-acid receptor β (Lu *et al.*, 1994; Berkers *et al.*, 1995; Toyoshiba *et al.*, 2004).

Despite the strong conservation of the AhR across species, gene polymorphisms, differences in co-activators and differences in downstream signalling following activation are all likely to modulate TCDD carcinogenicity (Ema *et al.*, 1994; Tuomisto *et al.*, 1999). These factors could explain the interindividual differences observed in the magnitude of the carcinogenic response after exposure to TCDD. For example, different AhR polymorphisms triggered a threefold difference in EROD activity in human lymphocytes (Smart & Daly, 2000).

4.2 Mechanisms of carcinogenicity

TCDD is not directly genotoxic and the tumorigenic activity is likely to be due to a fairly long half-life, especially in humans, resulting in a sustained activation of the AhR. TCDD half-life in the human body is estimated at 7.2 years (Milbrath *et al.*, 2009); long half-life in the environment and the ability to bio-accumulate in the food-chain are also reported (IARC, 1997). The sustained downstream signalling may trigger an adaptive biochemical and physiological response in the cell that can promote carcinogenesis (Biegel & Safe, 1990; Lu *et al.*, 1994; Berkers *et al.*, 1995; Schwarz & Appel, 2005), also by inducing mutations (Stohs *et al.*, 1990; Tritscher *et al.*, 1996; Shertzer *et al.*, 1998; Yoshida & Ogawa, 2000; Thornton *et al.*, 2001; Nebert *et al.*, 2004; Knerr *et al.*, 2006; Schlezinger *et al.*, 2006; Lin *et al.*, 2007; Green *et al.*, 2008). TCDD may also enhance – although it sometimes inhibits – the progression and invasiveness of initiated tumours (Marlowe & Puga, 2005; Peng *et al.*, 2009), but this topic will not be discussed in detail here.

The primary mechanism by which TCDD is thought to cause cancer is by altering the cellular ability to proliferate, migrate, apoptose, senesce and terminally differentiate (Safe, 2001; Marlowe & Puga, 2005; Ray & Swanson, 2009) in a multistep process focused on the accumulation of mutations and/or heritable epigenetic changes. Chemicals that inhibit apoptosis and increase proliferation usually increase cancer risk as well. TCDD has been shown to increase cellular proliferation both *in vivo* and *in vitro* in several tissues (Maronpot *et al.*, 1993; Barrett, 1995; Dere *et al.*, 2006) possibly through interactions with protein-kinase C signalling (Barrett, 1995), inhibition of senescence (Ray & Swanson, 2009) or activation of growth-signalling factors (Kohn, 1995). In initiation-promotion models, TCDD expanded the populations of preneoplastic foci in rat liver (Dragan *et al.*, 1992; Maronpot *et al.*, 1993; Tritscher *et al.*, 1995) and promoted carcinogenesis in liver, skin and lung in rodents (DiGiovanni *et al.*, 1977; Hébert *et al.*, 1990; Lucier *et al.*, 1991; Dragan *et al.*, 1992; Beebe *et al.*, 1995; Tritscher *et al.*, 1995; Tritscher *et al.*, 2000).

P.O.C. Diesel Exhaust

Finally, TCDD may upregulate drug-metabolizing enzymes, thus increasing the presence of highly reactive intermediates that form during metabolic activation and/or transformation of several key hormones. For example, CYP1A1, CYP1A2 and CYP1B1 induction is a major source of reactive oxygen species (ROS) formation in hepatocytes and this has been linked to the decoupling of the P450 catalytic cycle (Nebert *et al.*, 2004; Knerr *et al.*, 2006; Schlezinger *et al.*, 2006; Green *et al.*, 2008). A hormonal linkage with estrogen has been demonstrated through the increase in 8-oxo-deoxyguanosine (a marker of oxidative stress) in the liver of intact female

The carcinogenicity of mixtures of PCBs in rodents has also been clearly established through studies of various Aroclors (IARC, 1978; Mayes *et al.*, 1998; NTP, 2006c) yielding predominantly liver cancers (Cogliano, 1998). Two-year chronic exposure studies done by the US National Toxicology Program (NTP) on PCB 126 (NTP, 2006d) and PCB 118 (NTP, 2009), demonstrated tumour effects consistent with those seen for TCDD (hepatocellular adenomas, cholangiocarcinomas, gingival squamous cell carcinomas, and lung cystic keratonizing epitheliomas). Moreover, when equivalent TCDD doses where applied with the current TEF, a carcinogenic response equivalent to that predicted for TCDD from the NTP study (Walker *et al.*, 2005) was observed.

The set of DLC-28 (IARC, 1978, 1997; Milbrath *et al.*, 2009) have a long half-life similar to that of TCDD (estimated at 7.2 years in the human body) (Table 4.1). Many congeners have similar or longer half-lives (1,2,3,7,8-PeCDD, 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDD, 1,2,3,6,7,8- and 1.2.3.7.8.9-HxCDF, and PCBs 169, 114, 123, 156, 167 and 189) while most of the remaining half-lives are in excess of 1.4 years. Several authors report the presence of these compounds in human blood in the general population (Costopoulou *et al.*, 2006; Scott *et al.*, 2008; Zubero *et al.*, 2009) indicating a sustained, long-term exposure that, when coupled with the analyses for common pleiotropic response, argues in favour of the notion that all of the DLC-28s have the same carcinogenic potential in humans.

Experimental data on mechanism of carcinogenesis induced by DLC-28 are available for 2,3,4,7,8-PeCDF and PCB 126, in particular (Table 4.2), Both have been shown to bind to the AhR in humans and animals (IARC, 1978; Safe, 2001), to translocate into the nucleus and activate numerous metabolic enzymes *in vitro* (human and non-human cell lines) and *in vivo* in experimental animals (IARC, 1997; Safe, 2001; Vezina *et al.*, 2004; Haws *et al.*, 2006), to trigger changes in growth factors and signalling pathways related to cellular replication in rodents (Hemming *et al.*, 1995; Vondrácek *et al.*, 2005; N'Jai *et al.*, 2008). 2,3,4,7,8-PeCDF potential effect on cell replication is suggested in the NTP study (Walker *et al.*, 2007), and promotion in skin, liver and lung tissues is reported in initiation-promotion studies (Hébert *et al.*, 1990; Anderson *et al.*, 1991; Waern *et al.*, 1991). PCB 126 acts as a promoter of liver cancer in initiation-promotion studies (Hemming *et al.*, 1995; Haag-Grönlund *et al.*, 1998) with measured increases in cell-replication rate in the populations of initiated cells (Vondrácek *et al.*, 2005). PCB 126 and 2,3,4,7,8-PeCDF induce oxidative stress, the latter in a dose-dependent manner in brain and liver of rats (Hassoun *et al.*, 2002; Hennig *et al.*, 2002). These two compounds are carcinogenic in mixtures with TCDD (IARC, 1978; Hassoun *et al.*, 2001; NTP, 2006d) and by themselves in the NTP chronic bioassays in rats, where they increase hepatocellular adenomas, cholangiocarcinomas, gingival squamous-cell carcinomas, and, possibly, lung cystic keratonizing epitheliomas (NTP, 2006b, c, d).

4.4 Synthesis

There is strong evidence to support a receptor-mediated mechanism of action for TCDD-associated carcinogenesis in humans where the primary mechanism is the promotion of tumour development through the activation of cellular replication and the alteration in cellular senescence and apoptosis. Dioxin, through activation of an array of metabolic enzymes also increases the risk for oxidative stress, which serves as an indirect initiator of carcinogenesis. These events make dioxin a complete carcinogen. The conservation of the AhR and the related signalling pathways across species strongly support this mechanism in humans.

The receptor-mediated mechanism of action for TCDD-associated carcinogenesis in humans

Found in Structure Fires IARC 98 P.399

Known Prod. of combustion

Ethylene oxide

et al., 1983; Popp et al., 1994), which are partly converted to thio-diacetic acid (Scheick et al., 1997).

Concentrations of ethylene glycol were determined at the end of day 3 of a normal working week in blood samples from sterilization personnel exposed to ethylene oxide. TWA concentrations of ethylene oxide determined over eight hours ranged from 0.3 to 52 ppm [0.55–95.2 mg/m^3] (overall mean, 4.2 ppm [7.7 mg/m^3]). The mean concentrations of ethylene glycol in the blood of exposed subjects were twice as high (90 mg/L) as those in controls (45 mg/L) (Wolfs et al., 1983).

The concentration of thioethers excreted in urine collected at the end of sterilization processes was found to be twice as high in non-smoking personnel (10.2 mmol/mol creatinine) exposed to peak concentrations of 1–200 ppm [1.83–366 mg/m^3] ethylene oxide as the thioether concentration in unexposed workers (5.46 mmol/mol creatinine). The concentration of ethylene oxide in air was not monitored routinely (Burgaz et al., 1992).

The glutathione-*S*-transferase (GST) activity towards ethylene oxide in cytosolic fractions from human livers was low (too low to determine the Michaelis-Menten constant [Km] value). The maximum velocity (V_{max}) varied from 7.6 to 10.6 nmol/min/mg protein. Epoxide-hydrolase (EH) activity in the microsomal fraction of human liver averaged 1.8 nmol/min/mg protein. The Km for hydrolysis was estimated to be approximately 0.2 mM, but non-enzymatic hydrolysis was considerable and precluded accurate measurement (Fennell & Brown, 2001).

Metabolism of ethylene oxide to the GSH conjugate and ethylene glycol is generally considered to be the major pathway for the elimination of DNA-reactive ethylene oxide. However, strongly suggestive evidence *in vitro* was presented by Hengstler et al. (1994) that glycolaldehyde is formed by further metabolism of ethylene glycol and that this derivative leads to DNA–protein crosslinks and DNA strand-breaks (as measured with the alkaline elution assay) after in-vitro incubation with human mononuclear peripheral blood cells.

4.2 Genetic and related effects

4.2.1 GST polymorphisms

Ethylene oxide is a substrate of the GST iso-enzyme T1 (Hayes et al., 2005). This detoxifying enzyme is polymorphic and a relatively large proportion of the population (about 20% of Caucasians, almost 50% of Asians) has a homozygous deletion (*GSTT1*-null genotype) (Bolt & Thier, 2006). As expected, these individuals show a significantly higher amount of hydroxyethyl valine in their haemoglobin due to the presence of endogenous ethylene oxide (Thier et al., 2001). Nevertheless, the influence of this genetic trait on the formation of this type of adduct as a result of exposure to exogenous ethylene oxide at the workplace is much less clear, as discussed below.

In the cytoplasm of erythrocytes obtained from 36 individuals, ethylene oxide was eliminated three to six times faster in samples from so-called conjugators (defined by a standardized conjugation reaction of methyl bromide and GSH; 75% of the population) than in those from the remaining 25% (who lack this GST-specific activity). In the latter samples, the rate of disappearance did not differ from that of controls. In this experiment, the disappearance of ethylene oxide was investigated in the gas phase, in closed vials that contained GSH and cytoplasm of erythrocytes (Hallier et al., 1993).

Studies on the genotoxicity of ethylene oxide were reviewed in detail in *IARC Monograph* Volume 97 (IARC, 2008). Studies with peripheral blood of exposed workers have shown that exposure to ethylene oxide is associated with an elevated number of chromosomal aberrations including breaks, gaps, exchanges, and supernumerary chromosomes. An increased frequency

of sister chromatid exchange (SCE) in the peripheral lymphocytes of workers handling ethylene oxide was also reported.

4.2.2 DNA-adduct formation

In-vitro and in-vivo studies have shown that ethylene oxide can bind to cellular macromolecules, which results in a variety of DNA, RNA and protein adducts. The major DNA adduct recovered *in vivo* is *N*7-(2-hydroxyethyl) guanine (7-HEG), while some minor adducts such as *N*3-(2-hydroxyethyl)adenine (3-HEA) and O^6-(2-hydroxyethyl)guanine (O^6-HEG), are detected at much lower levels (Walker *et al.*, 1992). In-vitro studies indicate that other minor adducts can also be formed from the reaction of ethylene oxide with the *N*1 and N^6 positions of adenine and the *N*3 position of cytosine, uracil and thymine (IARC, 1994; Tates *et al.*, 1999; Kolman *et al.*, 2002).

Tompkins *et al.* (2009) suggested that the mutagenicity and carcinogenicity of ethylene oxide could be attributed to formation of multiple 2-hydroxyethyl (HE) DNA adducts such as 3-HEA and O^6-HEG. Boysen *et al.* (2009) argued that there is little evidence that 7-HEG adducts cause mutations since – unlike the *N*1, N^2, or O^6 positions of guanine – they do not participate in hydrogen bonding in the DNA double-helix and easily de-purinate. These authors conclude that the formation of *N*7-guanine adducts cannot be used in isolation as a quantitative biomarker for pro-mutagenic DNA lesions or mutagenic response. Marsden *et al.* (2009) used a dual-isotope approach to distinguish between endogenously formed background levels of 7-HEG and exogenously formed 7-HEG adducts in rats following exposure to [^{14}C]-labelled ethylene oxide. By combining liquid chromatography-tandem mass spectrometry and high-performance liquid chromatography/accelerator mass spectrometry analysis, both the endogenous and exogenous *N*7-HEG adducts were quantified in tissues of [^{14}C]ethylene oxide-treated rats. Levels of [^{14}C]-7-HEG induced in spleen, liver, and stomach DNA were insignificant compared with the measured background levels of *N*7-HEG naturally present.

The exact mechanism by which the other ethylene oxide-induced DNA adducts such as 3-HEA and O^6-HEG may lead to mutation is unknown. Several mechanisms could be involved, including the mispairing of altered bases or the formation of apurinic/apyrimidinic sites via DNA repair or chemical depurination/depyrimidination combined with the insertion of another base, which would typically be an adenine opposite an apurinic site (Tates *et al.*, 1999; Houle *et al.*, 2006). These lesions can also lead to the formation of DNA single-strand breaks and, subsequently, to chromosomal breakage. In addition, the putative ethylene oxide metabolite, glycolaldehyde, has been shown to form DNA–protein crosslinks and DNA single-strand breaks (Hengstler *et al.*, 1994).

4.2.3 Cytogenetic alterations and mutations

Studies of human exposure to ethylene oxide have focused on individuals employed in the operation of hospital- or factory-based sterilization units, and on workers who were involved in manufacturing or processing of ethylene oxide. The studies show that exposure to ethylene oxide results in chromosomal alterations that are related to both the level and duration of exposure, while a single study suggested that exposure to ethylene oxide causes gene mutations.

(a) Sister chromatid exchange

The induction of increased frequencies of sister chromatid exchange (SCE) has been found to be a sensitive indicator of genotoxic exposure to ethylene oxide in humans (Tates *et al.*, 1991). In several studies, significant differences were found in SCE frequencies in individuals and/or groups exposed to levels of ethylene oxide higher

translocations in the germ cells of exposed rodents, and a dose-related increase in the frequency of sister chromatid exchange, chromosomal aberrations and micronucleus formation in the lymphocytes of exposed workers.

Ethylene oxide is *carcinogenic to humans (Group 1).*

In making the overall evaluation, the Working Group considered that there is *sufficient evidence* for the carcinogenicity of ethylene oxide in experimental animals, and relied heavily on the compelling data in support of the genotoxic mechanism described above.

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were required to have been exposed to VCM for at least one year before 31 December 1972 and to have been employed in or after 1942. A second major update of this cohort was published by Wong *et al.* (1991). A third major follow-up included 10 109 subjects and provided an update of the vital status through to 31 December 1995 (Mundt *et al.*, 2000).

The European cohort study was conducted in four countries (Italy, Norway, Sweden and the United Kingdom). It included workers from 19 factories: 11 of these produced VCM/PVC, two produced VCM only, five produced PVC only and one was a PVC-processing plant. Male workers who had been employed for at least one year in 1942–1972 in jobs that entailed exposure to VCM were included (Simonato *et al.*, 1991). An update of the study (Ward *et al.*, 2001) analysed incidence and mortality through to the latest year for which data were available in each country, which ranged between 1993 and 1997.

2.1 Angiosarcoma of the liver

In both multicentre cohort studies (Mundt *et al.*, 2000; Ward *et al.*, 2001) a substantial excess of ASL in exposed workers was found (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.1.pdf). This tumour is extremely rare in the general population and it is not possible to calculate an SMR or SIR, because age- and calendar time-specific reference rates are not available. In the study from the US, 33 of the 80 deaths from cancer of the liver and biliary tract were identified from the death certificate as due to ASL. A total of 48 deaths due to ASL were identified by combining information from death certificates with that from a registry of ASL-cases that were related to exposure to VCM. This registry is maintained and updated by the Association of Plastics Manufacturers of Europe.

In the European study there were 53 deaths from primary liver cancer and 18 incident cases of liver cancer. This total of 71 cases comprised 37 ASL, 10 HCC, 7 cases of other known histology, and 17 cases of an unspecified type of liver cancer. [The Working Group noted that the authors searched for the best evidence for diagnosis of liver cancers by reviewing all available documentation, including death certificates, cancer-registry records, medical records, and listings of ASL from two registries.]

In both studies, the risk for ASL increased strongly with duration of exposure to vinyl chloride. In the European study, there was also a clear trend of higher risk with increasing cumulative exposure. Multiple cases of ASL were also reported in one smaller cohort study (Thériault & Allard, 1981). Two cases of ASL were reported among hairdressers and barbers who had been exposed to vinyl chloride for 4–5-year periods in the late 1960s and early 1970s, when it was used as a propellant in hairspray (Infante *et al.*, 2009).

2.2 Hepatocellular carcinoma

The assessment of vinyl chloride as a cause of HCC is complicated because many studies do not have histological or other definitive clinical information to discriminate HCC from ASL and/or secondary neoplasms (see Table 2.1 online). In the US multicentre study, mortality from cancers of the liver and biliary tract (ICD9-code, 155–156) was increased (SMR 3.6, 95%CI: 2.8–4.5; 80 deaths). Of the 80 deaths, 48 were identified as ASL. The diagnosis of HCC among the remaining deaths was not verified.

In an internal analysis of the European multicentre cohort (Ward *et al.*, 2001) based on 10 verified cases of HCC, the risk increased significantly and substantially with duration of employment and with cumulative exposure to vinyl chloride. The relative risk for workers with the longest duration of employment (> 26 years) was 35 (95%CI: 3.3–377) compared with workers with < 10 years of employment. An analysis of a single Italian plant with extended follow-up – that

was included in the European study – indicated 12 confirmed cases of HCC (Pirastu *et al.*, 2003). The maximal overlap between these two analyses was four cases, since only four HCC from Italy were included in the multicentre cohort. In this subcohort, the incidence of HCC again increased significantly with cumulative exposure to vinyl chloride. There was suggestive evidence that the risk for HCC from vinyl chloride is substantially higher among workers who are infected with hepatitis B virus (Wong *et al.*, 2003), or who report high levels of alcoholic beverage consumption (Mastrangelo *et al.*, 2004).

A meta-analysis of cohort studies of vinyl choride-exposed workers published up to 2002 (Boffetta *et al.*, 2003) was based on eight independent studies, i.e. two multicentric investigations (Mundt *et al.*, 2000; Ward *et al.*, 2001) and six additional, smaller studies (Thériault & Allard, 1981; Weber *et al.*, 1981; Smulevich *et al.*, 1988; Laplanche *et al.*, 1992; Huang, 1996; Wong *et al.*, 2002) (*P*-value for the test for heterogeneity was ≥ 0.01). Six of these eight studies reported results for liver cancer, but these were considered to be too heterogeneous to be included in a meta-analysis because for 'liver cancer overall' and for 'liver cancer other than ASL', the *P*-value for heterogeneity was < 0.001. For the two multicentre studies (Mundt *et al.*, 2000; Ward *et al.*, 2001), the lack of heterogeneity allowed calculation of summary estimates for liver cancer overall (meta-SMR, 2.96; 95%CI: 2.00–4.39; random effects model; *P*-value for heterogeneity = 0.03) and for liver cancer other than ASL (meta-SMR, 1.35; 95%CI: 1.04–4.39; random effects model; *P*-value for heterogeneity = 0.7).

[The Working Group noted that the meta-analysis did not evaluate the quality of the studies and that some heterogeneity between studies may have resulted from variable quality of the data. Excluding one study from the People's Republic of China, other studies reported SMRs that ranged from 1.78 (95%CI: 1.15–2.62) to 57.1 (95%CI: 24.6–113) for liver cancer overall and from 1.27 (95%CI: 0.84–1.83) to 10.1 (95%CI: 4.37–20.0) for liver cancer other than ASL.]

2.3 Cancer of the lung

Among workers exposed to vinyl chloride, there was no overall evidence of an increased risk for lung cancer (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.2.pdf). However, in PVC-packers and -baggers, the risk for lung cancer increased significantly with cumulative exposure to vinyl chloride (Ward *et al.*, 2001). [These workers are known to have had concomitant exposure to PVC-dust; the study did not allow attribution of the association to a specific agent or combination of agents.]

2.4 Malignant neoplasms of connective and soft tissue

Suggestive evidence was found for malignant neoplasms of connective and soft tissue (ICD9-code, 171). This derived from the multicentre study in the USA (Mundt *et al.*, 2000), in which a nearly threefold statistically significant overall increase in mortality from these neoplasms was observed (SMR 2.7, 95%CI: 1.4–4.7; 12 observed, 4.4 expected). The risk was higher for workers with longer duration of employment (i.e. 10–19 *vs* > 20 years) and for those first employed before 1960. Four of the 12 observed deaths were from angiosarcomas for which the site was unknown. The increased mortality from neoplasms of connective and soft tissue persisted even after exclusion of these four angiosarcomas. [This presumes that the malignant neoplasms of connective and soft tissue were mis-classified deaths from angiosarcoma of the liver.]

The findings mentioned above were not supported by results from the European multicentre study, in which the number of deaths from connective-tissue neoplasms was too small

for an evaluation of exposure–response (Ward *et al.*, 2001): there were six observed deaths from neoplasms of connective and soft tissue (SMR = 1.9, 95%CI: 0.7–4.1), but in a re-evaluation of the diagnoses three of the six deaths coded as tumours of the connective tissue were found to be ASL. [The Working Group noted that, although a statistically significant increase in mortality from neoplasms of connective and soft tissue was found in the US study, the discrepant results with the European study and the difficulties in arriving at a correct diagnosis and coding of the tumour site for this type of neoplasm, complicate an evaluation of these findings.]

2.5 Other cancers

The Working Group did not find strong evidence for associations of exposure to vinyl chloride with cancers of the brain or the lymphatic and haematopoeitic tissues, with melanoma of the skin (see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.3.pdf, Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.4.pdf, and Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.5.pdf). Although the associations found for these cancers in specific studies may reflect true increases in risk, the findings were inconsistent between studies, no clear exposure–response relationships were found in the European multicentre study (Ward *et al.*, 2001), and, for several of the sites, the numbers of observed/expected cases were small.

No conclusion could be reached for breast cancer since the available studies included too few women.

2.6 Synthesis

There is compelling evidence that exposure to vinyl chloride is associated with angiosarcoma of the liver, and strong evidence that it is associated with hepatocellular carcinoma. Together with the observation that vinyl chloride increases the risk for liver cirrhosis, which is a known risk factor for hepatocellular carcinoma, the findings from two large multicentre cohort studies provide convincing evidence that vinyl chloride causes hepatocellular carcinoma as well as angiosarcoma of the liver. There is contradictory evidence that exposure to vinyl chloride is associated with malignant neoplasms of connective and soft tissue, and inconsistent or scanty evidence that it is associated with cancers of the lung, brain, lymphohaematopoietic system, and breast, or with melanoma of the skin.

3. Cancer in Experimental Animals

The carcinogenicity of vinyl chloride has been studied intensively and repeatedly in experimental animals, with a wide range of concentrations, spanning orders of magnitude. The many studies consistently showed hepatic and extrahepatic angiosarcomas in mice and rats. Various other malignant neoplasms also occurred at several anatomical sites. However, the reporting of the results has often been incomplete, and the outcomes of many studies are available only from summary tables in the published literature, in which technical details are given in footnotes.

Studies of the carcinogenicity of vinyl chloride in experimental animals after oral administration, inhalation, subcutaneous injection, intraperitoneal injection, and transplacental and perinatal exposure have been reviewed in previous *IARC Monographs* (IARC, 1974, 1979, 1987, 2008). No studies have been published since the most recent evaluation (IARC, 2008). The

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of vinyl chloride. Vinyl chloride causes angiosarcoma of the liver, and hepatocellular carcinoma.

There is *sufficient evidence* in experimental animals for the carcinogenicity of vinyl chloride.

There is *sufficient evidence* in experimental animals for the carcinogenicity of chloroethylene oxide.

There is strong evidence that the carcinogenicity of vinyl chloride operates by a genotoxic mechanism that involves metabolic activation to reactive metabolites, binding of the metabolites to DNA, promutagenic action of these adducts leading to mutations in proto-oncogenes and tumour-suppressor genes. Many of these key events identified in experimental animals have also been demonstrated in humans.

Vinyl chloride is *carcinogenic to humans (Group 1).*

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workers. Exposed workers with the *GSTM1-null* genotype had a statistically significantly elevated CA frequency compared with controls and exposed workers with a *GSTM1*-positive genotype. Exposed workers with *XRCC1*^{194}*Arg/Trp* and *Trp/Trp* genotypes had statistically higher CA frequencies compared with those with the *XRCC1*^{194}*Arg/Arg* genotype. Also, there was an association between the *XRCC1*^{280}*Arg/Arg* and *XRCC3*^{241}*Thr/Thr* genotypes and a significant increase of CA frequency in exposed workers. The authors suggested that these wild-type genotypes may decrease the capacity to repair DNA single- and double-strand breaks and influence the formation of chromosomal aberrations (Hoyos-Giraldo *et al.*, 2009).

In most studies that measured a variety of cytogenetic end-points and markers of genotoxicity, elevated levels of genetic damage were reported in painters. Mechanistic data reviewed by ATSDR (1997, 2000a, b, 2007a, b) and by previous *IARC Monograph* evaluations on selected specific chemicals that had been or still are prevalent in exposures during painting, strongly support a role of these substances in the induction of haematopoietic malignancies (benzene, trichloroethylene), liver cancer (trichloroethylene), lung cancer (cadmium, chromium, PAHs) and bladder cancer (aromatic azo dyes).

4.4 Synthesis

The multiple genetic and cytogenetic effects observed among workers employed as painters or in the paint industry provide strong evidence in support of genotoxicity as one mechanism underlying the observed increase in cancer risk. However, due to the complexity and changing nature of the exposure mixtures and the potential interactions between exposures as a painter, other mechanisms are also likely. While it is clear that exposures to some agents in the paint industry have decreased over time, recent cytotoxicity studies and the ongoing exposures to multiple mutagens and carcinogens continue to raise concerns about cancer risks.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposure as a painter. Occupational exposure as a painter causes mesothelioma, and cancers of the urinary bladder and lung.

Also, a positive association has been observed between maternal exposure to painting (including pre-conception and during pregnancy) and childhood leukaemia in the offspring.

No data in experimental animals relevant to exposure as a painter were available to the Working Group.

The multiple genetic and cytogenetic effects observed among workers employed as painters and the information on individual chemicals to which painters are exposed provide strong evidence to support genotoxicity as a mechanism underlying the observed increase in cancer risk. However, due to the complexity and changing nature of the exposure mixtures and the potential interactions between exposures as painters, other mechanisms are also likely. While it is clear that exposures as a painter to some agents have been reduced over time, recent genotoxicity studies and the exposure to multiple mutagens and carcinogens continue to raise concerns about cancer risks.

Occupational exposure as a painter is *carcinogenic to humans (Group 1).*

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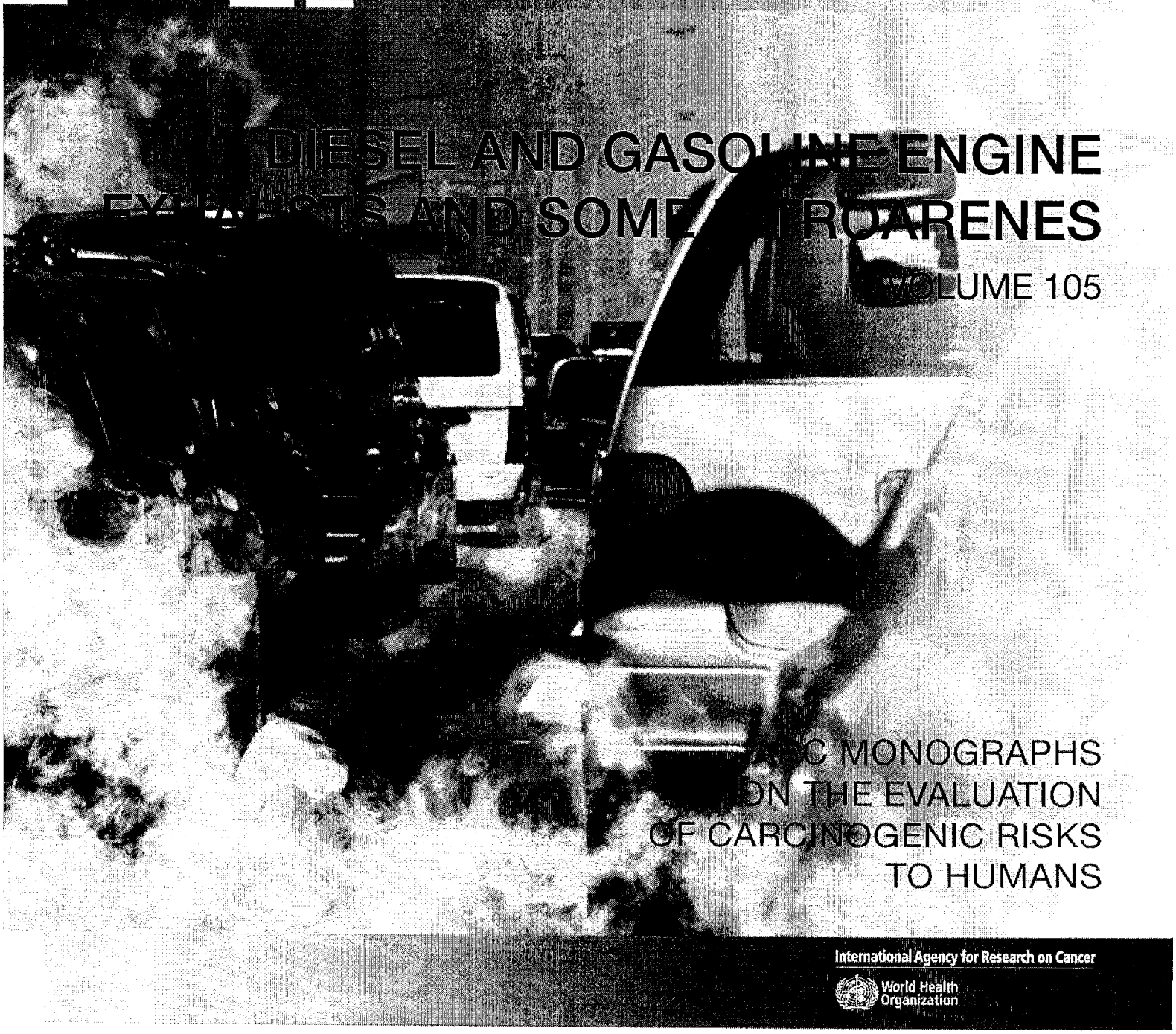
IARC MONOGRAPHS



DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

after optimization of the extraction method (Giammarise *et al.*, 1982).

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1.

3.1.1 *Intraperitoneal administration*

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 1,6-dinitropyrene (purity, > 99%; total dose, 200 nmol [58.7 μg]) or benzo[*a*]pyrene (purity, > 99%; total dose, 560 nmol [140 μg]) in 10, 20 and 40 μL of dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth or DMSO alone. At 25–27 days, when the mice were weaned, 25 males and 29 females in the treated group, 37 males and 37 females in the positive-control group, and 28 males and 31 females in the control group were still alive. All surviving mice were killed after 1 year. In the group injected with 1,6-dinitropyrene, 8 out of 25 (32%) male mice developed liver tumours (three adenomas, five carcinomas); this incidence was significantly greater than that in the vehicle controls ($P < 0.025$). No increase in the incidence of lung tumours or malignant lymphomas was observed in males or females compared with DMSO-treated animals (Wislocki *et al.*, 1986).

3.1.2 *Subcutaneous administration*

A group of 20 male BALB/c mice, aged 6 weeks, received subcutaneous injections of 0 (vehicle control) or 0.1 mg of 1,6-dinitropyrene (purity, > 99,9%) dissolved in 0.2 mL of DMSO once a week for 20 weeks (total dose, 2 mg). Animals were observed for 60 weeks or, for mice that developed tumours at the site of injection, until moribund. The first tumour in the 1,6-dinitropyrene-treated group was seen on day 112; 45 weeks after the first treatment, 10 out of 20 mice ($P < 0.002$) had developed tumours at the injection site that were diagnosed histologically as malignant fibrous histiocytomas [a term used as a specific diagnosis for some subcutaneous and intraperitoneal sarcomas]. No subcutaneous tumour was detected in the vehicle controls (Tokiwa *et al.*, 1984).

3.2 Rat

See Table 3.2.

3.2.1 *Oral administration*

A group of 36 female weanling Sprague-Dawley rats received intragastric intubations of 0 (vehicle control) or 10 μmol [3 mg]/kg body weight (bw) of 1,6-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 μmol [0.5 mg]/mL), three times a week for 4 weeks (average total dose, 16 μmol [4.7 mg]/rat) and were observed for 76–78 weeks. Two rats (6%) treated with 1,6-dinitropyrene and none of the controls developed leukaemia. Mammary adenocarcinomas and fibroadenomas were found in 11 out of 36 (31%) and 10 out of 36 (28%) treated animals, respectively, which was not statistically different from the incidence in controls (5 out of 35 (14%) adenocarcinomas and 9 out of 35 (26%) fibroadenomas). Adrenal and pituitary tumours were also observed in treated animals at an elevated but non-significant level compared with controls (King, 1988; Imaida *et al.*, 1991). [The Working Group noted the short duration of both treatment and observation periods and the use of a single dose.]

5. Summary of Data Reported

5.1 Exposure data

1,6-Dinitropyrene is produced by the nitration of 1-nitropyrene. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of dinitropyrenes. This leads to a content of 1,6-dinitropyrene in the range of 0.1–10% relative to the 1-nitropyrene content in diesel and gasoline exhaust particles and of ~1% in airborne particulate matter. 1,6-Dinitropyrene was present at a range of 1–10 ng/g in airborne particulate matter collected from ambient atmospheric samples. Air concentrations clearly declined from values in the 0.1–10 pg/m^3 range at urban locations to values in the 0.01–0.1 pg/m^3 range at suburban and rural locations.

1,6-Dinitropyrene is also generated by kerosene heaters. No data on occupational exposure were available to the Working Group.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,6-Dinitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and one study by subcutaneous injection, in rats in one study by oral administration, one study by intraperitoneal injection, two studies by implantation into the lung and two studies by subcutaneous injection, and in one study in hamsters by intratracheal instillation. Intraperitoneal injection of 1,6-dinitropyrene into newborn mice caused a significant increase in the incidence of liver carcinomas in males and its subcutaneous injection caused a significant increase in the incidence of malignant subcutaneous histiocytomas in males. In rats, oral intubation with 1,6-dinitropyrene caused a significant increase in the incidence of pituitary carcinomas in females; its intraperitoneal injection caused a significant increase in the incidence of malignant histiocytomas of the peritoneal cavity in females; its implantation into the lung (two studies) caused a significant increase in the incidence of squamous cell carcinomas of the lung in males; and its subcutaneous injection caused injection site sarcomas in males in one study and malignant histiocytomas and leukaemia in females in another study. In hamsters, intratracheal instillation of 1,6-dinitropyrene caused a significant increase in the incidence of lung adenocarcinomas and myeloid leukaemia in males and females.

5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism and excretion or genetic and related effects of 1,6-dinitropyrene in humans. Activation of this compound in bacteria or in mammalian cells occurs via the reduction of one nitro group initially to form a nitroso intermediate, that undergoes further reduction to the *N*-hydroxylamino derivative. 1,6-Dinitropyrene was strongly mutagenic in bacteria. It induced DNA-adduct formation and caused mutation in the splenic T lymphocytes of rats, and induced chromosomal aberrations in human fibroblasts. The mutagenicity of 1,6-dinitropyrene is related to the ability of its corresponding hydroxylamino derivative to bind to DNA. *O*-Acetylation of the *N*-hydroxylamino group by acetyltransferases followed by removal of the acetoxy group yields the active electrophilic nitrenium ion, which reacts with deoxyguanosine at the C8 position

'Long-flow' furnace black was first used in photocopy toners in 1967; its manufacture involved an oxidation process whereby some nitration of pyrene also occurred. A carbon black sample manufactured before 1979 was reported to contain 23.4 mg/kg 1,8-dinitropyrene (Sanders, 1981); another 'long-flow' furnace carbon black sample was also found to contain this compound (Ramdahl & Urdal, 1982). Subsequent changes in the production technique reduced the total extractable nitropyrene content from uncontrolled levels of 5–100 ng/mg to below 0.3 ng/mg (Rosenkranz *et al.*, 1980; Sanders, 1981; Butler *et al.*, 1983). Toners produced from a new type of carbon black since 1980 had no detectable levels of mutagenicity, and hence of nitropyrenes (Rosenkranz *et al.*, 1980; Butler *et al.*, 1983). A sample of carbon black made in 1980 contained 0.16 mg/kg 1,8-dinitropyrene after optimization of the extraction method (Giammarise *et al.*, 1982).

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 *Intraperitoneal administration*

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 1,8-dinitropyrene (total dose, 200 nmol [58.7 μg]; purity, > 99%) or benzo[*a*]pyrene (total dose, 560 nmol [140 μg]; purity, > 99%) in 10, 20 and 40 μL of dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth or DMSO alone. At 25–27 days, when the mice were weaned, 31 males and 33 females in the treated group, 37 males and 27 females in the positive-control group and 28 males and 31 females in the vehicle-control group were still alive. All surviving mice were killed after 1 year. In the group injected with 1,8-dinitropyrene, 5 out of 31 (16%) males developed liver tumours compared with 2 out of 28 (7%) controls. No increase in the incidence of lung tumours or malignant lymphomas was observed in males or females compared with DMSO-treated animals (Wislocki *et al.*, 1986). [The Working Group noted the short observation period.]

3.1.2 *Subcutaneous administration*

A group of 20 male BALB/c mice, aged 6 weeks, received subcutaneous injections of 0.05 mg of 1,8-dinitropyrene (purity, > 99.9%) dissolved in 0.2 mL DMSO (total dose, 1 mg) once a week for 20 weeks. A positive-control group of 20 males received injections of 0.05 mg of benzo[*a*]pyrene, and a further group of 20 mice served as controls. [It was unclear whether the animals were untreated or injected with DMSO.] Animals were observed for 60 weeks or until moribund. After 60 weeks, 6 out of 15 (40%) mice injected with 1,8-dinitropyrene had developed subcutaneous tumours; no such tumours were found in controls ($P < 0.05$). All of the subcutaneous tumours were diagnosed histologically as malignant fibrous histiocytomas [a term used as a specific diagnosis for subcutaneous sarcomas]. Some animals in the 1,8-dinitropyrene-treated group developed tumours of the lung or liver (Otofuji *et al.*, 1987). [The Working Group noted the small number of animals used.]

3.2 Rat

See Table 3.2

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

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

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

3.1 Mouse

See Table 3.1

3.1.1 Intraperitoneal administration

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

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

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

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

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

An assay was conducted in male and female newborn HSD:ICR mice to compare the potency of 6-nitrochrysene with that of isomers of nitrochrysene in the induction of tumour formation. A total dose of 100 nmol/mouse of 6-nitrochrysene in DMSO was administered by intraperitoneal injection to 38 males and 24 females on days 1, 8 and 15 after birth. A group that received DMSO alone served as a vehicle control. Animals were killed and analysed histologically for lung and liver tumours 30 weeks after the last dose. The lung tumour incidence was 100% in males and 96% in females, and was statistically significantly increased compared with that in controls (12% and 3%, respectively; $P < 0.001$). Liver tumours were observed in 64% ($P < 0.001$) of the treated males, none of the treated females, 8% of the control males and none of the control females. The types of tumour were not specified. 6-Nitrochrysene was significantly more potent than the isomers of nitrochrysene, which did not show a significant induction of tumours (El-Bayoumy *et al.*, 1992).

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

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

5.3 Animal carcinogenicity data

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

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

5.4 Mechanistic and other relevant data

6-Nitrochrysene is metabolically activated via nitroreduction, ring-oxidation or a combination of both pathways, leading to the formation of DNA adducts. When administered orally or by intramammary injection to rats, 6-nitrochrysene induced mammary adenocarcinomas; its carcinogenic activity in the rat mammary gland exceeds that of benzo[*a*]pyrene – classified as a Group 1 human carcinogen by the IARC. Furthermore, 6-nitrochrysene was found to induce lung, liver and skin tumours in mice. Human hepatic and pulmonary microsomes and human mammary epithelial cells metabolized 6-nitrochrysene to reactive metabolites that caused DNA damage. 6-Nitrochrysene induced the same DNA adducts in human mammary epithelial cells as those detected in the mammary gland – the target organ – of rats. Haemoglobin adducts derived from 6-nitrochrysene have been identified in humans exposed to diesel engine exhaust. The mutation spectrum induced by 6-nitrochrysene in mammary tissue in *lacZ*-transgenic mice was dominated by mutations at G and A residues and could be linked to the mutation profile of 1,2-dihydro-dihydroxy-6-hydroxyamino-chrysene, a metabolite that is formed by a combination of nitroreduction and ring-oxidation. The formation of its major adduct, 5-(deoxyguanosin-N^2-yl)-1,2-dihydro-dihydroxy-6-amino-chrysene, in the rat mammary gland supports the hypothesis that 1,2-dihydro-dihydroxy-6-hydroxyamino-chrysene is the ultimate carcinogen. Mutations in the H-*Ras* and K-*Ras* oncogenes were observed in lung tumours from mice exposed to 6-nitrochrysene.

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

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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