BITUMENS AND BITUMEN EMISSIONS

Bitumens and bitumen emissions were considered by previous IARC Working Groups in 1984 and 1987 (IARC, 1985, 1987). Since then, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent: definitions and classifications

1.1.1 Introduction

Bitumens are engineering materials produced by the distillation of crude oil during petroleum refining and exist in numerous forms and types. Bitumens are dark viscous liquids or semi-solids that are non-volatile at ambient temperatures and soften gradually when heated. In North America, bitumen is commonly known as "asphalt cement" or "asphalt binder". "Asphalt" is the term used for a mixture of small stones, sand, filler and bitumen (~5%), which is used as a road-paving material. Bitumen emissions are defined as the complex mixture of aerosols, vapours, and gases from heated bitumen and products containing bitumen. Although the term "bitumen fume" is often used in reference to total emissions, bitumen fume refers only to the aerosolized fraction of total emissions (i.e. solid particulate matter, condensed vapour, and liquid bitumen droplets). Accordingly, the term "bitumen emissions" is more appropriate for referring to total content of bitumen in air.

Different grade specifications of bitumen, based on physical properties, can be achieved for specific applications either directly via refining or by blending. For example, the basic product is often referred to as "straight-run" bitumen and is commonly used in road-paving applications. This basic product can be further processed by blowing air through it at elevated temperatures to produce "oxidized" bitumen, which is commonly used in roofing applications. While these are the two products most commonly used in industry, there are four additional classes that are produced to achieve specific physical characteristics by modification of the production process (see Section 1.1.3).

Bitumens should not be confused with coal-derived products such as coal tars or coal-tar pitches, which are distinctly different substances. While bitumens are derived from petroleum, coal-tar products are derived from the high-temperature carbonization of bituminous coals (> 1000 °C) and are by-products of gas and coke production. Coal-tar products contain much higher concentrations of polycyclic aromatic hydrocarbons (PAHs) than bitumens, particularly in the three- to seven-ring size range. In contrast, bitumens contain higher concentrations of paraffinic and napthenic hydrocarbons

Table 1.1 Ranges of PAH concentrations in bitumens and coal-tar pitches, and in fume (BSM) from bitumen and from coal-tar pitch

PAH	Bitumens (μg/g)	Coal-tar pitches (μg/g)	Bitumen fume (ppm) 160–250 °C	Coal-tar pitch fume (ppm) 160–210 °C
Phenanthrene	0.32–7.3	19850–25700	107–842	$2.0–2.5 \times 10^5$
Anthracene	0.01–0.32	4600–7310	3.6–22	$0.56–0.76 \times 10^5$
Fluoranthene	0.1–0.72	29000–36000	13–32	$0.76–0.92 \times 10^5$
Pyrene	0.17–1.5	21300–27200	15–134	$0.44–0.55 \times 10^5$
Chrysene	0.8–3.9	11200–22670	33–157	$0.056–0.11 \times 10^5$
Perylene	0.04–3.9	2770–3500	1.7–15	119–456
Benzo[*a*]anthracene	0.14–1.1	20400–24510	12–40	$0.059–0.12 \times 10^5$
Benzo[*k*]fluoranthene	ND–2.2	5250–6010	ND–2.6	377–1216
Benzo[*a*]pyrene	0.22–1.8	11360–15170	2.9–8.5	553–2022
Benzo[*g,h,i*]perylene	1.2–5.7	3430–3530	6.0–15	34–200
Anthanthrene	ND–0.11	1231–1728	ND	9–69
Dibenzo[*a,i*]pyrene	ND–0.6	127–164	ND–0.5	ND–0.6
Coronene	ND–0.4	ND–120	ND–11	ND

BSM, benzene-soluble matter; ND, not detected, below the limit of detection; PAH, polycyclic aromatic hydrocarbon
From Brandt & de Groot (1985)

and their derivatives, whose large size and viscosity result in limited solubility (Table 1.1). Puzinauskas & Corbett (1978) provide a concise review of the differences between bitumens and coal-tar products (IARC, 2010).

Similarly, bitumen should not be confused with petroleum pitch, which is the highly aromatic residue produced by thermal cracking (i.e. extreme heat treatment) of selected petroleum fractions. The properties and chemical composition of petroleum pitch are therefore quite different from those of refined bitumen. While the term "petroleum pitch" is not used consistently (this term is used to describe different materials in different areas), petroleum pitches are principally used as binders in the manufacture of metallurgical electrodes (IARC, 2010).

1.1.2 Chemical properties and physical characteristics of bitumens

Bitumens contain a complex mixture of aliphatic compounds, cyclic alkanes, aromatic hydrocarbons, PAHs and heterocyclic compounds containing nitrogen, oxygen and sulfur atoms, and metals (e.g. iron, nickel, and vanadium). However, most of the available analytical data are focused on the characterization of PAHs. Table 1.2 lists the PAHs and volatile organic compounds present in bitumens or in bitumen emissions that have been evaluated by IARC. Elemental analyses indicate that most bitumens contain primarily hydrocarbons, i.e. carbon, 79–88%; hydrogen, 7–13%; sulfur, traces to 8%; oxygen, 2–8%; nitrogen, 3%; and the metals vanadium and nickel in parts per million (Speight, 2000). The exact chemical composition of a bitumen varies depending on the chemical complexity of the original crude petroleum and the manufacturing processes. In addition, the products of other refining processes, e.g. flux or solvent from petroleum distillate, may be blended with bitumen to achieve the desired performance specifications. Consequently, no two bitumen products are chemically identical, and chemical analysis cannot be used to define the exact chemical structure or chemical composition of bitumens.

PAHs are present in crude oils (Bingham *et al.*, 1979) and generally in lower amounts in bitumens

Table 1.2 IARC evaluation of compounds identified in bitumens or their emissions

Agent	Levelof evidence		IARC Group	Volume	Year of publication
	Humans	Animals			
Acenaphthene	I	I	3	92	2010
Anthanthrene	I	L	3	92	2010
Anthracene	I	I	3	92	2010
Benzene	S	S	1	29, Sup 7, 100F	2011
Benz[*a*]acridine	I	I	3	103	2011
Benz[*c*]acridine	I	L	3	103	2011
Benzo[*a*]anthracene	I	S	2B	92	2010
Benzo[*a*]fluorene	I	I	3	92	2010
Benzo[*a*]pyrene	I	S	1[a]	92, 100F	2011
Benzo[*b*]fluoranthene	I	S	2B	92	2010
Benzo[*b*]fluorene	I	I	3	92	2010
Benzo[*b*]naphtha[*2,1-d*]thiophene	I	L	3	103	2011
Benzo[*e*]pyrene	I	I	3	92	2010
Benzo[*k*]fluoranthene	I	S	2B	92	2010
Carbazole	I	S	2B	103	2011
Chrysene	I	S	2B	92	2010
Coronene	I	I	3	32, Sup 7	1987
Dibenz[*a,h*]acridine	I	S	2B	103	2011
Dibenz[*a,j*]acridine	I	S	2A[a]	103	2011
Dibenz[*c,h*]acridine	I	L	2B[a]	103	2011
7H-Dibenzo[*c,g*]carbazole	I	S	2B	103	2011
Dibenzo[*a,i*]pyrene	I	S	2B	92	2010
Dibenzo[*a,l*]pyrene	I	S	2A[a]	92	2010
Dibenzothiophene	I	I	3	103	2011
Ethylbenzene	I	S	2B	77	2000
Fluoranthene	I	L	3	92	2010
Fluorene	I	I	3	92	2010
Indeno[*1,2,3-cd*]pyrene	I	S	2B	92	2010
1-Methylphenanthrene	I	I	3	92	2010
3-Methylchrysene	I	L	3	92	2010
4-Methylchrysene	I	L	3	92	2010

Table 1.2 (continued)

Agent	Levelof evidence		IARC Group	Volume	Year of publication
	Humans	Animals			
5-Methylchrysene	I	S	2B	92	2010
Naphthalene	I	S	2B	82	2002
Perylene	I	I	3	92	2010
Phenanthrene	I	I	3	92	2010
Phenol	I	I	3	47, 71	1999
Picene	I	L	3	92	2010
Pyrene	I	I	3	92	2010
Styrene	L	S	2B	60, 82	2002
Tetrachloroethylene	L	S	2A	63	1995
Toluene	I	ESLC	3	47, 71	1999
Triphenylene	I	I	3	92	2010
Xylene [*m*+*p*-]	I	I	3	47, 71	1999
Xylene [*o*-]	I	I	3	47, 71	1999

[a] Upgraded based on strong mechanistic evidence

ESLC, evidence suggesting lack of carcinogenicity; I, inadequate evidence; L, limited evidence; S, sufficient evidence

(Brandt & Molyneux, 1985; Brandt *et al.*, 1985a, b). This is because the principal refinery process used for the manufacture of bitumens, namely vacuum distillation, removes the majority of compounds of lower relative molecular mass with lower boiling-points, including PAHs with three to seven fused rings, and because the maximum temperatures involved in the production of vacuum residue range from 350 °C to 450 °C and are not high enough to initiate significant PAH formation. Although most of the PAHs are removed during the manufacturing process, residues of two- to seven-ring PAHs are found both in solid bitumens and bitumen emissions. Bitumen emissions tend to contain proportionally more two-ring PAHs, such as naphthalene, and less five-ring PAHs, such as benzo[*a*]pyrene, than solid bitumens.

Bitumen products are tailored to needs on the basis of required physical properties rather than on chemical composition. Bitumens are soluble in carbon disulfide, chloroform, ether and acetone, partially soluble in aromatic organic solvents, and insoluble in water at 20 °C (IPCS, 2004). Until the 1990s, bitumen specifications in both Europe and the USA relied primarily on mechanical tests of hardness and viscosity. At that time, the Strategic Highway Research Program (SHRP) introduced the performance grade (PG) system, which replaced the penetration and viscosity grading systems for both conventional, unmodified bitumens and polymer-modified bitumens in the USA. The PG system is used to assess and designate engineering properties at temperatures that are representative of the climatic conditions in which the bitumens will be used (Asphalt Institute & Eurobitume, 2011). Conventional notation for PG binders is a two-number system where the first number represents the maximum pavement design temperature (°C), while the second number represents the minimum likely pavement design temperature (°C) that can be used without failure (e.g. PG 64–28). Table 1.3 summarizes the ASTM requirements by performance grade.

Older specification systems are still recognized alongside the newer generation of performance-based systems. The important characteristics of bitumen production are summarized below.

(a) Penetration

The penetration test (or "pen" test) is used to measure the hardness of bitumens, lower penetration indicating greater hardness. In testing, a container of bitumen is kept at the standard test temperature, 25 °C, in a temperature-controlled water bath. A steel needle of specified dimensions is allowed to bear on the surface of the bitumen for 5 seconds under a load of 100 g (British Standards Institution, 1974). The distance that the needle penetrates, in tenths of a millimetre (dmm), is the penetration measurement. Specifications for penetration-graded bitumens typically state the penetration range for a grade (e.g. 50/70). On the basis of this test, bitumens have been classified into five standard grades of penetration (from hardest to softest): 40–50, 60–70, 85–100, 120–150, and 200–300 dmm (NIOSH, 2001a).

(b) Softening-point

In the softening-point test, the temperature of a sample of bitumen in the form of a disc is raised at 5 °C per minute while being subjected to loading by a small steel ball. As the temperature rises, the bitumen softens and the particular temperature at which the disc of bitumen is deformed by a distance of 1 inch [2.54 cm] is recorded as the softening-point in °C (British Standards Institution, 1983a).

(c) Viscosity

The viscosity of bitumen can be measured in several ways. For example, vacuum capillary viscometers are used for definition by grade; for products of relatively low viscosity, simple

Table 1.3 Bitumen specifications by performance grade

	PG 46	PG52	PG 58	PG 64	PG 70	PG 76	PG 82
Grade range	**–34 to –46**	**–10 to –46**	**–16 to –40**	**–10 to –40**	**–10 to –40**	**–10 to –34**	**–10 to –34**
Average 7-day maximum pavement design temperature (°C)	< 46	< 52	< 58	< 64	< 70	< 76	< 82
Minimum pavement design temperature (°C)	>–34 to >–46	>–10 to >–46	>–16 to >–40	>–10 to >–40	>–10 to >–40	>–10 to >–34	>–10 to >–34
Original binder							
Flash-point temperature, D92; min. (°C)	230	230	230	230	230	230	230
Viscosity, D 4402: max. 3 Pa × s, test temperature (°C)	135						
Dynamic shear, D7175: G*/sinδ, min. 1.00 kPa; 25 mm plate, 1 mm gap; test temperature at 10 rad/s (°C)	46	52	58	64	70	76	82
Rolling thin film oven residue (T 240)							
Mass loss, max. %	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dynamic shear, D7175: G*/sinδ, min. 2.20 kPa; 25 mm plate, 1 mm gap; test temperature at 10 rad/s (°C)	46	52	58	64	70	76	82
Pressure ageing vessel residue (PP 1)							
PAV ageing temperature (°C)	90	90	100	100	100 (110)	100 (110)	100 (110)
Dynamic shear, D7175: G* × sinδ, max. 5000 kPa; 8mm plate, 2 mm gap; test temperature at 10 rad/s (°C)	10 to 4	25 to 7	25 to 13	31 to 16	34 to 19	37 to 25	40 to 28
Creep stiffness, D 6648: S, max. 300 MPa; m-value, min. 0.300; test temperature at 60 s (°C)	–24 to –36	0 to –36	–6 to –30	0 to –30	0 to –30	0 to –24	0 to –24
Direct tension, D6723: failure strain, min. 1.0%; test temperature at 1.0 mm/min. (°C)	–24 to –36	0 to –36	–6 to –30	0 to –30	0 to –30	0 to –24	0 to –24

PAV, pressure ageing vessel; PG, performance grade
Adapted from ASTM (2008)

orifice-type viscometers are generally used (British Standards Institution, 1983b). Viscosity is typically calculated from the time required for the bitumen binder to flow between two successive marks. The viscosity grade of bitumen (AC-2.5, AC-5, AC-10, AC-20, AC-30, and AC-40) or bitumen residue (AR-4000, AR-8000, and AR-16 000) indicates viscosity in hundreds of poises (gram per centimetre per second) at 60 °C (NIOSH, 2001a).

(d) Temperature susceptibility

Tests of ductility are used to determine the ability of bitumen to stretch at intermediate temperatures (4–25 °C). "Dog-bone" shaped specimens are pulled at a constant rate until the sample breaks. Penetration index is used as an indication of temperature susceptibility.

(e) Solubility

The solubility test ASTM D2042 (ASTM, 2010) is used to measure the purity of the bitumen. Active cementitious constituents will be soluble in trichloroethylene while non-cementing matter is not. As a prerequisite of eligibility to be graded in the Superior Performing Asphalt Pavements (Superpave) system, insoluble matter cannot exceed 1%.

(f) Flash-point

The flash-point is the temperature at which bitumen fume may flash, or spark. As an example, this temperature is usually 230 °C or higher for common paving bitumens. The flash-point provides an indication of fire hazard and the test is frequently used to indicate whether a given product has been contaminated with materials of lower flash-point.

1.1.3 Classification of refined petroleum bitumens

For the purposes of this *Monograph*, bitumens have been categorized into six classes. Examples of typical specifications for straight-run bitumens (class 1), oxidized bitumens (class 2) and cutback bitumens (class 3) are provided in the tables in this Section. Emulsion bitumens (class 4), modified bitumens (class 5) and thermal ly cracked bitumens (class 6) are used in a range of applications with specifications that vary depending on the intended use, but are not as widely used as classes 1 and 2.

(a) Straight-run or paving bitumens (class 1)

Straight-run or paving bitumens (CAS No. 8052-42-4; EINECS No. 232-490-9) are usually produced from the residue from atmospheric distillation of petroleum crude oil by applying further distillation under vacuum, solvent precipitation, or a combination of these processes (see Section 1.2.1). In Australia, class 1 bitumens are called "viscosity-graded asphalt cements"; in the USA, they are called "asphalt binders". An additional straight-run bitumen product includes residues obtained via further separation by a de-asphalting process (Asphalt Institute & Eurobitume, 2011). The types and levels of PAHs found in bitumens of class 1 are shown in Table 1.4 and Table 1.5.

In Europe, straight-run bitumens are defined by the upper and lower limits of penetration values. For example, a nominal 200-PEN grade has a range of 170–230 in the British Standards Specification (British Standards Institution, 1982a, b). Penetration grades commonly used vary from 15-PEN to 450-PEN, though terminology varies by country. In France, the 180–220 grade has limits of 180–220, while in Germany the B200 grade may vary from 160 to 210 in penetration value. Specific ranges of softening-point are required for particular straight-run bitumens to ensure that the penetration index (PI, a measure

Table 1.4 Content of PAHs (mg/kg) in eight samples of straight-run bitumens (class 1)

PAH	n[a]	Bitumen[b]							
		A	B	C	D	E	F	G	H
Anthracene	3	ND	ND	ND	ND	ND	ND	ND	ND
Phenanthrene	3	2.3	0.4	3.5	1.3	0.6	35*	1.1	2.3*
Pyrene	4	0.6	1.8	4.0	8.3	0.9	38	0.3	0.08
Fluoranthene	4	+	+	2.0	+	+	5	ND	ND
Benzofluorenes	4	+	+	+	+	+	+	+	ND
Benz[*a*]anthracene	4	0.15	2.1	1.1	0.7	0.9	35	0.2	ND
Triphenylene	4	0.25	6.1	3.1	3.4	3.8	7.6	1.0	0.3
Chrysene	4	0.2	8.9	2.3	3.9	3.2	34	0.7	0.04
Benzo[*a*]pyrene	5	0.5	1.7	1.3	2.5	1.6	27	0.1	ND
Benzo[*e*]pyrene	5	3.8	13	2.9	3.2	6.5	52	1.6	0.03
Benzo[*k*]fluoranthene	5	+	ND	+	+	+	ND	ND	ND
Perylene	5	ND	39	2.2	6.1	2.9	3.0	0.1	ND
Anthanthrene	6	ND	Tr	Tr	Tr	+	1.8	ND	ND
Benzo[*g,h,i*]perylene	6	2.1	4.6	1.0	1.7	2.7	15	0.6	Tr
Indeno[*1,2,3-cd*]pyrene	6	Tr	ND	Tr	Tr	Tr	1.0	ND	ND
Picene	6	+	+	+	+	+	1.0	+	ND
Coronene	7	1.9	0.8	0.5	0.2	0.9	2.8	0.9	ND

[a] Number of aromatic rings
[b] Estimate includes alkyl derivatives
+, not estimated but present in small amount; ND, not detected; Tr, trace
Adapted from Wallcave *et al.* (1971)

Table 1.5 Content of 14 PAHs (mg/kg) in some straight-run (class 1) and oxidized (class 2) bitumens[a]

PAH	n[b]	Class 1			Class 2			
		80/100	80/100	50/60	80/100	85/40	110/30	95/25
Phenanthrene	3	7.3	5.0	1.7	5.0	0.32	1.7	2.4
Anthracene	3	0.32	0.27	0.015	0.17	0.01	0.03	0.07
Fluoranthene	4	0.72	0.46	0.41	0.39	0.15	0.4	0.46
Pyrene	4	1.5	1.0	0.26	1.1	0.17	0.3	0.29
Chrysene	4	1.5	3.3	0.47	3.9	0.90	1.0	0.80
Benz[*a*]anthracene	4	1.1	0.89	0.14	0.63	0.33	0.3	0.23
Perylene	5	3.3	0.69	0.044	0.25	0.14	0.08	0.20
Benzo[*k*]fluoranthene	5	0.19	ND	0.024	ND	0.051	0.10	0.04
Benzo[*a*]pyrene	5	1.8	0.92	0.22	1.1	0.49	0.35	0.48
Benzo[*g,h,i*]perylene	6	4.2	2.3	1.67	2.7	1.3	1.2	2.0
Anthanthrene	6	0.11	0.04	0.006	0.02	0.01	ND	0.03
Dibenzo[*a,l*]pyrene	6	ND	ND	ND	ND	ND	ND	ND
Dibenzo[*a,i*]pyrene	6	0.50	ND	0.05	0.60	ND	0.3	0.10
Coronene	7	ND	ND	0.40	ND	ND	ND	ND

[a] Bitumens obtained from a range of crude oils originating from the Middle East, Venezuela and Mexico.
[b] Number of aromatic rings
ND, not detected; PAH, polycyclic aromatic hydrocarbon
From Brandt & de Groot (1985)

Table 1.6 Specifications for straight-run bitumens (class 1) by penetration grade

Property		Test method	Penetration grade									
			15 PEN	25 PEN	35 PEN	40 PEN HD	50 PEN	70 PEN	100 PEN	200 PEN	300 PEN	450 PEN
Penetration at 25 °C		BS 4 691	15 ± 5	25 ± 5	35 ± 7	40 ± 10	50 ± 10	70 ± 10	100 ± 20	200 ± 30	300 ± 45	450 ± 65
Softening-point (°C)	Min.	BS 4 692	63	57	52	58	47	44	41	33	30	25
	Max.		76	69	64	68	58	54	51	42	39	34
Loss on heating for 5 h at 163 °C		BS 2000:Part 45										
Loss by mass (%)	Max.		0.1	0.2	0.2	0.2	0.2	0.2	0.5	0.5	1.0	1.0
Drop in penetration (%)	Max.		20	20	20	20	20	20	20	20	25	25
Solubility in trichloroethylene by mass (%)	Min.	BS 4 690	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5

Max., maximum; Min., minimum; PEN, penetration grade
From IARC (1985)

Table 1.7 Specifications for hard bitumens (class 1)

Property		Test method	Grade	
			H 80/90	H 100/120
Softening-point (°C)	Min.	BS 4692	80	100
	Max.		90	120
Penetration at 25 °C	Min.	BS 4691	6	2
	Max.		12	10
Loss on heating for 5 h at 163 °C by mass (%)	Max.	BS 2000: Part 45	0.05	0.05
Solubility in trichloroethylene by mass (%)	Min.	BS 4690	99.5	99.5

Max., maximum; Min., minimum
From IARC (1985)

of change in penetration with temperature) does not vary by more than the allowable amount. Table 1.6 summarizes the specifications for class 1 bitumens by penetration grade.

Under the American PG system, PG 64–22 bitumens, for example, provide enough stiffness to prevent permanent deformation or rutting at pavement temperatures as high as 64 °C and low-temperature cracking at temperatures as low as –22 °C. The standard PG specifications are described in AASHTO M320–04 and ASTM D6373 (Asphalt Institute & Eurobitume, 2011).

Hard bitumens are a subset of straight-run bitumens that have low penetration values (i.e. < 15) and are generally designated by the prefix H (HVB in Germany) combined with the softening-point range, e.g. H 80/90. Hard bitumens are brittle in nature and are commonly used in mastic applications. While the nomenclature is derived from the softening-point range, each grade also has a defined penetration range, giving these materials a PI of 0 to +2.0. Table 1.7 summarizes the specifications of hard bitumens by grade.

In the past decade, warm-mix asphalt technologies have been developed for use in road-paving applications, allowing application temperatures to be lowered to 100–140 °C rather than the higher temperatures associated with the application of conventional paving bitumen (140–160 °C). Warm-mix asphalts are produced by adding one of three additives to straight-run bitumens: water (application temperature of 129–135 °C), organics/waxes or chemicals/surfactants (application temperature of 116–121 °C). The resulting road-paving materials allow for lower mixing temperatures, improved coating of the mineral aggregate, better compaction, and lower application temperatures (Prowell *et al.*, 2011).

(b) Oxidized bitumens (class 2)

Oxidized bitumens or blown bitumens (CAS No. 64742-93-4; EINECS No. 265-196-4) are produced by passing air through hot, soft bitumens under controlled temperature conditions, a process that reduces temperature susceptibility and increases resistance to stress. Intense oxidation produces a fully-oxidized product, which has a PI of +2.0 to +8.0 and is used in roofing applications (Asphalt Institute & Eurobitume, 2011). Mild oxidation (i.e. a mild degree of air blowing) produces air-rectified (semi-blown) bitumen, a different product that has a PI ≤ +2.0 and applications similar to those for class 1 bitumens (Asphalt Institute & Eurobitume, 2011). In the USA, oxidized bitumens are also known as "air-blown asphalts" or "roofing asphalts". The types and levels of PAHs found in class 2 bitumens are shown in Table 1.5.

Oxidized bitumens are classified by the ranges of allowable values for penetration and softening-point. For instance, a common grade

Table 1.8 Specifications for oxidized bitumens (class 2)

Property		Test method	Grade					
			75/30	85/25	85/40	95/25	105/35	115/15
Softening-point (°C)	Min.	BS 4692	70	80	80	90	100	110
	Max.		80	90	90	100	110	120
Penetration at 25 °C		BS 4691	30 ± 5	25 ± 5	40 ± 5	25 ± 5	35 ± 5	15 ± 5
Loss on heating for 5 h at 163 °C by mass (%)	Max.	BS 2000: Part 45	0.2	0.2	0.5	0.2	0.5	0.2
Solubility in trichloroethylene by mass (%)	Min.	BS 4 690	99.5	99.5	99.5	99.5	99.5	99.5

Max., maximum; Min., minimum
From IARC (1985)

such as 85/25 has a mean value of 85 for the permissible softening-point range of 80–90 °C and a mean value of 25 for the penetration range of 20–30. In the USA, the specifications for oxidized bitumens are based on softening-point and penetration tests at three temperatures (0 °C, 25 °C and 46 °C). Common grades in this class of bitumens are 85/25, 85/40, 100/40, 105/35, 105/13 and 115/15. Oxidized bitumens are somewhat rubbery in nature and exhibit low-temperature dependence. Table 1.8 outlines the specifications for class 2 bitumens by grade.

(c) Cutback bitumens (class 3)

Cutback bitumens or fluxed bitumens are produced by adding an agent to straight-run bitumens or oxidized bitumens for the purpose of reducing (i.e. "cutting back") viscosity and rendering the products more fluid for ease of handling. Since cutback bitumens include different combinations of multiple products (blends), there is no CAS No. available for cutback bitumens. Examples of agents that are suitable for blending include solvent extracts (aromatic by-products from the refining of base oils), thermally-cracked residues, or certain heavy petroleum distillates with final boiling points > 350 °C. Coal-tar products are also sometimes used as fluxes (see IARC, 2010). When volatile diluents from petroleum crudes (i.e. white spirit, naphtha, kerosene or gas oil) are used, the initial properties of bitumens are recovered when the diluent evaporates. However, when non-volatile agents such as coal tar are used, there is limited evaporation (Asphalt Institute & Eurobitume, 2011). In the USA, cutback bitumens are sometimes referred to as "road oils".

Grades of cutback bitumens are designated by a value in seconds required for a given quantity of the product to flow through a standard orifice at a fixed temperature. Typical products used in road applications are made by cutting back 100-PEN bitumens with 8–14% kerosene. They are designated by the midpoints of the viscosity limits adopted. European specifications for typical products are given by the British Standards Institution (1982a), while specifications in the USA are given by the ASTM. Cutback bitumens are viscous to highly fluid materials at ambient temperatures. Table 1.9 outlines the specifications for class 3 bitumens by grade.

(d) Bitumen emulsions (class 4)

Bitumen emulsions are fine dispersions of bitumen droplets in water, primarily of straight-run bitumens (class 1), although cutback bitumens (class 3) and modified bitumens (class 5) can also be used. Accordingly, there is no CAS No. available for bitumen emulsions. Bitumen emulsions are manufactured using high-speed

Table 1.9 Specifications for cutback bitumens (class 3)

Property		Test method	Grade		
			50 s	100 s	200 s
Viscosity (STV) at 40 °C, 100-mm cup		BS 2000: Part 72	50 ± 10	100 ± 20	200 ± 40
Distillation to 225 °C (% by volume)	Max.	BS 2000: Part 72	1	1	1
Distillation to 360 °C (% by volume)	Max.		8 to 14	6 to 12	4 to 10
Penetration at 25 °C of residue from distillation to 360 °C		BS 4691	100 to 350	100 to 350	100 to 350
Solubility in trichloroethylene by mass (%)	Min.	BS 4690	99.5	99.5	99.5

Max., maximum; Min., minimum; STV, standard tar viscometer
From IARC (1985)

shearing devices, such as colloid mills. The bitumen content can range from 30% to 70% by weight. They can be anionic, cationic or non-ionic depending on the surfactant used (Asphalt Institute & Eurobitume, 2011). In the USA, they are referred to as "asphalt emulsions".

(e) Modified bitumens (class 5)

Modified bitumens contain appreciable quantities (typically 3–15% by weight) of special additives, such as polymers, crumb rubber, elastomers, sulfur, polyphosphoric acid and other products used to modify their properties. This is a variable class of bitumens that are modified for use in specialized applications (Asphalt Institute & Eurobitume, 2011). Accordingly, there is no CAS No. available for modified bitumens.

(f) Thermally-cracked bitumens (class 6)

Thermally-cracked bitumens (CAS No. 92062-05-0; EINECS No. 295-518-9) are produced by extended high-temperature distillation of a petroleum residue (440–500 °C). The thermally-cracked residue produced by this process is vacuum-distilled and further treated to create a hard material used in blending bitumens (Asphalt Institute & Eurobitume, 2011). Thermally-cracked bitumens may contain levels of PAHs of up to 272 µg/kg (Yanysheva *et al.*, 1963). Thermally-cracked bitumens are not produced in the USA.

1.1.4 PAH composition of class 1 and class 2 products and their emissions

Trumbore *et al.* (2011) evaluated the effect of oxidation on the concentrations of PAHs in bitumens. Five samples of straight-run bitumen were laboratory-oxidized to a range of softening-points used for common roofing products. This resulted in a reduction of four- to six-ring PAHs in the oxidized products. [Since workers are exposed to bitumen emissions generated at temperatures that vary by product, it is important to consider the concentration and composition of bitumen emissions generated from different products when heated to the temperatures at which they are typically applied.]

Cavallari *et al.* (2012a) characterized temperature-dependent emissions from 20 samples of straight-run bitumens (typically used in paving) and five samples of oxidized bitumens (typically used in roofing), obtained directly from contractors. Emissions were generated in a laboratory at eight different temperatures ranging from 120 °C to 315 °C. Two of the evaluated temperatures (120 °C, 150 °C) were consistent with those used in paving applications and showed that emissions from straight-run bitumens included two to three-ring PAHs but rarely four- to six-ring PAHs. In comparison, three of the temperatures evaluated (180 °C, 205 °C, 230 °C) were consistent with those used in hot-appplied roofing applications and showed that emissions from oxidized

bitumens included two- to three-ring PAHs and four- to six-ring PAHs at much greater frequency and significantly higher concentrations. In multivariate models, PAHs were found to significantly increase with increasing temperature, with a stronger effect for oxidized bitumens than for straight-run bitumens. Table 1.10 summarizes the PAH results in laboratory-generated emissions by bitumen type and temperature.

While the above experiment was conducted to characterize the chemical composition under occupational conditions, bitumen emissions are also generated for experimental purposes. Accordingly, emission-generation systems are developed to produce emissions in a laboratory setting that are similar to those in the field. For example, Binet *et al.* (2002) analysed PAHs and sulfur-containing PAHs in laboratory-generated emissions from straight-run bitumen samples at 170 °C, selected to represent the upper range of temperatures used in paving applications (Table 1.11).

1.1.5 Naturally occurring bitumens

Natural bitumens form from petroleum as a result of the evaporation of light fractions and of oxidation under the influence of hypergenesis. The petroleum first changes into thick and highly viscous maltha, then into hard and easily fusible bitumens. Further change in natural bitumens usually leads to the formation of asphaltite. Natural bitumens can be recovered for specialized industrial purposes.

1.2 Methods of analysis

1.2.1 Bitumens

The chemical composition of bitumens depends on the chemical complexity of the original crude petroleum and the manufacturing processes, and can be determined by global methods based on their spectrometric properties, or by class separation following chromatography coupled with mass spectrometry (MS) detection for identification of individual chemical compounds. Using solvent precipitation and adsorption chromatography, the chemical characterization of bitumens is based on their separation into four broad classes of compounds: asphaltenes, resins, cyclic compounds, and saturates (IARC, 1985).

(a) Fourier transform infrared

This technique is used to detect and analyse the oxygenated species (ketones, acids, bases) contained in bitumen. With modified bitumens, it is used to identify and quantify added polymers such as styrene–butadiene type copolymers (Masson *et al.*, 2001).

(b) Simulated distillation

Simulated distillation is a type of gas chromatography in which the results are expressed as the boiling-point of the products. It can be used to detect the presence of compounds that are volatile at 100–300 °C. This method (ASTM D2887) is used particularly for class 3 and 4 bitumens and is also useful as a mean of monitoring changes in volatile products in the pavement (ASTM, 2009).

(c) Gel-permeation chromatography

Gel-permeation chromatography is useful for separating compounds with very different molecular sizes (Jennings *et al.*, 1993), and can also be used to identify polymers that have been added to the bitumen (class 5).

(d) Class separation by adsorption chromatography

Adsorption chromatography is a technique to separate bitumens into fractions – asphaltenes, resins, cyclic compounds and saturates – using an alumina column or silica-gel chromatography. The method ASTM D2007-11 may be used (ASTM, 2011).

Table 1.10 Concentrations of PAHs (μg/m³) in laboratory-generated emissions, by temperature, for straight-run bitumens (*n* = 20 samples, *n* = 1600 measurements) and oxidized bitumens (*n* = 5 samples, *n* = 400 measurements)

	Temperature regime 1[a]				Temperature regime 2[a]					
	120 °C		150 °C		180 °C		205 °C		230 °C	
	% BDL	GM (GSD)	% BDL	GM (GSD)	% BDL	GM (GSD)	% BDL	GM (GSD)	% BDL	GM (GSD)
Two-ring PAHs										
Acenaphthene	85	[b]	0	1.80 (1.66)	0	57.9 (1.46)	0	89.9 (1.37)	0	108.2 (1.42)
Fluorene	100	[b]	48	[b]	0	7.62 (5.54)	0	18.3 (2.93)	0	27.6 (2.44)
2-Methylnaphthalene	5	1.20 (1.59)	0	4.51 (2.07)	0	25.3 (1.64)	0	77.5 (1.49)	0	110.5 (1.41)
Naphthalene	0	1.32 (1.43)	0	3.77 (1.63)	0	47.4 (1.51)	0	89.8 (1.56)	0	81.0 (1.87)
Three-ring PAHs										
Anthracene	97	[b]	0	0.81 (5.36)	48	[b]	38	6.66 (25.7)	0	17.2 (10.1)
Fluoranthene	88	[b]	55	[b]	0	8.29 (1.66)	0	14.7 (1.37)	0	20.6 (1.44)
Phenanthrene	100	[b]	0	2.26 (1.53)	0	54.4 (1.46)	0	78.4 (1.45)	0	100.5 (1.42)
Four-ring PAHs										
Benz[*a*]anthracene	97	[b]	17	0.23 (2.23)	0	5.84 (1.84)	0	12.1 (1.44)	0	20.0 (1.39)
Benzo[*b*]luoranthene	97	[b]	85	[b]	33	0.24 (3.07)	0	2.39 (2.81)	0	4.71 (3.11)
Benzo[*k*]fluoranthene	96	[b]	94	[b]	70	[b]	24	0.67 (7.42)	0	1.53 (8.03)
Chrysene	99	[b]	100	[b]	17	0.35 (1.68)	10	0.66 (1.78)	0	1.46 (1.36)
Pyrene	99	[b]	81	[b]	0	5.21 (3.36)	0	6.71 (3.32)	0	8.86 (3.21)
Triphenylene	98	[b]	97	[b]	58	[b]	10	0.31 (1.84)	10	0.55 (2.11)
Five-to six-ring PAHs										
Benzo[*a*]pyrene	96	[b]	97	[b]	80	[b]	38	0.13 (1.86)	0	0.49 (1.54)
Benzo[*e*]pyrene	97	[b]	94	[b]	93	[b]	39	0.12 (1.92)	15	0.19 (1.97)
Dibenz[*a,h*]anthracene	97	[b]	97	[b]	96	[b]	50	[b]	49	[b]
Dibenzo[*g,h,i*]perylene	98	[b]	97	[b]	94	[b]	50	[b]	50	[b]
Indeno[*1,2,3-cd*]pyrene	97	[b]	97	[b]	98	[b]	93	[b]	19	0.14 (1.63)

[a] Samples were evaluated over two temperature regimes: standard application temperatures for warm-mix asphalt (100–140 °C) during paving (regime 1), and standard application temperatures for type II, III, and IV build-up roofing applications (175–240 °C) (regime 2).

[b] GM (GSD) were not calculated for samples with >40% below detection limit (BDL)

% BDL, percent below the detection limit; GM, geometric mean; GSD, geometric standard deviation; PAH, polycyclic aromatic hydrocarbon

From Cavallari *et al.* (2012a)

Table 1.11 Concentrations of PAHs and S-heterocyclic PAHs in laboratory-produced bitumen emissions (class not reported); TPM concentration, 5 mg/m³; temperature, 170 °C

PAH	N[a]	Fume (n = 3)		Vapours (n = 3)	
		µg/m³	% of TPM	µg/m³	% of TPM
Naphthalene	2	0.10	0.002	53	1.06
Methylnaphthalenes	2	0.05	0.001	134	2.68
1-Ethylnaphthalene	2	ND	ND	20	0.4
Dimethylnaphthalenes	2	0.41	0.008	104	2.08
2,3,5-Trimethylnaphthalene	2	0.27	0.005	27	0.54
Biphenyl	2	ND	ND	4.3	0.086
2-Methylbiphenyl	2	ND	ND	0.8	0.016
Acenaphthylene	3	ND	ND	1.7	0.034
Acenaphthene	3	ND	ND	4.1	0.082
Anthracene	3	0.01	< 0.001	0.5	0.01
Fluorene	3	0.14	0.003	12.1	0.24
Phenanthrene	3	1.85	0.037	7.7	0.15
1-Methylphenanthrene	3	1.62	0.032	1.4	0.028
2-Methylphenanthrene	3	1.58	0.032	1.3	0.026
3,6-Dimethylphenanthrene	3	0.16	0.003	ND	ND
Benzo[*a*]anthracene	4	0.06	0.001	ND	ND
Benzo[*a*]fluorene	4	0.17	0.003	ND	ND
Benzo[*b*]fluorene	4	0.01	< 0.001	ND	ND
Chrysene	4	0.12	0.002	ND	ND
Fluoranthene	4	ND	ND	ND	ND
Pyrene	4	0.14	0.003	ND	ND
Methylchrysenes	4	0.23	0.005	ND	ND
1-Methylpyrene	4	0.19	0.004	ND	ND
Benzo[*a*]pyrene	5	0.04	< 0.001	ND	ND
Benzo[e]pyrene	5	0.06	0.001	ND	ND
Benzo[*k*]fluoranthene	5	0.06	0.001	ND	ND
Benzo[*b*]naphtho[1,2-*d*]thiopene	4	0.10	0.002	ND	ND
Benzo[*b*]naphtho[2,1-*d*]thiopene	4	0.33	0.007	ND	ND
Benzo[*b*]naphtho[2,3-*d*]thiopene	4	0.04	0.001	ND	ND
Dibenzothiopene	3	2.64	0.053	ND	ND
Sum of two-ring PAHs		ND	ND	343	6.86
Sum of three-ring PAHs		13	0.26	29	0.58
Sum of four–five ring PAHs		1.08	0.022	ND	ND
Sum of *S*-PAHs		3.12	0.062	ND	ND

[a] Number of aromatic rings
ND, not detected; TPM, total particulate matter
From Binet *et al.* (2002)

(e) Analysis of PAHs

Due to the existence of numerous structural isomers of the PAHs, chromatographic separation either by gas chromatography (GC) coupled with universal flame ionization detection (FID), MS or high-performance liquid chromatography (HPLC) coupled with ultraviolet or fluorescence detection (FD) is generally employed for isomer-specific identification and quantification. Different official methods for PAH analysis have been proposed: National Institute for Occupational Safety and Health (NIOSH) Method 5506 (NIOSH, 1998) for PAHs by HPLC-FD; NIOSH Method 5515 (NIOSH, 1998) for PAHs by GC-FID; and Method 5800 (NIOSH, 1998) for PAHs by HPLC-FD with supplementary clean-up. However, these methods present a poor clean-up scheme, limited theoretical plate-separation power and weak selectivity of FID to achieve the reliable determination of PAHs in such complex matrices. HPLC provides a useful fractionation technique for isolating PAHs from complex sample mixtures and allows quantification with selective detectors after further separation, for example, by GC-MS (Vu-Duc *et al.*, 1995, 2007). Individual PAHs in bitumen emissions may be analysed using intensive clean-up procedures followed by GC-ion trap MS (Huynh *et al.*, 2007).

The development of standard reference materials with certified values for PAHs in complex environmental matrices allows evaluation of new analytical techniques (Wise *et al.*, 1993; Vu-Duc *et al.*, 1995; Schubert *et al.*, 2003). Intensive clean-up procedures followed by GC-MS analytical methods were proposed to overcome the difficulties of quantification of PAHs in bitumens (Vu-Duc *et al.*, 1995, 2007; Sauvain *et al.*, 2001; Huynh *et al.*, 2007). Sulfur-containing PAHs were also identified and quantified by such methods (Binet *et al.*, 2002; Vu-Duc *et al.*, 2007) (see *Monograph on Some* N- *and* S-*heterocyclic polycyclic aromatic hydrocarbons*, in this volume).

1.2.2 Bitumen emissions

Bitumen emissions are defined as complex mixtures of aerosols, vapours and gases from heated bitumens and products that contain bitumen. Although the term "bitumen fume" is often used in reference to total emissions, bitumen fume refers here only to the aerosolized fraction of total emissions (i.e. solid particulate matter, condensed vapour and liquid bitumen droplets). Accordingly, the term "bitumen emissions" is more appropriate for referring to total content of bitumens in air.

A variety of methods for sample collection and analysis are available for evaluating bitumen emissions. Originally, methods focused on inhalable particulates (aerosols fraction) and its solvent extractable fraction. More recent methods address both the aerosol fraction and the vapour fraction. The chemical composition (e.g. PAHs) of the collected fractions can then be determined.

All the following methods use an active sampling technique with personal air pumps to draw air through a sampling medium. The standardized NIOSH Method 5042 has been developed for determination of total particulate matter (TPM) and benzene-soluble fraction (BSF) or cyclohexane-soluble fraction collected on filters only (NIOSH, 1998). Each fraction is analysed separately by gravimetric analysis. A sampling method based on infrared spectrophotometric absorption of aerosols and vapours of bitumen emissions (filter plus XAD-2 cartridge) was developed in Germany (IFA method 6305). This method uses bitumen condensate as a reference standard (DFG, 2011). In the USA, NIOSH method 5506 uses a filter followed by an XAD-2 tube to capture the vapour fraction. This method uses HPLC fluorescence to analyse the PAHs (NIOSH, 1998).

A field study was performed to compare the IFA method 6305 and a modified NIOSH 5506 method using GC-time of flight-MS instead of

HPLC (Kriech *et al.*, 2010). The resulting concentrations from the aerosol, vapours, and aerosol plus vapour fractions showed strong correlation, but the absolute values were higher with the NIOSH method.

Individual PAHs in bitumen emissions may be analysed using an intensive clean-up procedure followed by GC-ion trap MS (Huynh *et al.*, 2007). Luminescence spectroscopy was used as an alternative method to quantify, without identification, a subset of PACs in condensates of bitumen fume (Osborn *et al.*, 2001).

1.2.3 Dermal exposure to PAHs

Unlike for air sampling, there are no standard methods for the assessment of dermal exposure. Dermal exposure to PAHs from bitumens can be measured based on sampling by hand washing or pads on the skin followed by PAH analysis by HPLC or GC-MS.

In the hand-washing method, hands are washed before and after the working shift with 3 mL sunflower oil which is rubbed on the hands for 1 minute. The oil is then wiped with a cleaning tissue, which is extracted with dichloromethane. PAHs are analysed with HPLC-FD (Jongeneelen *et al.*, 1988).

In the exposure-pad method, polypropylene filters are attached to both wrists of the worker for the whole working shift. After sampling the filters are extracted with a mixture of cyclohexane and dichloromethane. PAHs are analysed by GC-MS (Jongeneelen *et al.*, 1988).

1.2.4 Biomonitoring of PAHs

Uptake of PAHs by inhalation and dermal contact can be monitored by measuring the concentration of metabolites of PAHs in the urine of exposed workers.

Urinary 1-hydroxypyrene (1-OHP) is a metabolite of pyrene, a compound commonly detected in bitumen emissions. Urine samples are hydrolysed enzymatically, purified in solid-phase and 1-OHP is analysed by HPLC-FD (Jongeneelen *et al.*, 1988; Lintelmann *et al.*, 1994).

The same method can be used to determine the main metabolites (i.e. 1-, 2-, 3-, 4- and 9-hydroxyphenanthrenes) of phenanthrene, another major PAH in bitumen emissions.

Another major PAH in bitumen emissions is naphthalene, which is metabolized to 1- and 2-naphthol. Urine samples are hydrolysed with an acid, purified in the solid phase, and naphtols are analysed by GC-MS (Keimig & Morgan, 1986). Alternatively, enzymatic hydrolysis and HPLC-FD can be used (Hansen *et al.*, 1992).

Unmetabolized PAHs (naphthalene, phenanthrene and anthracene) can be found in urine and may be analysed by head-space solid-phase microextraction coupled with GC-MS (Sobus *et al.*, 2009a).

1.3 Production and use

1.3.1 Production volumes

The widespread availability of bitumens resulting from oil refining is a comparatively modern development. Bitumens have been produced in the USA by vacuum distillation of crude petroleum since 1902, when 18 000 tonnes were produced. By 1907, the quantity made from this source equalled the amount recovered from sources of natural bitumen (IARC, 1985). By 1938, annual consumption had grown to 5 million tonnes (Chipperfield, 1984). Production in the USA reached 29 million tonnes in 1978, but dropped steadily to 20 million tonnes in 1982 (IARC, 1985). By 2000, bitumen production had risen again, reaching 30 million tonnes for paving and non-paving applications (IPCS, 2004). Several European countries were producing substantial quantities of bitumens by the 1920s and experienced similar increases thereafter. Table 1.12 summarizes the estimated quantity of bitumens used between 1960 and

Table 1.12 Annual use of bitumens by country (million tonnes)

Country	Year				
	1960	1976	1980	1982	2004
Austria	0.1	0.6	0.6	0.5	NA
Belgium, the Netherlands, Luxembourg	0.3	1.3	1.1	0.8	NA
Canada	1.5	2.8	3.4	NA	3.0
Finland	NA	NA	NA	0.4	0.3
Denmark, Norway, Sweden	0.4	1.3	1.0	1.3	0.9
France	1.2	3.1	2.8	2.4	4.2
Germany	1.4	3.9	3.4	3.0	2.3
Italy	0.6	NA	1.9	1.9	NA
Japan	NA	NA	4.7	4.4	3.0
New Zealand	0.1	0.1	0.1	0.1	0.2
South Africa	0.1	0.3	0.3	0.3	0.3
United Kingdom of Great Britain and Northern Ireland	1.1	1.9	1.8	2.0	2.3
United States of America	18.9	25.5	27.3	23.2	27.4

NA, not available
From IARC (1985) and IBEF (2006)

2004 in selected countries. Table 1.13 provides a more complete overview of global consumption by country in 2004 and 2005 (estimated). As of 2007, approximately 85% of bitumens were being used in paving applications, 10% in roofing applications, and 5% in other specialized applications such as waterproofing, insulation, and pipe coatings (Asphalt Institute & Eurobitume, 2011). Table 1.14 provides an estimate of the pattern of bitumen use by class. It is estimated that the current annual world use of bitumens is more than 102 million tonnes (Asphalt Institute & Eurobitume, 2011).

1.3.2 Production processes

Bitumens are derived from the distillation of crude petroleum oils that give substantial amounts of heavy residue, typically from 10–50%, although crude oils giving a greater yield of residue are sometimes used. While the manufacturing process can alter the physical properties of bitumens, the chemical properties do not change unless thermal cracking [breakage of bitumen molecules at high temperatures] occurs as in the production of class 6 bitumens (NIOSH, 2001a).

The processes used in bitumen production are summarized below and illustrated in Fig. 1.1 (for a more detailed summary of these processes, see Chipperfield, 1984; NIOSH, 2001a).

(a) Distillation

The first stage in oil refining is atmospheric distillation. Crude petroleum is heated to 340–400 °C (644–752 °F) and introduced at atmospheric pressures into a distillation tower in which the most volatile components vapourize. More volatile components rise higher in the tower than less volatile components. When the temperature drops below the boiling-point of a specific component, that component condenses and is collected in a tray. The remaining residuum is called "straight-reduced bitumen" (Roberts *et al.*, 1996; Speight, 2000). Raising the temperature to 400–560 °C increases the likelihood of cracking and causes the more volatile components (and even the components with higher boiling-points) to be released from the residuum.

Table 1.13 Annual consumption of straight-run bitumens (class 1) and bitumen emulsions (class 4)

Country	Annual consumption (tonnes)			
	2004		Estimation 2009[a]	
	Paving bitumens	Bitumen emulsions	Paving bitumens	Bitumen emulsions
Afghanistan	NR	NR	500	NR
Algeria	NR	NR	950 000	35 000
Angola	NR	NR	45 000	3 000
Argentina	328 000	20 000	500 000	74 000
Australia	NR	NR	[b]849 000	[b]80 000
Austria	NR	20 500	[b]500 000	[b]20 000
Bangladesh	NR	NR	50 000	NR
Belgium	209 000	9 300	200 000	6 700
Benin	34 400	650	[b]10 000	[b]1 500
Botswana	30 000	12 000	20 000	4 000
Brazil	1 200 000	400 000	1 600 000	[b]440 000
Bulgaria	132 000	5 014	[b]140 000	[b]5 000
Cambodia	11 000	5 200	24 000	9 000
Cameroon	NR	NR	16 000	NR
Canada	2 675 000	350 000	3 450 000	[b]250 000
Chile	180 000	18 000	325 000	[b]30 000
China	NR	NR	15 200 000	418 000
Colombia	200 000	15 000	342 000	[b]55 000
Congo	NR	NR	15 000	1 500
Croatia	NR	NR	[b]200 000	5 000
Czech Republic	347 650	36 950	430 000	35 000
Democratic Republic of the Congo	NR	NR	18 000	NR
Denmark	180 000	22 000	140 000	14 000
Ecuador	180 000	10 000	236 000	23 000
Egypt	NR	NR	820 000	NR
El Salvador	36 000	3 200	31 000	1 800
Estonia	60 000	15 000	69 300	31 500
Finland	306 000	8 100	[b]300 000	[b]10 000
France	3 186 707	990 520	3 040 000	950 000
Gabon	NR	NR	5 000	2 500
Germany	2 170 000	120 000	3 800 000	170 000
Greece	NR	25 000	300 000	[b]10 000

Table 1.13 (continued)

Country	Annual consumption (tonnes)			
	2004		Estimation 2009[a]	
	Paving bitumens	Bitumen emulsions	Paving bitumens	Bitumen emulsions
Guatemala	60 000	3 500	63 000	3 500
Honduras	18 000	1 500	20 000	2 000
Hungary	160 000	5 310	160 000	15 000
Iceland	30 000	1 442	22 000	1 800
India	3 500 000	90 000	4 959 000	224 000
Indonesia	600 000	15 000	805 000	20 100
Iran, Islamic Republic of	NR	NR	2 400 000	45 000
Ireland	600 000	130 000	600 000	120 000
Israel	NR	NR	230 000	15 000
Italy	NR	NR	2 016 000	115 000
Japan	2 730 000	223 985	1 768 000	192 000
Kenya	6 500	2 000	13 000	4 000
Korea, Republic of	1 807 310	57 073	1 941 400	77 600
Lao People's Democratic Republic	7 500	444	12 000	3 000
Latvia	55 100	13 500	[b]30 000	5 000
Lithuania	NR	NR	3 700	2 000
Madagascar	NR	4 500	[b]3 000	[b]1 000
Malaysia	857 000	36 000	650 000	45 000
Mauritius	10 000	1 500	11 200	1 000
Mexico	1 400 000	620 000	1 851 000	650 000
Morocco	NR	62 000	363 000	78 000
Mozambique	25 000	1 500	10 000	2 500
Myanmar	NR	NR	10 000	1 000
Namibia	12 000	12 000	15 000	1 000
Nepal	NR	NR	30 000	12 000
Netherlands	NR	NR	300 000	30 000
New Zealand	160 000	20 000	171 000	14 000
Nicaragua	27 000	1 500	[b]30 000	2 000
Nigeria	NR	NR	186 000	NR
Norway	264 000	6 800	360 000	8 000
Oman	NR	NR	200 000	NR
Pakistan	NR	NR	324 000	4 200

Table 1.13 (continued)

Country	Annual consumption (tonnes)			
	2004		Estimation 2009[a]	
	Paving bitumens	Bitumen emulsions	Paving bitumens	Bitumen emulsions
Peru	NR	NR	7 300	1 700
Philippines	NR	NR	70 000	4 000
Poland	800 000	85 000	1 300 000	130 000
Portugal	611 608	66 171	450 000	40 000
Romania	290 000	37 000	270 000	48 000
Russian Federation	NR	NR	3 441 000	300 000
Saudi Arabia	NR	NR	500 000	75 000
Singapore	5 000	1 000	45 000	3 000
Slovakia	8 580	153	117 000	6 800
South Africa	250 000	80 000	415 000	75 000
Spain	1 200 000	347 623	1 950 000	265 000
Sri Lanka	NR	15 000	58 000	2 000
Sweden	375 000	64 000	507 000	64 000
Switzerland	NR	NR	160 000	17 000
Thailand	861 150	147 070	585 000	119 000
Tunisia	20 000	17 000	[b]160 000	[b]15 000
Turkey	133 000	80 000	2 000 000	56 020
United Arab Emirates	NR	NR	400 000	3 000
United Kingdom	2 196 000	149 842	1 370 000	135 000
United Republic of Tanzania	NR	NR	30 000	3 000
United States of America	25 000 000	2 400 000	[b]20 352 000	2 250 000
Uruguay	60 000	3 000	[b]60 000	[b]3 000
Venezuela	120 000	12 000	300 000	[b]10 000
Viet Nam	30 000	18 000	495 000	14 000
Zambia	8 000	2 400	10 000	3 300
Zimbabwe	2 500		8 000	100
Total	**57 463 005**	**7 031 247**	**88 238 400**	**2 263 000**

[a] Data not consolidated, presented at World Congress on Emulsion, October 2010.
[b] Estimation based on incomplete information from that country.
NR, not reported
From IBEF (2006)

Table 1.14 Bitumen use pattern (%) by class

Class	Western Europe	Japan	USA
Straight-run bitumens [class 1]	74	86	70
Oxidized bitumens [class 2]	12	6	13
Cutback bitumens [class 3]	8	7[a]	10
Bitumen emulsions [class 4]	4		7
Modified bitumens [class 5]	< 2	< 1	0

[a] Classes 3 and 4 combined
Adapted from IARC (1985)

The atmospheric residue of very heavy crude oils is sometimes used for bitumen production and is generally distilled further to yield various products. Atmospheric distillation followed by vacuum distillation of the residuum helps separate remaining volatile components with higher boiling points. The use of vacuum distillation prevents thermal degradation of distillates and residue by reducing pressure. The resulting products are called "vacuum-processed bitumens" (NIOSH, 2001a).

Vacuum residues from particular crude oils meet specification requirements for straight-run bitumens (class 1). Steam is sometimes injected into the residue to aid distillation in a process known as steam stripping, and bitumens produced in this way are referred to as "vacuum-processed, steam-refined" (class 1).

(b) Air blowing

Oxidized bitumens (class 2) are produced by extended air blowing of vacuum residues, propane-precipitated bitumens, or mixtures of vacuum residues and atmospheric residues or waxy distillates. Catalysts such as ferric chloride (0–2%) and phosphorus pentoxide (0–4%) are used in a few refineries to speed the reaction or to modify the properties of the resultant bitumens, referred to as "catalytic air-blown bitumens" (class 2) (Speight, 2000). The blowing process dehydrogenates the residue, resulting in oxidation and condensation polymerization. The content of asphaltenes is considerably increased, while the content of cyclics is decreased (IARC, 1985).

Limited air blowing, known as "air rectification", may be used to produce bitumens for paving or industrial uses with properties similar to those of class 1 (Asphalt Institute & Eurobitume, 2011).

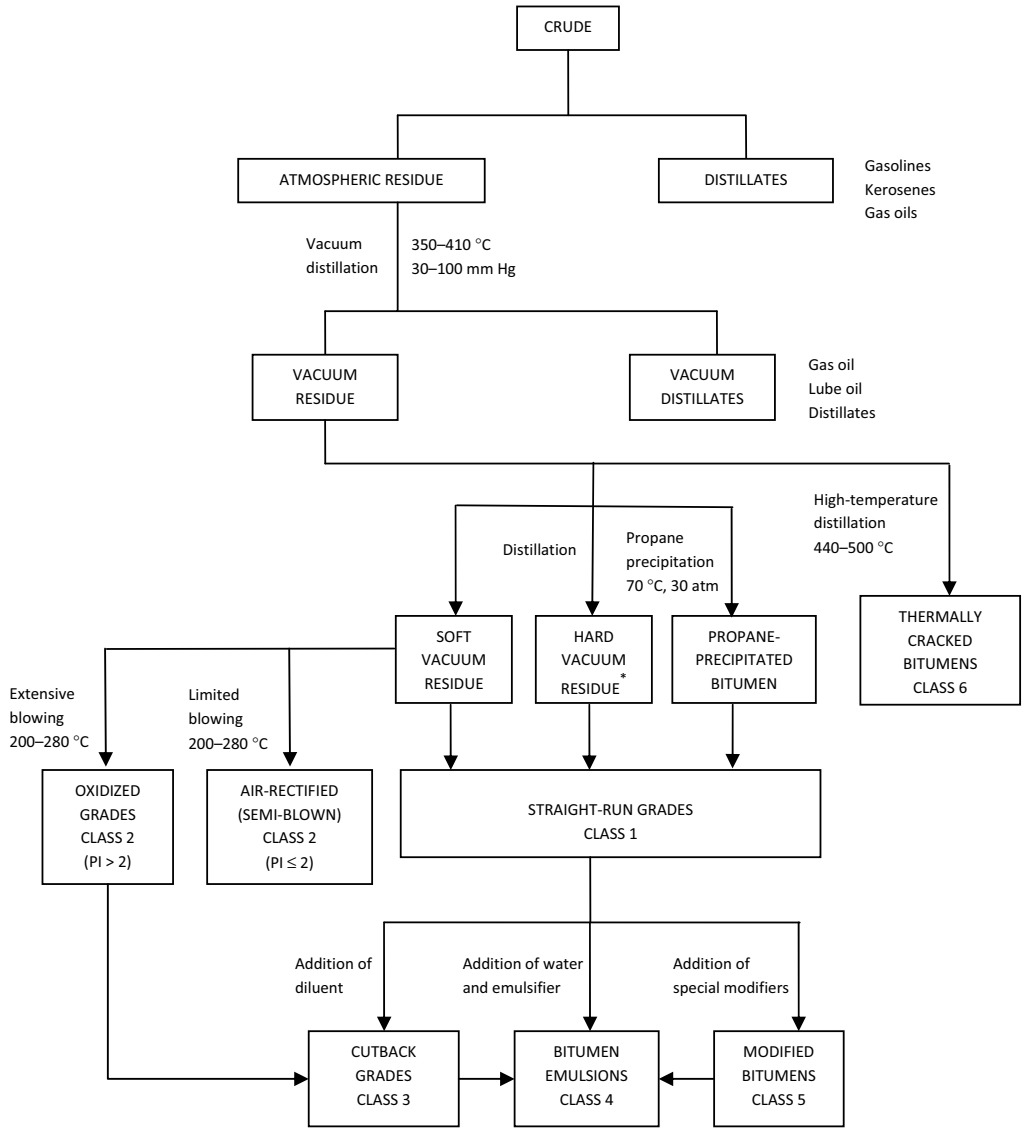
(c) Solvent precipitation

Some crude oils contain components of high boiling-point that are difficult to recover even when high vacuum is used. Such materials are therefore separated from the vacuum residue using solvent precipitation, usually with propane or butane. The resulting product precipitated is called "propane-precipitated bitumen", although in a strict sense this is a class 1 bitumen as defined in this *Monograph*. In the USA, propane-precipitated bitumens are also referred to as "solvent-refined asphalt" or "propane-derived asphalt". Solvent-precipitated bitumens, which are harder and less resistant to temperature changes than other bitumens, have a higher content of asphaltenes than the vacuum residues from which they are produced, but a lower content of saturates than would be obtained by distillation of the vacuum residue (IARC, 1985).

(d) Transportation and storage

Class 1 and class 2 bitumens are normally delivered in bulk by pipeline, tanker truck, or railcar, in liquid form at temperatures of 90–230 °C, depending on the type of bitumen and local practice. Cutback bitumens are usually

Fig. 1.1 Main processing methods in the production of bitumens



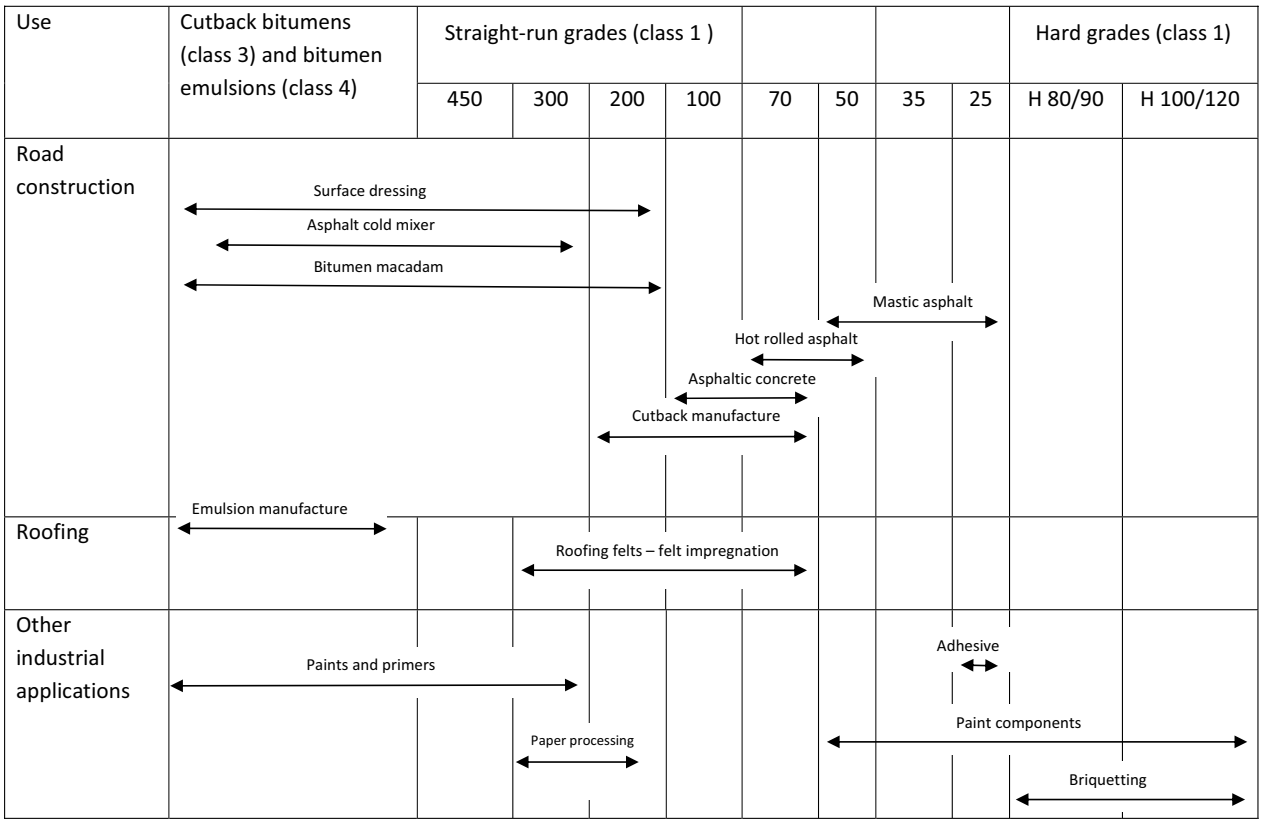
* Used for mastic asphalt
Compiled by the Working Group

stored at 50–80 °C, although storage temperatures of up to 230 °C have been noted. Lower temperatures are usually maintained with steam coils in the tanks. Emulsions are stored and transported between 20 °C and 90 °C. Saturated and coating bitumens are normally stored at 200–260 °C (NIOSH, 2001b; NAPA & EAPA, 2011).

1.3.3 Uses

The major applications of bitumen are in paving for roads and airfields, hydraulic uses (such as dams, water reservoirs and sea-defence works), roofing, flooring and protection of metals against corrosion. More than 80% of bitumens are used in the many different forms of road construction and maintenance. Fig 1.2a describes the principal uses of cutback and

Fig. 1.2a Principal uses of cutback, straight-run and hard bitumens, and bitumen emulsions



Compiled by the Working Group

emulsions, straight-run and hard bitumens, and Fig 1.2b describes the principal uses of oxidized bitumens.

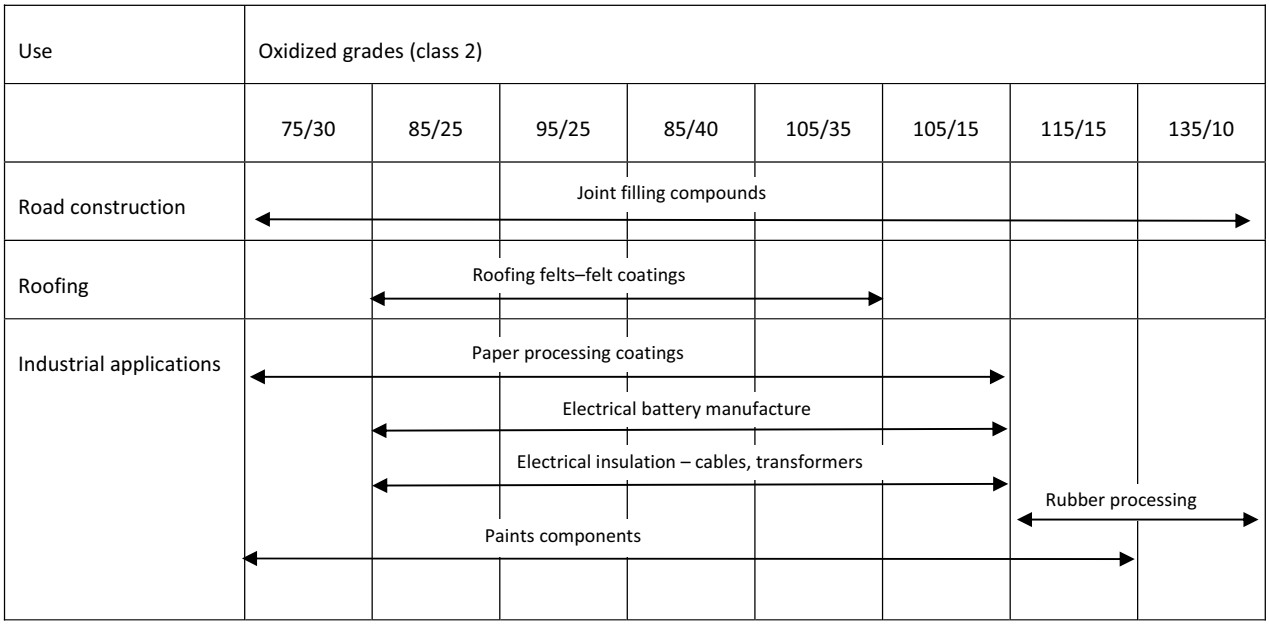
(a) Manufacture of products containing bitumen

The manufacture of roofing felt is based on the use of hot straight-run bitumens (class 1, typically 200-PEN) to impregnate, during immersion, a dry felt made from waste paper or rags. A surface coating is then applied to both sides of the saturated felt using oxidized bitumens (class 2, e.g. 85/40 or 105/35), which sometimes contains added filler. Impregnated felts are also used for damp-proof courses in masonry. Oxidized bitumens are also used in roofing applications such as shingles.

(b) Paving

Asphalt mixes are manufactured by heating and drying mixtures of graded crushed stone, sand and filler (the mineral aggregate) and mixing with straight-run or air-rectified bitumens (typically 4–10% by weight), which serve mainly as a binder to hold the aggregate together. At the construction site, the asphalt mix is fed through a mechanical laying machine, which spreads and compacts the mix. The application temperature of the hot mix asphalt is usually between 112 °C and 162 °C (NIOSH, 2001a). Asphalt mixes include asphaltic concrete, bitumen macadams, and hot rolled asphalts. Special techniques can be adopted to mix aggregate or sands with cutback bitumens (class 3) or emulsions (class 4). These may be carried out with only minor heating or at

Fig. 1.2b Principal uses of oxidized bitumens



Compiled by the Working Group

ambient temperature and are therefore referred to as "asphalt cold mixes".

Although straight-run and air-rectified bitumens are the main types used in paving asphalt mixes, as described above, cutback (class 3) and emulsified (class 4) bitumens are commonly used to provide a waterproof layer under new pavement surfaces and sometimes to improve bonding between various layers of asphalt pavement. They are also used in some surface sealing applications and to produce a cold-mix patching material (NAPA & EAPA, 2011).

Bitumens are laid onto roads by a placement and compaction crew of about five to nine people. These jobs, as pictured in Fig. 1.3, include paver operators, screed operators, labourers/rakers and roller operators. Paver operators (pavers, paving machine operators) drive the paver machine, which receives asphalt from delivery trucks and distributes it on the road in preparation for the roller machine. Screed operators work behind the paver, controlling the even spread of the asphalt mat with a spreading augur before compaction. Mobile rakers work behind the paver, shovelling and raking excess asphalt material to fill in voids and prepare joints for rolling. Labourers often work as rakers, but also handle other tasks that may be more removed from the asphalt fume. Roller operators (rollers) drive the machinery that compacts the asphalt mat and have the mobility to work at varying distances from the paving machine. A foreman supervises the crew, often coming into close proximity to the screed (NAPA & EAPA, 2011).

In place of new mineral aggregates and bitumens, reclaimed asphalt pavement is commonly added to asphalt mix for use in highway pavements and other applications. The proportion of reclaimed asphalt pavement used depends on several factors, but can contribute to as much as 30% of highway mixtures and 60% in other applications (NAPA & EAPA, 2011). Specifications vary in the amount of reclaimed asphalt pavement allowed for particular pavements. Regulations prohibit the recycling of reclaimed asphalt pavement with a given coal-tar

Fig. 1.3 Typical job composition of a road paving crew

Courtesy of the National Asphalt Pavement Association

content in most European countries and in the USA (see Section 1.5.2).

Surface dressing and surface treatments are used to seal minor roads (i.e. low traffic volume) or to maintain road surfaces that have suffered abrasion and loss of skid resistance. Straight-run bitumens (class 1), cutbacks (class 3) or emulsions (class 4) are sprayed onto the surface being treated to give a uniform film to which chippings are applied, followed by light rolling.

Coal tar, which is similar in appearance to bitumen, was used in global paving industries until the 1990s. Coal tar is a by-product of processing coal by thermal degradation in a coking plant and of making oil from coal in the Fischer–Tropsch process. As a result of these two processes, coal tar has a much higher PAH content than bitumen, which is produced by petroleum refining. The differential use of coal tar in different countries worldwide was based mostly on economics and on the availability of bitumen. In Europe, for example, coal tar was blended with bitumen and used in all layers of paving until oil production increased and coal fell out of economic favour in the 1970s and 1980s. Coal tar was eventually phased out in Europe in the 1990s and controls were put in place to prevent coal tar from re-entering pavement as a result of recycling. Coal tar has not been widely used in the USA since the Second World War

Fig. 1.4a Example of cold-applied bitumen

Courtesy of the National Roofing Contractors Association

and is limited to a few non-road applications, such as a sealer in airfield pavement (NAPA & EAPA, 2011). More information on use of coal tar is available in *IARC Monograph* Volume 92 (IARC, 2010).

(c) Roofing

The roofing industry primarily uses oxidized bitumens (class 2) in applications that vary widely according to the type of roofing product and application temperature. Bitumen roofing products can be cold-applied (e.g. bitumen shingles on steep-sloped roofs of residential buildings), soft-applied (e.g. bitumen membranes on low-sloped roofs), or hot-applied (i.e. hot liquid bitumens as the bonding agent on gently sloping roofs) (Asphalt Roofing Manufacturers Association, 2011). Over the past 20 years, cold-applied roofing systems have largely replaced hot-applied roofing. In Europe and North America, cold-applied bitumen accounts for 81% of the production of bitumen roofing, while soft-applied (13%) and hot-applied (6%) bitumen are much less common (Asphalt Roofing Manufacturers Association, 2011).

In cold-applied roofing applications, workers install bitumen shingles using fasteners, typically roofing nails or staples. In soft-applied roofing applications, workers use either propane torches or hot-air welders to heat the polymer-modified bitumen membranes as the material is unrolled to ensure adequate adhesion to the other elements of the system (Asphalt Roofing Manufacturers Association, 2011). Fig 1.4a and

Fig. 1.4b Example of soft-applied bitumen

Courtesy of the National Roofing Contractors Association

Fig. 1.4b show roofing workers applying these types of roofing system.

In hot-applied roofing, bitumens are typically heated on-site in a kettle (180–230 °C) and pumped to the roof via a supply line or brought to the roof in buckets. A worker remains on the ground and tends the kettle (i.e. filling, fluid and temperature checks, and skimming-off debris), while other workers tear off the old roof and put down the new roof. The rooftop workers first tear off the old roof, which is typically an old bitumen roof that may or may not contain coal tar. Once the bitumen has been delivered to the rooftop, it may be drawn directly into mop carts or buckets for manual installation jobs in which it is applied much like mopping a floor. Fig 1.4c and Fig. 1.4d show workers applying a hot bitumen roof (Asphalt Roofing Manufacturers Association, 2011).

(d) Mastic-asphalt applications

Mastic asphalt is a mixture of relatively hard-grade, straight-run bitumens (class 1), coarse aggregate, and/or sand, and/or limestone fine aggregate, and/or filler. Mastic asphalt may also contain additives (polymers, natural bitumens, wax or pigments) (class 5). Its application temperature is high, usually 200–250 °C. It is pourable, spreads well when hot, and forms a waterproof and durable surface (Fig. 1.5; European Mastic Asphalt Association, 2009).

Mastic asphalts are used in Europe, but are practically nonexistent in the USA and Canada. Their use in Asia has begun to grow recently. They are used in bridge decks, as flooring in houses and industrial buildings, in heavy traffic motorways, rooftop car parks, hydraulic constructions (canal slopes, riverbanks) and in flat-roof waterproofing (European Mastic Asphalt Association, 2009).

Fig. 1.4c Example of hot-applied bitumen (kettleman)

Courtesy of the National Roofing Contractors Association

Fig. 1.4d Example of hot-applied bitumen (mopper)

Courtesy of the National Roofing Contractors Association

Fig. 1.5 Example of mastic-asphalt application

Courtesy of European Mastic Asphalt Association

Mastic asphalt was often manufactured in the past in mobile mixing plants at the work-site. Nowadays it is manufactured in specially designed stationary plants. From there it is transported to the processing location in mixers with a heating system mounted on a truck or chassis of a trailer. From the transportation mixer it is transferred into dumpers or carts. If necessary, it may also be poured into metal or wooden buckets or wheelbarrows to reach the actual processing site. Recently special pumps have been developed for the transfer of mastic asphalt to the application site. Both interior and exterior applications are often done manually. Mastic asphalt is hand-spread to the desired thickness and levelled with a wooden float or screed. Mechanical pavers are used for large surfaces, e.g. in the paving of highways. As a rule, the surface of mastic asphalt is coated with sand or aggregates. In road construction, aggregate pre-coated with bitumen is usually spread evenly and pressed into the still warm mastic asphalt (European Mastic Asphalt Association, 2009).

The term "stone-mastic asphalt" describes a paving mixture with a high stone content, used

in some countries. It should not be confused with the "mastic-asphalt application" described here.

(e) Other specialized applications

(i) Waterproofing

For waterproofing operations, polymer-modified bitumen membranes and bitumen paints that often contain a specialized cutback-bitumen product integrated with relatively small amounts of other materials are used. Emulsified bitumens that can be applied at lower or ambient temperatures have largely replaced cutback bitumens for this application (NAPA & EAPA, 2011).

(ii) Electrical and sound insulation

The electrical properties of bitumens (primarily class 2) enable them to be used in wrappings and jointing compounds for heavy-duty cables. Bitumens (classes 2 and 5) find wide use for sound insulation, e.g. in car bodies and floor mats, and in floor mountings for factory machinery (NAPA & EAPA, 2011).

(iii) Pipe coatings

To protect pipelines for oil, water, etc. coatings of bitumen enamel are applied to the cleaned, primed, metal surface. The enamel is made of oxidized bitumens (class 2, e.g. 115/15) with the addition of up to 30% of an inert filler, such as slate dust. The primer is a cutback (class 3) of the oxidized bitumens with a volatile solvent (white spirit/mineral or white spirit) (NAPA & EAPA, 2011).

(iv) Briquettes

Briquetting was previously a process by which fine materials (e.g. coal dusts, metal tailings) were mixed with a bitumen binder to form conveniently handled blocks or pellets for use as fuel in the metal industry and in power plants. The most suitable bitumens for this purpose were hard and of a low PI, e.g. 85/2 or 90/1 (softening-point/penetration at 25 °C). Other grades, such as 15-PEN or H 80/90, may also have been used (NAPA & EAPA, 2011).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

(a) Natural occurrence

Natural bitumens are widespread in regions where oil-bearing rocks occur on or not far below the Earth's surface, and seep spontaneously to the surface. Bitumen products occur naturally as rock asphalt deposits such as uintahite (from Utah, USA) and as lake asphalt (e.g. in Trinidad). Deposits of natural bitumen occur around the world, including Pitch Lake, Trinidad; the Dead Sea; Venezuela; and Switzerland (IPCS, 2004) (see Section 1.1.5).

(b) Air

Releases from bitumens into the air occur in the vicinity of hot-mix asphalt plants and road-laying operations, and near factories. Bitumen-producing refineries are also a source of releases into the air. In the production of roofing felts, emissions of particulates including bitumen fume were found to be 1.35 mg/g bitumens for controlled and 3.15 mg/g bitumens for uncontrolled conditions. In a bitumen-blowing operation, releases of particulates into the air ranged from 0.29 to 3.65 mg/g bitumens for well controlled and uncontrolled operations, respectively (EPA, 1978).

Kebin *et al.* (1996) reported on the percentage of polar, aromatic and saturated fractions measured in air samples collected 2–84 m from a highway in Denmark. [The Working Group noted that diesel and gasoline exhaust from traffic, and residues from tyre abrasion, were likely to have contributed to the composition of these fractions.]

(c) Soil and sediment

PAHs and trace elements were found in creek-bed sediment near a seal-coated parking lot in Austin, Texas.

In an experimental setting, parking lots and test plots sealed with coal tar, with "asphalt", or left unsealed, and unsealed concrete [asphalt] pavements were sprayed with distilled deionized water to simulate rainfall and the wash-off was collected for analyses. The highest PAH levels were reported for effluent from pavement sealed with coal-tar emulsion, followed by bitumen-sealed and unsealed pavements (US Department of the Interior, 2004).

(d) Water

Bitumen emissions can end up in water through surface runoff from land, and fallout and rainout from the atmosphere. Concentrations of PAHs and selected heavy metals were determined in water samples collected from water draining from road surfaces and from water upstream and downstream from the point of discharge from road surfaces into stream sites in California, USA. The concentrations of PAHs in all stream and road runoff samples were below the detection limit of 0.5 µg/L (Cooper & Kratz, 1997).

Leaching tests of bitumen-based materials have been conducted in laboratories. Six samples of paving bitumen and four samples of roofing bitumen were leached according to the United States Environmental Protection Agency (EPA) method SW846–1311 (Kriech *et al.*, 2002a). None of the roofing samples tested leached any of the 29 PAHs analysed. Four of the paving samples did not leach any of the 29 PAHs, and the leachates of two paving samples contained detectable amounts of naphthalene and phenanthrene. The levels were below the detection limit of 0.1 µg/L, except for naphthalene with a value of 0.18 µg/L.

Leaching tests on samples of reclaimed asphalt pavement from Florida, USA, detected none of 16 EPA-priority pollutant PAHs in the leachates of these samples (Brantley & Townsend, 1999). The concentrations of PAHs with more than two rings in leachate water from ten samples of bitumen and asphalt were 4–50 ng/L (Brandt & de Groot, 2001).

(e) Food vegetation

Kebin *et al.* (1996) reported on the percentage of polar, aromatic and saturated fractions measured in plant samples collected at 5–10 m from a main road in Denmark. [The Working Group noted that diesel and gasoline exhaust from traffic, and residues from tyre abrasion, were likely to have contributed to the composition of these fractions.]

1.4.2 Occupational exposures

(a) Number of workers exposed

No reliable estimates were available to the Working Group concerning the number of workers exposed to bitumen. It is most likely that the largest number of workers is exposed in road-paving and roofing operations. Furthermore, occupational exposures occur in the production of bitumen, production of roofing material and in asphalt-mixing plants. Conservative estimates stemming from the early 2000s for western Europe mention 4000 asphalt-mixing plants and 100 000 paving crewmen (Boffetta & Burstyn, 2003). In 2007 the estimates of number of workers employed in the hot-mix asphalt industry in the USA, as presented by the National Asphalt Pavement Association, totalled 300 000 individuals. The number of mixing plants was estimated at 4000 (Acott, 2007). In Delhi, India, alone some 25 hot-mix plants are currently in operation (Chauhan *et al.*, 2010). Paving is a worldwide activity that is reflected in the presence of 400 mixing plants in Mexico, 60 in South Africa and 45 in New Zealand. In China, more than 6500 small plants exist that produce about one third of the volume produced in Europe (NAPA & EAPA, 2011).

In Europe, the statistical office Eurostat provides figures for the number of workers employed in construction of roads and railways (NACE 42.1), construction of utility projects (42.3) and roofing activities (NACE 43.91) (Eurostat, 2010). For the first quarter of 2011, these numbers totalled more than 10 million workers. Of these workers, approximately 10% were women.

(b) Occupational exposure to bitumen and its emissions

Different sampling methods (e.g. different inhalable samplers used), analytical methods and measurement strategies (short-duration, shift-long, personal *versus* static sampling) have been used across countries and over time, which complicates interpretation of time trends and differences between regions and countries. In the following sections, recent exposures to bitumen emissions and its specific ingredients are described for workers involved in bitumen-production plants, in the production of bitumen-containing materials, and for workers applying bitumen and bitumen products in road paving, roofing, and more specialized applications such as mastic flooring and waterproofing. Data on historical exposure up to 1984 are reported in a previous *IARC Monograph* (IARC, 1985).

A review by NIOSH (2001a) presents a detailed picture of exposure to bitumens in the USA. Analysis of data from a large number of studies of exposure suggested that personal exposures to bitumen fume (measured as TPM) were highest during asphalt flooring and waterproofing activities, followed by manufacture of roofing products, bitumen refining, roofing application and at asphalt-mixing plants, with lowest exposures to bitumen fume encountered during road-paving activities. The BSF of the collected fume showed a similar pattern.

In Europe, Rühl *et al.* (2006) reported on 1272 samples (mainly 2-hour task-based measurements) collected in Germany between 1991 and 2005. Higher inhalation exposures to bitumen emissions were observed for workers engaged in (indoor) laying of mastic asphalt. Roofing work was next, followed by hot-mix paving and work in the mixing plant. Production of bitumen and industrial production of bitumen-containing products (e.g. sheets and shingles) showed median concentrations of bitumen fume that were mainly < 1 mg/m^3.

Results from personal measurements of inhalation and dermal exposure of workers exposed to bitumen and its emissions during production and use of bitumen are presented in Tables 1.15 and Table 1.16, respectively, by industry and application. Table 1.17 summarizes the results of biomonitoring of urinary 1-OHP.

(i) Production and transport of bitumens

Boogaard (2007) reported the exposure of process operators at a refinery producing bitumen monitored in three separate surveys. The arithmetic mean urinary concentrations of 1-OHP of the operators were relatively low and varied between 0.12 and 0.17 µmol/mol creatinine. A recent study from France focusing on workers transporting bitumen showed (model based on task-based measurements of exposure during loadings) average concentrations in fumes as follows: total PAHs, 3.51 ng/m^3; benzo[*a*]pyrene, 2.3 ng/m^3; and pyrene, 5.7 ng/m^3 (Deygout *et al.*, 2011).

(ii) Production of bitumen-containing materials

In the USA, a study among workers in 19 plants manufacturing bitumen-roofing products involved the use of bitumens of class 1 and class 2 (Calzavara *et al.*, 2003). The reported average concentration of TPM was relatively high at 2.47 mg/m^3, but the average BSF concentration was relatively low (0.11 mg/m^3).

(iii) Road paving

For a large European multicentre epidemiological study, Burstyn *et al.* (2000a, b) built a large exposure-measurement database

Table 1.15 Inhalation exposure of workers exposed to bitumens and bitumen fume by inhalation, by type of production and application

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Bitumen production										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	64			0.5	10	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	6			0.2	2.9	mg/m³
Hicks (1995)		USA	TPM	7–9 h	44		0.18 (3.7)			mg/m³
			RPM	7–9 h	8		0.26 (3.6)			mg/m³
			BSF	7–9 h	44		0.16 (3.7)		13	mg/m³
			PYR	7–9 h	9		All < LOD			µg/m³
			BaP	7–9 h	44		0.15 (1.3)			µg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	4	0.4		< 0.1	1.0	mg/m³
			11-PAHs	109–437 min	4	33		3.8	95	ng/m³
Deygout *et al.* (2011)		France	16-PAHs	466 min	6	0.31	0.23			µg/m³
			NAP	466 min	6	2 755	1 894			ng/m³
			PYR	466 min	6	5.7	5.4			ng/m³
Posniak (2005)		Poland	PYR	Full shift	3	0.003		ND	0.01	µg/m³
			BaP	Full shift	3	0.007		ND	0.013	µg/m³
Deygout *et al.* (2011)		France	BaP	466 min	6	2.3	2.2			ng/m³
Roofing material manufacturing										
Hicks (1995)		USA	TPM	405 min	34		1.4 (3)		13	mg/m³
			BSF	405 min	34		0.27 (4.4)		3.7	mg/m³
Gamble *et al.* (1999)		USA	TPM	405 min	77		0.6		6.2	mg/m³
			BSF	405 min	77		0.08		1.3	mg/m³
Calzavara *et al.* (2003)		USA	TPM	405 min	58		2.47 (2.51)	< 0.03	13.3	mg/m³
			BSF	405 min	58		0.11 (0.08)	< 0.01	0.42	mg/m³
Asphalt mixing and transport										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	80			0.25	45	g/m³

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Hicks (1995)		USA	TPM	7–9 h	33		0.78 (2.8)			mg/m³
			RPM	7–9 h	6		0.24 (3.1)			mg/m³
			BSF	7–9 h	33		0.15 (2.8)			mg/m³
			PYR	7–9 h	8		All < LOD			µg/m³
			BaP	7–9 h	33		All < LOD			µg/m³
Conventional paving										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	298			0.12	15.5	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	12			0.2	15.1	mg/m³
Burstyn *et al.* (2000a)	1960–90	Europe	TPM	6–8 h	1 193	1.91	0.28	< LOD	260	mg/m³
			Vapoursz	6–8 h	510	7.59	1.86	< LOD	290	mg/m³
			BaP	6–8 h	487	95.8	8.58	< LOD	8 000	ng/m³
Heikkilä *et al.* (2002)	1992–96	Finland	Bitumen fume	6–8 h	70			0.01	3.9	mg/m³
			TPM	6–8 h	70			0.2	4.2	mg/m³
Deygout & Le Coutaller (2010)	2000–06	France	TPM	177–506 min	46	[0.43]	0.26 (2.73)	0.01	2.61	mg/m³
Hugener *et al.* (2009)		Switzerland	TPM					ND	3.8	µg/m³
Hicks (1995)		USA	TPM	7–9 h	37		0.37 (1.7)			mg/m³
Watts *et al.* (1998)	1994	USA	TPM	16 h				111	345	ng/m³
Burr *et al.* (2002)	1994–97	USA	TPM	8 h	78			0.01	0.89	mg/m³
Kriech *et al.* (2002b)		USA	TPM		44		0.23	0.09	0.64	mg/m³
Mickelsen *et al.* (2006)		USA	TPM	8 h	132	0.35		0.26	0.75	mg/m³
Hicks (1995)		USA	RPM	7–9 h	7		0.18 (1.5)			mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	11			0.1	0.5	mg/m³
Deygout & Le Coutaller (2010)	2000–06	France	BSF	177–506	45	[0.21]	0.13 (2.66)	< LoQ	3.35	mg/m³
Jongeneelen *et al.* (1988a)	1986	The Netherlands	CSM		27		0.2–0.6			mg/m³

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Hicks (1995)		USA	BSF	7–9 h	37		0.24 (3)		3.7	mg/m^3
Burr *et al.* (2002)	1994–97	USA	BSF	8 h	32			0.01	0.82	mg/m^3
Kriech *et al.* (2002b)		USA	BSF		44		0.06	0.06	0.31	mg/m^3
Mickelsen *et al.* (2006)		USA	BSM	8 h	132	0.13		0.04	0.56	mg/m^3
Deygout & Le Coutaller (2010)	2000–06	France	Vapour fraction	177–506 min	37	[1.52]	0.75 (3.29)	0.05	11.13	mg/m^3
Kriech *et al.* (2002b)		USA	TOM		45		1.23	0.33	8.32	mg/m^3
Heikkilä *et al.* (2002)	1992–96	Finland	Total PAHs	6–8 h	65			0.15	52.6	µg/m^3
Väänänen *et al.* (2003)	1999–2000	Finland	Total PAHs	8 h	35		5.7	0.87	46	µg/m^3
Heikkilä *et al.* (2002)	1992–96	Finland	4–6 ring PAHs	6–8 h	65			< 0.05	0.93	µg/m^3
Campo *et al.* (2006)	2003	Italy	15 PAHs	4 h	147			127	2 973	ng/m^3
Hugener *et al.* (2009)		Switzerland	16 PAHs					36	510	µg/m^3
Burr *et al.* (2002)	1994–97	USA	2–3 ring PAHs	8 h	48			0.01	191	µg/m^3
			4–7 ring PAHs	8 h	48			0.01	25	µg/m^3
McClean *et al.* (2004a)	1999–2000	USA	Total PAHs	Full shift	109			0.3	40	µg/m^3
Campo *et al.* (2006)	2003	Italy	NAP	4 h	147			2	2 319	ng/m^3
Heikkilä *et al.* (2002)	1992–96	Finland	PYR	6–8 h	66	< 0.01–0.12				µg/m^3
Väänänen *et al.* (2003)	1999–2000	Finland	PYR	8 h	35			0.01	1.2	µg/m^3
Campo *et al.* (2006)	2003	Italy	PYR	4 h	147			< 0.6	282.2	ng/m^3
Posniak (2005)		Poland	PYR	Full shift	13	0.043		nd	0.24	µg/m^3

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Hicks (1995)		USA	PYR	7–9 h	9		0.17 (1.3)			µg/m³
Watts *et al.* (1998)	1994	USA	PYR	16 h				1.6	4.2	ng/m³
McClean *et al.* (2004a)	1999–2000	USA	PYR	Full shift	109			0.01	1.7	µg/m³
Heikkilä *et al.* (2002)	1992–96	Finland	BaP	6–8 h	66	< 0.01			< 0.01	µg/m³
Väänänen *et al.* (2003)	1999–2000	Finland	BaP	8 h	14	0.01–0.07	0.03	< 0.01	0.32	µg/m³
Campo *et al.* (2006)	2003	Italy	BaP	4 h	147			< 0.003	40.25	ng/m³
Posniak (2005)		Poland	BaP	Full shift	13	0.006		ND	0.034	µg/m³
Hugener *et al.* (2009)		Switzerland	BaP					0.003	0.2	µg/m³
Hicks (1995)		USA	BaP	7–9 h	37	all < LOD	NA			µg/m³
Watts *et al.* (1998)	1994	USA	BaP	16 h				0.9	4.4	ng/m³
McClean *et al.* (2004a)	1999–2000	USA	BaP	Full shift	109			0.01	0.03	µg/m³
Heikkilä *et al.* (2002)	1992–96	Finland	VOC	6–8 h	70			0.2	65.5	mg/m³
Modified asphalt paving										
Väänänen *et al.* (2006)	2003	Finland	Bitumen fume	4 h	20	0.13	0.11	0.05	0.29	mg/m³
			TPM	4 h	20	0.4	0.4	< LOQ	1.1	mg/m³
Watts *et al.* (1998)	1994	USA	TPM	16 h				164	389	ng/m³
Burr *et al.* (2002)	1994–97	USA	TPM	8 h	87			0.01	0.91	mg/m³
	1994–97	USA	BSF	8 h	37			0	0.75	mg/m³
Väänänen *et al.* (2006)	2003	Finland	Vapours	4 h	20	0.9	0.9	0.4	1.9	mg/m³
			Total PAHs	4 h	18	1.6	1.42	0.54	3.45	µg/m³
Burr *et al.* (2002)	1994–97	USA	2–3 ring PAHs	8 h	60			0.6	540	µg/m³
			4–7 ring PAHs	8 h	60			0.1	27	µg/m³
Väänänen *et al.* (2006)	2003	Finland	PYR	4 h	18		< 0.015	< 0.015	0.038	µg/m³
Watts *et al.* (1998)	1994	USA	PYR	16 h				4.5	57	ng/m³

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Väänänen *et al.* (2006)	2003	Finland	BaP	4 h	18				< 0.01	µg/m³
Watts *et al.* (1998)	1994	USA	BaP	16 h				0.9	3.5	ng/m³
Roofing										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	182			0.25	18.2	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	9			0.5	6.4	mg/m³
Hicks (1995)		USA	TPM	7–9 h	38		0.55 (2.5)			mg/m³
Kriech *et al.* (2004)		USA	TPM	5.1 h	35		0.94		10	mg/m³
Hicks (1995)		USA	RPM	7–9 h	6		0.15 (2.2)			mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	9			0.2	5.4	mg/m³
Hicks (1995)		USA	BSF	7–9 h	38		0.25 (3.4)			mg/m³
Kriech *et al.* (2004)		USA	BSF	5.1 h	35		0.33		9.6	mg/m³
Wolff *et al.* (1989)	1987	USA	Total PAHs	Full shift	13	5.8–23.0				µg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	11 PAHs	109–437 min	3			24	364	ng/m³
Wolff *et al.* (1989)	1987	USA	PYR	Full shift	18	2.6–5.4				µg/m³
Hicks (1995)		USA	PYR	7–9 h	18		0.21–0.34			µg/m³
			BaP	7–9 h	72		0.16			µg/m³
Wolff *et al.* (1989)	1987	USA	BaP	Full shift	18	0.9–1.5				µg/m³
Mastic										
Spickenheuer *et al.* (2011)	2001–08	Germany	Aerosols and vapours, tunnel	315 min		6	7.84			mg/m³
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	608			0.13	77	mg/m³
Breuer *et al.* (2011)	2001–08	Germany	Aerosols and vapours	315 min	320		3.46	LOQ	41.68	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	12			2.9	18.2	mg/m³

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Breuer *et al.* (2011)	2001–08	Germany	TPM	315 min	320			LOQ	33.02	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	12			1.8	13.6	mg/m³
			11 PAHs	109–437 min	7			285	2 971	ng/m³
Heikkilä *et al.* (2002)	1992–96	Finland	BaP	6–8 h	2	0.01				µg/m³
			PYR	6–8 h	2	0.19				µg/m³
Joint filling										
Rühl *et al.* (2006)	1991–05	Germany	Aerosols and vapours	2 h			20	0.32	5.5	mg/m³

AM, arithmetic mean; BaP, benzo[*a*]pyrene; BSF, benzene-soluble fraction; CSM, cyclohexane-soluble matter; h, hour; LOD, limit of detection; LOQ, limit of quantification; Max., maximum; Min., minimum; min, minute; NA, not applicable; NAP, naphthalene; ND, not detected; PAHs, polycyclic aromatic hydrocarbons; PYR, pyrene; RPM, respirable particulate matter; TOM, total organic matter; TPM, total particulate matter; VOC, volatile organic compounds

Table 1.16 Dermal exposure of workers exposed to bitumens and bitumen fume, by type of production and application

Reference	Time period	Country	Agent	*n*	AM	GM/ Median	Min.	Max.	Unit
Mixing and conventional paving									
Väänänen *et al.* (2005)	1999–2000	Finland	Total native PAHs	22		4.6–10	1.8	78	ng/cm^2
			Total PAHs	30		7.8	0.71	63	ng/cm^2
Väänänen *et al.* (2006)	2003	Finland	Total PAHs	18	1.6	1.4	0.71	3.5	ng/cm^2
McClean *et al.* (2004a)	1999–2000	USA	Total PAHs, paving	59		89	< 38	751	ng/cm^2
			Total PAHs, milling	39		< LOD	< 38	757	ng/cm^2
			Total PAHs, road construction	11		45	< 38	246	ng/cm^2
			PYR, paving	59		3.5	< 2.6	25	ng/cm^2
			PYR, milling	39		< LOD	< 2.6	7.1	ng/cm^2
			PYR, road construction	11		< LOD		2.6	ng/cm^2
Väänänen *et al.* (2005)	1999–2000	Finland	PYR	22		0.45–2.9	0.07	24	ng/cm^2
			PYR	30		0.88	< 0.05	24	ng/cm^2
Väänänen *et al.* (2006)	2003	Finland	PYR	18	0.19	0.12	< 0.09	0.48	ng/cm^2
Jongeneelen *et al.* (1988a)	1986	the Netherlands	PYR, wrist pad	39		< 10–24			ng
			PYR, on hands	35		37.4–216			µg
McClean *et al.* (2004a)	1999–2000	USA	BaP	59		< LOD	< 0.6	2.5	ng/cm^2
			BaP	39		< LOD	< 0.6	1.2	ng/cm^2
			BaP	11		< LOD	< 0.6	1.2	ng/cm^2
Väänänen *et al.* (2005)	1999–2000	Finland	BaP	22		< LOD–0.04	< 0.02	0.11	ng/cm^2
Roofing									
Wolff *et al.* (1989)	1987	USA	Total PAHs	7	600 (544)				ng

Table 1.16 (continued)

Reference	Time period	Country	Agent	*n*	AM	GM/ Median	Min.	Max.	Unit
McClean *et al.* (2007a)	1998	USA	Total PAHs, roof work	71		898 (4.5)	48	34 014	ng/cm^2
			Total PAHs, tear-off	41		886 (4.6)	49	33 538	ng/cm^2
			Total PAHs, put-down	56		344 (3.6)	48	21 437	ng/cm^2
			Total PAHs, kettleman	19		299 (3.7)	40	4 558	ng/cm^2
			PYR, roof work	71		11 (5.5)	< 2.4	221	ng/cm^2
			PYR, tear-off	41		11.5 (4.8)	< 2.4	168	ng/cm^2
			PYR, put down	55		3.8 (5.4)	< 2.4	150	ng/cm^2
			PYR, kettleman	19		4.5 (5.5)	< 2.4	34	ng/cm^2
Wolff *et al.* (1989)	1987	USA	PYR	10	171 (197)				ng
McClean *et al.* (2007a)	1998	USA	BaP, roof worker	71		3.3 (12)	< 0.5	59	ng/cm^2
			BaP, tear-off	41		4.6 (5.8)	< 0.5	84	ng/cm^2
			BaP, put-down	54		1 (11)	< 0.5	59	ng/cm^2
			BaP, kettlemen	18		0.9 (18)	< 0.5	20	ng/cm^2
Wolff *et al.* (1989)	1987	USA	BaP	10	83 (88)				ng

AM, arithmetic mean; BaP, benzo[*a*]pyrene; LOD, limit of detection; Max., maximum; Min., minimum; PAH, polycyclic aromatic hydrocarbon; PYR, pyrene

Table 1.17 Urinary concentrations of 1-hydroxypyrene (1-OHP) in workers exposed to bitumens and bitumen fume, by type of production and application

Reference	Time period	Country	Time of sampling	*n*	AM	GM/ Median	Min.	Max.	Unit
Bitumen production									
Boogaard (2007)		Europe	Post-shift	> 121	0.12–0.17		< LOD	2.18	µmol/mol creatinine
Preuss *et al.* (2003)		Germany	Post-shift	70 NS		0.08	0.02	0.35	µg/g creatinine
Mixing and conventional paving									
Väänänen *et al.* (2006)	2003	Finland	Pre-shift	8 NS	0.17	0.15	0.06	0.26	µmol/mol creatinine
			Post-shift	8 NS	0.27 (0.15)	0.22	0.06	0.55	µmol/mol creatinine
Szaniszló & Ungváry (2001)		Hungary	Post-shift	< 10 NS	0.2 (0.23)				µmol/mol creatinine
Cavallo *et al.* (2003)		Italy	Pre-shift	18	609	381	222	2 460	ng/g creatinine
			Post-shift	18	680	516	59	1 774	ng/g creatinine
Campo *et al.* (2006)	2003	Italy	Pre-shift	56 NS		252	< 50	2 114	ng/L
			Post-shift	56 NS		506	< 50	3 799	ng/L
Cavallo *et al.* (2006)		Italy	Pre-shift	19 (9 S, 10 NS)	0.27				µg/g creatinine
			Post-shift	19 (9 S, 10 NS)	0.81				µg/g creatinine
Loreto *et al.* (2007)		Italy	Post-shift	16	0.89 (0.43)				ng/mL urine
Jongeneelen *et al.* (1988b)		The Netherlands	Pre-shift	3	0.5				µmol/mol creatinine
			Post-shift	3	0.6				µmol/mol creatinine
Järvholm *et al.* (1999)	1990	Sweden	Pre-shift	28 NS		0.96	0.04	3.8	µmol/L
			Post-shift	28 NS		0.96	0.23	4	µmol/L
Burgaz *et al.* (1992)		Turkey	Post-shift	39 (18 NS)	0.61 (0.38)				µmol/mol creatinine
Burgaz *et al.* (1998)	1997	Turkey	Post-shift	28 (12 NS)	0.78 (0.46)		0.32	2.20	µmol/mol creatinine
Karakaya *et al.* (1999)		Turkey	Post-shift	16	0.92 (0.14)				µmol/mol creatinine
Karakaya *et al.* (2004)		Turkey	Post-shift	6 S 4 NS	0.65 0.38				µmol/mol creatinine

Table 1.17 (continued)

Reference	Time period	Country	Time of sampling	*n*	AM	GM/ Median	Min.	Max.	Unit
Karaman & Pirim (2009)	2008	Turkey	Pre-shift	26	0.18 (0.07)				µmol/mol creatinine
			Post-shift	26	0.39 (0.21)				µmol/mol creatinine
Heikkilä *et al.* (2002)	1992–96	Finland	Pre-shift	15 NS	4		< 0.1	26.4	nmol/L
			Post-shift	15 NS	3.6		< 0.1	33	nmol/L
Väänänen *et al.* (2003)	1999–2000	Finland	Pre-shift	25 NS		0.15	< 0.06	3.9	µmol/mol creatinine
			Post-shift	26 NS		0.24	< 0.06	2.2	µmol/mol creatinine
McClean *et al.* (2004b)		USA	Pre-shift Post-shift	64 51		0.4–1.4 1.6–2			µg/g creatinine
Modified asphalt paving									
Väänänen *et al.* (2006)	2003	Finland	Pre-shift	7 NS	0.45	0.39	0.1	0.64	µmol/mol creatinine
			Post-shift	7 NS	0.46	0.45	0.3	0.64	µmol/mol creatinine
Paving with bitumen and coal tar									
Jongeneelen *et al.* (1988b)		The Netherlands	Pre-shift	[58]	0.8–2.3				µmol/mol creatinine
			Post-shift	[55]	0.9–3.2				µmol/mol creatinine
Sellappa *et al.* (2011)	2010	India	Post-shift	20 S 16 NS	2.09 (1.23) 1.13 (0.95)				µmol/mol creatinine
Roofing									
McClean *et al.* (2007a)	1998	USA	Pre-shift	54 (62% S)		0.5–1			µmol/mol creatinine
			Post-shift	63 (62% S)		0.6–1.3			µmol/mol creatinine
Mastic									
Pesch *et al.* (2011)	2001–08	Germany	Pre-shift	120 NS		193	94	385	ng/L
			Post-shift	120 NS		419	216	678	ng/L
Milling									
McClean *et al.* (2004b)		USA	Post-shift	10	0.4–0.5	0.31–0.62			µg/g creatinine

AM, arithmetic mean; GM, geometric mean; Max., maximum; Min., minimum; NS, non-smokers; S, smokers

(Asphalt Worker Exposure database). This database contained almost 1200 measurements of bitumen fume (measured as TPM or inhalable dust) and 500 measurements of organic vapour. PAH concentrations were also available for almost 500 measurements. The database covered 30 years from the late 1960s to 1999.

The arithmetic mean exposure was 1.9 mg/m^3 (range, < limit of detection [LOD] – 260 mg/m^3), 7.6 mg/m^3 (range, < LOD – 290 mg/m^3) and 95.8 ng/m^3 (< LOD – 8000 ng/m^3) for bitumen fume, bitumen vapour and benzo[*a*]pyrene respectively. Concentrations in the solvent-extracted fraction were strongly correlated with inhalable dust levels; 93% of inhalable dust emitted in road paving consisted of organic particulate matter. The correlation between vapour and fume concentrations appeared to be low.

Time trends from multivariate empirical (statistical) models in which differences in strategy, sampling and analytical methods, and use of coal tar (benzo[*a*]pyrene model only) were accounted for, showed that exposures decreased by a factor of two to three each decade (Burstyn *et al.*, 2003a). For road pavers from eight European countries, the model predicted a decrease in exposure to bitumen fume from 1.2–2.0 mg/m^3 in 1960 to 0.2–0.5 mg/m^3 in the mid-1990s (see Fig. 1.6) A temporary increase was estimated when recycling of old asphalt (which often contains coal-tar layers) started in the mid-1960s. Since the mid-1970s, however, exposures have again come down steadily, due to banning of recycling of asphalt layers with coal tar. For organic vapour and benzo[*a*]pyrene, the decreasing trends were slightly steeper (see Fig. 1.7 and Fig. 1.8).

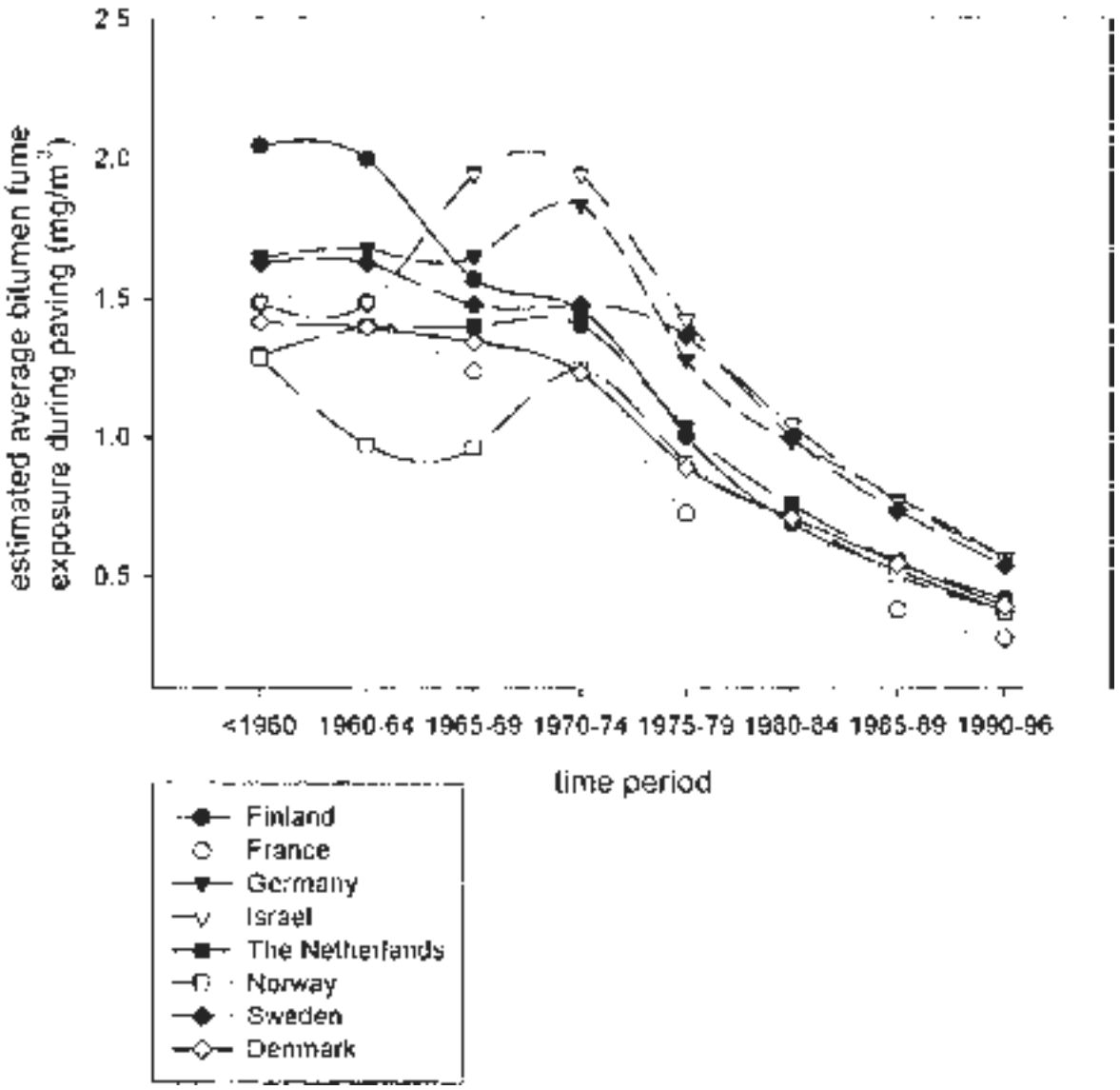
Studies carried out in 1999–2000 among road pavers working in resurfacing (n = 20), as well as millers (n = 12) and road-construction workers (n = 6) not exposed to hot-mix asphalt (McClean *et al.*, 2004a) showed that inhalation exposures varied considerably by task, crew, use of recycled asphalt product and work rate. Geometric mean breathing-zone concentrations of total PAHs reached 4.1 µg/m^3 among pavers, 1.4 µg/m^3 for millers, and was hardly detectable (among 36% of workers) for road-construction workers. Benzo[*a*]pyrene concentrations did not exceed 0.03 µg/m^3. Geometric mean dermal exposure levels to total PAHs measured with patches on the wrists reached 89 ng/cm^2 among pavers, 45 ng/cm^2 among road-construction workers and was only detected in 26% of the samples from millers. Applying lag models to urinary 1-OHP concentrations measured in the same workers, the impact of exposure to total PAHs on uptake of pyrene was estimated to be eight times greater with dermal exposure than with inhalation (McClean *et al.*, 2004b). Re-analyses of these data addressing other urinary biomarkers of exposure, such as naphthalene and phenanthrene, showed that toxicokinetic processes probably have less influence on variance in urinary biomarkers than dermal exposure and effects of covariates such as smoking (Sobus *et al.*, 2009b).

Kriech *et al.* (2002b) carried out a study among 45 pavers at 11 hot-mix paving sites across the USA. Geometric mean exposures were 0.23 mg/m^3 for bitumen fume, 0.06 mg/m^3 for BSF, and 1.23 mg/m^3 for total organic matter (TOM). Mixture temperatures varied between 120 and 165 °C.

Another recent study on 12 paving workers during four working weeks (144 worker-days) assessed inhalation and dermal exposure to PAHs under different scenarios. This intervention study found that lower application temperatures and dermal protection reduced inhalation and dermal exposure to bitumen-derived PAHs (Cavallari *et al.*, 2012a, b).

A recent study by Kriech *et al.* (2011) looked at the effect of substitution of hot-mix by warm-mix asphalt. Of the six warm-mixes, five resulted in 30–60% reductions in concentrations of TOM in the pavers' breathing zones.

Fig. 1.6 Assessed time trend in average exposure to bitumen fume (pavers only)

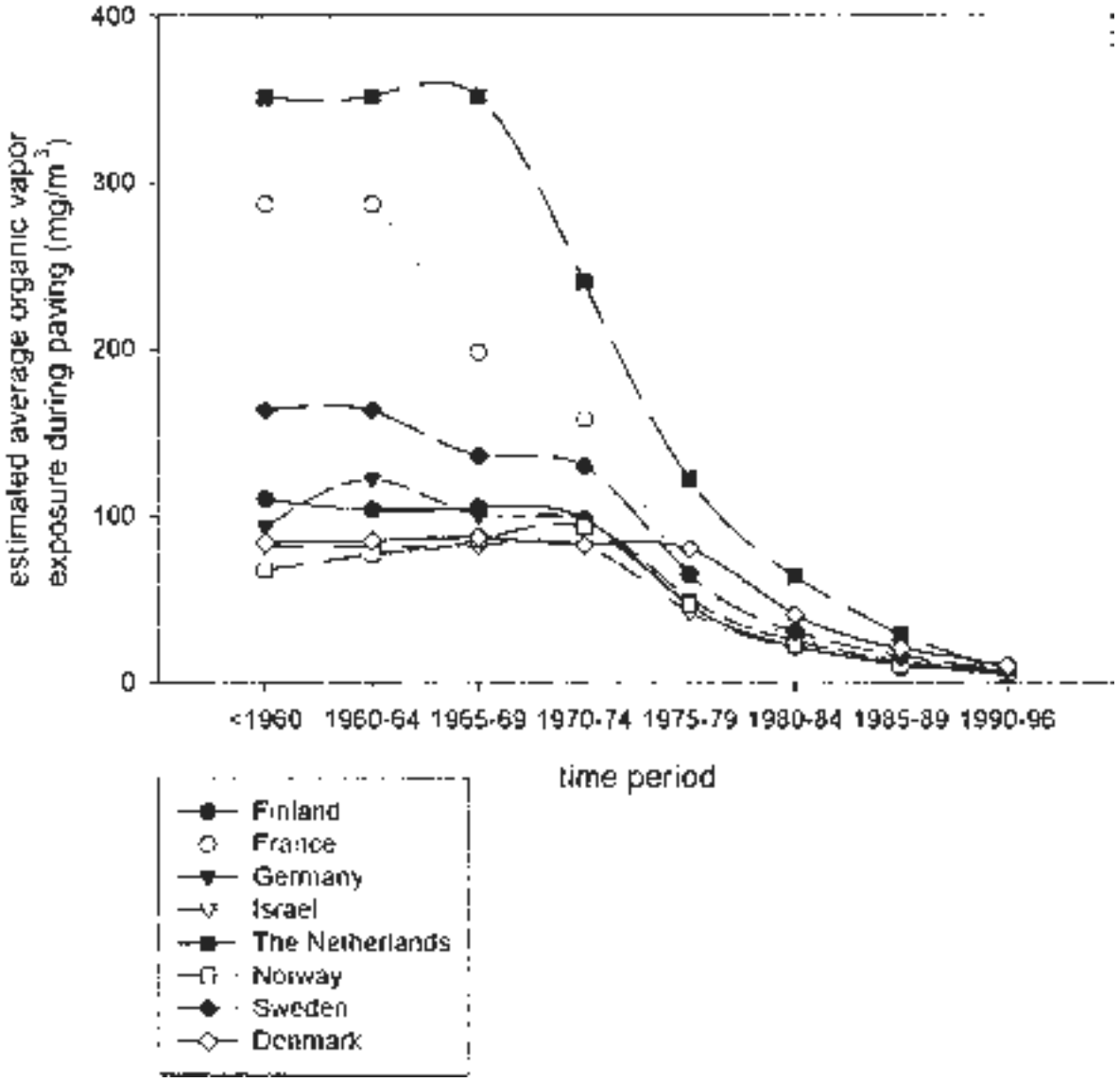


From Burstyn *et al.* (2003a)

Heikkilä *et al.* (2002) measured pre- and post-shift concentrations of urinary 1-OHP in 32 road pavers at 13 paving sites in Finland. The workers had been exposed to 11 different asphalt mixtures. Post-shift concentrations of 1-OHP were significantly higher ($P < 0.05$) among pavers (AM, 6.6 nmol/L; standard deviation [SD], 9.8) than among controls (AM, 1.6 nmol/L; SD, 2.6), and twice as high among pavers who were smokers (AM, 7.4 nmol/L; SD, 9.0) compared with non-smokers (AM, 3.6 nmol/L; SD, 8.3) ($P < 0.05$).

Also in Finland, Väänänen *et al.* (2006) investigated the exposure of road pavers to asphalt containing waste plastic and tall-oil pitch. Exposure was monitored over one working day at four paving sites among 16 road pavers who used mixtures of conventional asphalt or mixtures containing waste material. The concentrations of 11 aldehydes in air were 515 µg/m³ and

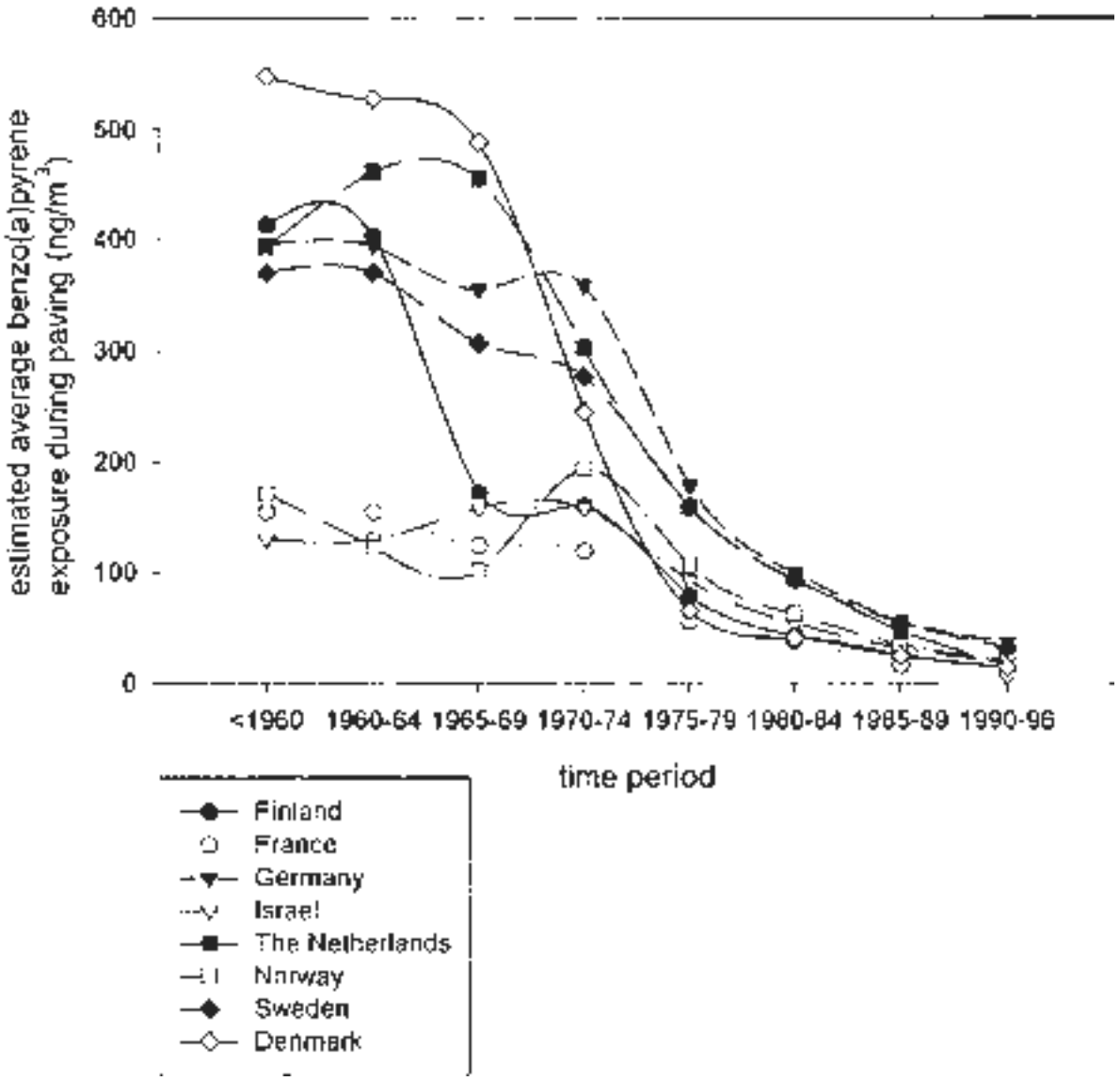
Fig. 1.7 Assessed time trend in average exposure to organic vapour (pavers only)



From Burstyn *et al.* (2003a)

902 µg/m^3 for asphalt and stone-mastic asphalt, respectively, at the worksites where tall-oil and waste plastic were used, being around 4 and 14 times greater than at the corresponding work-sites where conventional asphalt was used. Eight hydroxy-PAHs were measured, and the parent PAHs naphthalene, phenanthrene and pyrene were quantified in urine samples collected before and after the working shift. The post-shift concentrations (mean ± SD, µmol/mol creatinine) in workers using conventional asphalt of 1-naphthol/2-naphthol, combined 1-, 2-, 3-, 4- and 9-phenanthrol, and 1-OHP were 18.1 ± 8.0, 2.41 ± 0.71 and 0.66 ± 0.58 for smokers; and 6.0 ± 2.3, 1.70 ± 0.72 and 0.27 ± 0.15 for non-smokers, respectively. For asphalt workers using mixtures that contained waste material, the concentrations were 22.0 ± 9.2, 2.82 ± 1.11 and 0.76 ± 0.18 (smokers); and 6.8 ± 2.6, 2.35 ± 0.69 and 0.46 ± 0.13 (non-smokers). Similarly, PAH

Fig. 1.8 Assessed time trend in average exposure to benzo[*a*]pyrene (pavers only)



From Burstyn *et al.* (2003a)

concentrations were significantly higher in smokers than in non-smokers.

In Italy, Campo *et al.* (2006) compared the exposure from asphalt workers exposed to bitumen fume and diesel exhausts (n = 100) with road-construction workers (n = 47) exposed to diesel exhausts only. Total PAHs and 15 individual PAHs were monitored; median concentrations of individual PAHs ranged from 0.33 to 426 ng/m^3. 1-OHP concentrations increased during the day and over the working week. Work activities contributed in the same order of magnitude as cigarette smoking to the observed concentrations of 1-OHP. Campo *et al.* (2007) reported urinary unmetabolized PAHs. Median concentrations of urinary naphthalene, phenanthrene, fluoranthene and pyrene in end-shift samples were 117, 50, 8 and 6 ng/L among the asphalt workers and 104, 19, 5 and 4 ng/L among the road-construction workers. From the same

study, Buratti *et al.* (2007) reported on hydroxy-PAH concentrations quantified in urine samples collected at three different time-points during the week. The urinary concentrations of hydroxy-PAH increased with time: median concentrations of 2-hydroxyfluorene, 3-hydroxyphenanthrene and 1-hydroxypyrene in non-smokers were 0.29, 0.08 and 0.18 ng/L at baseline; 0.50, 0.18 and 0.29 ng/L pre-shift; and 1.11, 0.44 and 0.44 ng/L post-shift, respectively. Each hydroxy-PAH showed a characteristic profile, reflecting differences in half-lives. In non-smokers, positive correlations were found between vapour-phase PAHs and hydroxy-PAHs, both in pre- and post-shift samples. Concentrations of hydroxy-PAHs in smokers were two to five times higher than those in non-smokers. A recent study of dermal exposure among 24 road pavers in the same country showed dermal exposure in the range of a few nanograms per cm^2 (Fustinoni *et al.*, 2010).

In a recent study among road pavers in France, exposure data were collected for 2000–06. Geometric mean concentrations of 0.26, 0.13 and 0.75 mg/m^3 [arithmetic means, 0.43, 0.21 and 1.52 mg/m^3] were reported for TPM, BSF and bitumen vapours, respectively (Deygout & Le Coutaller, 2010).

Sellappa *et al.* (2011) reported on a study among pavers laying "tar bitumens", which, according to the authors, are increasingly being used in India and are applied hot. This binder consists of 70% bitumen and 25–30% tar. Mean urinary concentrations of 1-OHP among the road pavers was high (1.68 ± 0.93 µmol/mol creatinine) and significantly higher than among controls (0.55 ± 0.42 µmol/mol creatinine).

(iv) Roofing

In a study in the USA, 26 bitumen roofers employed in removing old roofs, putting down new roofs and operating the bitumen kettle were monitored to evaluate the effect of dermal exposure to PAHs on urinary concentrations of 1-OHP (McClean *et al.*, 2007a). Dermal concentrations of PAHs were about four times higher for workers tearing off old roofs than for those attending the kettle (812 ng/cm^2 versus 181 ng/cm^2). These concentrations were 2–20 times higher than those reported for road pavers. Exposure to coal tar was associated with an 35-times increase in dermal exposure to benzo[*a*]pyrene. As with the study in pavers (McClean *et al.*, 2004b), a distributed lag model showed that dermal exposure had a significant effect on urinary concentrations of 1-OHP. The presence of coal-tar pitch appeared to be the primary determinant of dermal exposure to PAHs and particularly for benzo[*a*]pyrene; when controlling for exposure to coal-tar pitch, dermal exposure to bitumen also had an effect.

Full-shift breathing-zone measurements for TPM, BSF and PAHs were significantly higher for roofers exposed to coal tar than for roofers not exposed to coal tar (Toraason *et al.*, 2001, 2002). Similarly, urinary concentrations of 1-OHP were higher in roofers exposed to coal tar than in roofers not exposed to coal tar. TPM or BSFs were not associated with urinary 1-OHP, but PAH levels were highly correlated with urinary 1-OHP.

A study by Kriech *et al.* (2004) of 26 roofing workers using built-up roofing asphalt (BURA) type III (class 2) at six sites in the USA found exposures for TPM that ranged from 0.29 to 10.3 mg/m^3. The BSF ranged from 0.02 to 9.63 mg/m^3, and TOM from 0.49 to 11.8 mg/m^3. At the six sites, the higher values were observed for the men operating the bitumen kettle.

(v) Specialized applications as mastic flooring and waterproofing

Using the Asphalt Worker Exposure database described above (Burstyn & Kromhout, 2000), the concentrations of bitumen fume and benzo[*a*]pyrene during mastic-laying operations were estimated to be higher, on average, than those in road paving by a factor of six and of eight, respectively. A study on dermal exposure with an observational assessment method indicated

again higher exposures for mastic workers versus road pavers, by a factor of two to five (Agostini *et al.*, 2011).

The German Human Bitumen study monitored a non-random sample of 320 bitumen-exposed workers and 69 non-exposed construction workers from 2001 to 2008 at 57 construction sites in Germany. With the main focus being whether respiratory effects would be noticeable among workers exposed at concentrations above the former German exposure limit of 10 mg/m^3, the selection of workers and type of application studies was biased towards worst-case exposure situations (e.g. > 90% were indoor mastic applications) (Breuer *et al.*, 2011; Raulf-Heimsoth *et al.*, 2011a; Spickenheuer *et al.*, 2011). Shift-long median exposures to bitumen fume (measured as inhalable dust) were relatively high at 3.1 mg/m^3 (based on bitumen condensate reference). Median concentrations of bitumen aerosols and vapours combined reached 5.1 mg/m^3. The concentrations of urinary 1-OHP and of the sum of 1-, 2+9-, 3- and 4-hydroxyphenanthrene (OHPhe) in 317 exposed non-smoking workers increased during a shift, from 193 to 419 ng/L and from 618 to 1414 ng/L, respectively (Pesch *et al.*, 2011).

(c) Coexposures

In addition to bitumen emissions, road-paving workers and roofers may also be exposed to other chemical agents. Coal tars have been used on their own or mixed with bitumens in road paving in many countries worldwide, and may still be in use in some places.

Chemical components of coal tar, namely benzo[*a*]pyrene, may occur in emissions when old asphalt containing coal tar is recycled. Also, removal of old roofing materials containing coal tar can result in considerable dermal and inhalation exposure to coal tar (McClean *et al.*, 2007a).

Road-paving workers are exposed to diesel exhaust because paving machines are usually powered by diesel fuel. They may also be exposed to diesel and gasoline engine exhaust from background traffic. Diesel fuel, gas oil, kerosene and organic solvents have also been used to clean equipment. Organic solvents and aliphatic amines are also used as components of some application mixes (Burstyn *et al.*, 2000c). Substitution of biodiesel for diesel oil as a cleaning agent was shown to reduce inhalation and dermal exposure to PAHs (Cavallari *et al.*, 2012a, b).

Mineral dusts including crystalline silica from gravel or sand may occur in air in mixing plants, paving sites and during milling of existing asphalt roads. Mineral dusts may also be generated due to (mechanical) sweeping of roads and roadsides. There is also evidence that asbestos and lime have been added infrequently to paving mixtures (Burstyn *et al.*, 2000c).

The modification of bitumen with recycled or waste materials such as crumb rubber, sulfur, ground roofing shingles, foundry sand, fly ash, contaminated soil, plastics, and waste fibres, gives rise to some specific exposures. The thermal degradation products of polyethylene and polypropylene include aldehydes, ketones, hydrocarbons, formic acid and acetic acid. Styrene and alcohols may be emitted from polystyrene, and hydrogen chloride and phthalates from polyvinyl chloride (Väänänen *et al.*, 2006).

(d) Synopsis

The characterization of occupational exposure to bitumens and their emissions is very complex. Exposure to particulate matter, volatile organic compounds and various polycyclic aromatic compounds is common among bitumen workers; however, the concentration and composition of exposure is highly variable and depends on where and under which circumstances the bitumen and bitumen-containing products are being used. The highest exposures to fume and vapours have been described for mastic-asphalt workers and roofers applying hot bitumen, while mixing-plant workers and pavers are exposed at lower concentrations.

In field studies, it is difficult to determine the extent to which such differences in exposure are due to differences in bitumen or differences in application practices, although there is strong evidence that higher application temperatures are associated with higher exposures. For example, the typical temperature ranges used in mastic applications (200–250 °C) and in hot-bitumen roofing applications (180–230 °C) are higher than those in paving applications (110–170 °C). Studies have shown consistently that inhalation and dermal exposures among mastic workers are higher than among conventional paving workers.

Coexposure to coal-tar pitch can significantly confound measurements of exposure to bitumens. Among roofing workers, exposure to coal tar was associated with increases of thirty-five times in dermal exposure to benzo[*a*]pyrene and six times in dermal exposure to PAHs. Similarly, exposure to benzo[*a*]pyrene by inhalation among road pavers was estimated to be a factor of five higher when coal tar was present.

Data on time trends are primarily available for road paving in Europe, where exposures have decreased by a factor of two to three each decade since 1970 for bitumen fume, bitumen vapour and benzo[*a*]pyrene.

1.5 Regulations and guidelines

1.5.1 Limits for occupational exposure

Limits for occupational exposure to bitumen emissions have been set in more than 50 countries or regions (Table 1.18), although many countries do not have limits. The legal status of such limits varies between binding regulatory limits and voluntary guidelines. They are based on different analytical metrics and measurement methods. More than half of the regulated countries use a limit of 5 mg/m^3 for the 8-hour time-weighted average (TWA) concentration, typically as TPM. Slightly fewer than half use a TWA limit concentration of 0.5 mg/m^3, measured typically as BSF of the inhalable fraction of bitumen fume. Ten countries have set a limit for short-term exposure in addition to the TWA-based limit.

Limits for occupational exposure have also been set in many countries for some agents that may occur in fume or vapours emitted from hot bitumens. Such agents include PAHs, naphthalene, benzo[*a*]pyrene and aldehydes.

No regulations or guidelines specifically concerning bitumens or bitumen emissions in ambient air, drinking-water or food were available to the Working Group.

1.5.2 Regulation and policies for reclaimed asphalt pavement

(a) France

A threshold for PAHs in reclaimed asphalt material containing coal tar has been set at 50 mg/kg (French Government, 2010).

(b) Sweden

The Swedish Road Administration has developed guidelines on how to handle recycling of asphalt containing tar (SRA, 2004). The asphalt is chemically identified as "containing coal tar" when the concentration of the 16 EPA-priority PAHs is > 70 mg/kg of solid matter.

(c) Switzerland

Coal tar was used for paving in Switzerland until 1991. Regulations have limited the use of materials containing coal tar (BAFU, 2006). Reclaimed asphalt material containing PAHs at concentrations < 5000 mg/kg can be recycled into both hot (asphalt plant) or cold (*in situ*) applications, as well as in unbound form, storable in landfill for inert waste. It is also possible to recycle asphalt crust containing PAHs at concentrations of up to 20 000 mg/kg, but only for cold applications. At concentrations above 20 000 mg/kg, the waste is directed to a landfill (Hugener *et al.*, 1999, 2010).

Table 1.18 National occupational exposure limits (OELs) for bitumen emissions

Country	OEL (mg/m³)	Basis	Analytical metric
Australia	5		Bitumen fume
Belgium	5	TWA	TPM
Canada-Quebec	5	TWA	TPM
Denmark	1	TWA	Cyclohexane-soluble fraction
	2	STEL	Cyclohexane-soluble fraction
European Union	No limit		
Finland	5	TWA	Organic dust (used also for bitumen fume)
France	No limit		
Germany	No limit		
Hungary	No limit		
Ireland	0.5	TWA	Benzene-soluble fraction of aerosol
	10	STEL (15 min)	Benzene-soluble fraction of aerosol
Italy	No limit		
Japan	No limit		
New Zealand	5	TWA	
Norway	5	TWA	TPM
Poland	5	TWA	TPM
	10	STEL	TPM
Portugal	0.5	TWA	Benzene-soluble inhalable particulates
Republic of Korea	0.5	TWA	
Singapore	5	TWA	TPM
Spain	0.5	Daily limit	Benzene-soluble inhalable particulates
Switzerland	10	TWA	Total hydrocarbons
United Kingdom of Great Britain and Northern Ireland	5	TWA	TPM
	10	STEL (10 min)	TPM
USA			
NIOSH	5	STEL (15 min)	TPM
ACGIH	0.5	TWA	Benzene-soluble inhalable particulates

ACGIH, American Conference of Governmental Industrial Hygienists; min, minute; NIOSH, National Institute of Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; STEL, short-term exposure limit; TPM, total particulate matter; TWA, 8-hour time-weighted average

From NAPA & EAPA (2011), GESTIS (2011)

(d) The Netherlands

If intended for recycling, reclaimed asphalt material containing coal tar should not contain 10 specified PAHs (anthracene, benzo[*a*]anthracene, benzo[*a*]fluoranthene, benzo[*g,h,i*]perylene, benzo[*k*]fluoranthene, chrysene, fluoranthene, indeno[1,2,3-*c,d*]pyrene, naphthalene and phenanthrene) in excess of a concentration of 75 mg/kg (The Netherlands, 2000).

(e) USA

In the USA there are no regulations for reclaimed asphalt pavement (RAP) at the federal level, and specifications vary by state (Mundt *et al.*, 2009). Currently, most state departments of transportation limit the percentage of RAP allowed in the mix; maxima range between 10% (e.g. Iowa, Washington) and 30% (e.g. Florida, Pennsylvania), with 20% most commonly reported. In some states, the amount of RAP used in the mix has decreased over time (e.g. Kansas, Ohio, and Washington),

while it has increased in others (e.g. Indiana and Pennsylvania). Restrictions for RAP use include: no RAP in rubber asphalt (Florida); no recycling of pavements containing coal tar (Minnesota); no poor quality or "dirty" RAP (Texas and Virginia); and RAP to be used only from known sources (Washington). Six states (Kansas, New York, Ohio, Pennsylvania, California, and Utah) specifically reported no current restrictions on use of RAP.

2. Cancer in Humans

2.1 Introduction

2.1.1 Introduction to studies of cancer in humans

Studies designed to evaluate cancer incidence or mortality attributable to exposure to bitumens and bitumen emissions have employed cohort and case–control designs. There have also been several occupational surveys that used death certificates or other routinely collected administrative data to provide information on this issue. These studies, which serve primarily as hypothesis-generating exercises in the early stages of evaluation of a scientific issue, are typically analysed using proportionate mortality methods. Such studies are less valuable when hypothesis testing is required because of the limitations of routine data and of the proportionate mortality ratio as an indicator of association. Every attempt was made, however, to obtain and review articles that specifically indicated a focus on cancer and possible exposure to asphalt or bitumen.

2.1.2 Strengths and limitations of epidemiological studies

The potential for confounding from other occupational exposures can bias estimates of relative risk and this is a particular problem in the study of bitumen because many occupations linked to bitumen exposure may also involve exposure to coal tars, which are established human carcinogens. Studies that obtained information on both exposures for direct adjustment can be used to evaluate the potential for such confounding, not only in that study, but in other studies of similar occupational groups, by providing an indication of how likely confounding from occupational exposure to coal tars, or other occupational exposures, may be.

Studies that characterize exposure to bitumens only on the basis of broad occupational titles (such as roofer or road-construction worker) may experience exposure misclassification because workers classified under a given job title may have different exposures if they perform different job tasks. Moreover, some studies are based on exposure data obtained from a census or death certificates at a single point in time, which does not reflect job mobility. Other studies combine several occupations to form the exposure group, which may lead to even more serious misclassification. All these methods can lead to non-differential misclassification of exposure, which may in turn detract from the ability to detect associations between occupational exposure and cancer.

Exposure assessment in some studies is only qualitative, and sometimes as crude as "ever *versus* never". This approach can be useful, but can also encompass considerable misclassification of exposure. In hypothesis-testing studies, quantitative assessment of exposure is necessary to identify exposure–effect patterns.

Publication bias can occur when positive findings are published preferentially. Such publication bias could create a literature that appears to indicate an abundance of positive associations. This can be a particular problem for case–control studies where many occupations/exposures can be evaluated, but where details regarding exposure are weak. Publication bias may be a more serious problem during the early years of

concern about a health issue. However, as the issue grows in importance and visibility, results from all studies, regardless of the findings, are more likely to make their way into the scientific literature. There was no information available regarding publication bias on the risk of cancer associated with exposure to bitumen; however, it has been an important concern since bitumens and their emissions were first evaluated by IARC in 1984 (IARC, 1985).

Studies of the risk of cancer associated with bitumens and bitumen emissions have mainly focused on workers employed in roofing, paving and mastic-asphalt operations. These operations occur in very different locations and have different histories regarding the materials used and potential coexposures. In all, coal tars have been used at some time (see Section 1).

Cohort and case–control studies can have different strengths and limitations. The quality of each study depends upon how reliably and accurately the disease of interest is characterized, the quality of exposure assessment, and controls for confounding. These factors must be evaluated for each study and cannot be arbitrarily determined by study type. In general, it is easier to assess occupational exposures in cohort studies because they are grounded in industry, which provides considerable information on work practices and exposure. Case–control studies can usually deal with confounding more easily because information is typically solicited directly from study subjects, which is not the case with occupational cohort studies. Nested case–control studies within an occupational cohort are also used to obtain information directly from participants, or surrogate respondents.

2.2 Cohort studies

2.1.2 IARC multicentre cohort study

See Table 2.1

The findings from the IARC multicentre cohort study are reviewed in the following three sections. The first reviews the findings of the cohort study by occupational group (Boffetta *et al.*, 2001, 2003a) and the findings obtained by applying a job–exposure matrix to investigate disease risks related to exposure to specific substances (Boffetta *et al.*, 2003b; Burstyn *et al.*, 2000a, 2003a). In the second section, analyses of several subcohorts published separately are reviewed (Bergdahl & Järvholm, 2003; Burstyn *et al.*, 2003b, 2007; Hooiveld *et al.*, 2003; Randem *et al.*, 2004; Behrens *et al.*, 2009). In the third section, findings from the nested case–control study are reviewed (Olsson *et al.*, 2010). The nested case–control study focused on cancer of the lung, included cases of cancer of the lung diagnosed from 1980 onwards, and additional incident cases of cancer of the lung.

(a) IARC multicentre study

The IARC multicentre cohort study of European workers exposed to bitumen comprised 79 822 workers from the asphalt industry, roofing industry, and related trades in Denmark, Finland, France, Germany, Israel, the Netherlands, Norway and Sweden. [The studies by Engholm & Englund (1995) and Pukkala (1995) overlapped with the present multicentre study. It is unclear to what extent the studies by Hansen (1989a, b) also overlapped with the present study. The study by Boffetta *et al.* (2003a, b) provided additional assessment of exposure that was superior to that in the individual studies.]

For all countries except Sweden, study participants were identified from records of road-paving and asphalt-mixing companies. For Sweden, participants were identified from the records of the Swedish construction industry's Organisation for Working Environment Safety and Health (*Bygghälsan*); all road pavers and asphalt mixers, as well as a sample of other construction workers, were included. In all countries, all male workers active for at least one

Table 2.1 IARC multicentre cohort study and risk of cancer

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Boffetta *et al.* (2001, 2003a) IARC Multicentre cohort study in Denmark, Finland, France, Germany, Israel, the Netherlands, Norway, and Sweden	29 820 bitumen workers, employed for at least one season in the bitumen industry.	Beginning 1953–79 and ending 1995–2000	Job tasks obtained from company records. A job-exposure matrix was constructed to assess exposure to bitumen fume and other potential occupational carcinogens	Lung cancer	Bitumen workers (SMR)	330	1.17 (1.04–1.30)	Age, calendar period, country
					Bitumen workers (RR using construction workers as ref.)		1.09 (0.89–1.15)	No smoking data available. No trend with duration, cumulative exposure or average exposure to bitumen was present. In a subgroup of road paving workers, the risk of lung cancer increased with average exposure level (P = 0.02)
					Road paving workers	189	1.17 (1.01–1.35)	
					Roofers/ waterproofers	14	1.33 (0.73–2.33)	
					Bitumen workers		*SMR*	
				All causes (001–999)		3987	0.96 (0.93–0.99)	
				All malignant neoplasms (140–180)		1016	0.95 (0.90–1.01)	
				Upper aerodigestive tract (140–150, 161)		92	1.27 (1.02–1.56)	
				Oral cavity and pharynx (140–149)		35	1.21 (0.84–1.68)	
				Oesophagus (150)		37	1.29 (0.91–1.78)	
				Stomach (151)		70	0.99 (0.77–1.25)	
				Colon (153)		55	0.71 (0.54–0.93)	
				Rectum (154)		43	0.89 (0.64–1.20)	
				Liver (155)		17	0.73 (0.43–1.17)	
				Pancreas (157)		43	0.76 (0.55–1.02)	
				Nose and nasal sinuses (160)		160	0.92 (0.25–2.34)	
				Larynx (161)		20	1.34 (0.82–2.07)	
				Lung (162)		330	1.17 (1.04–1.30)	
				Pleura (163)		5	0.72 (0.23–1.68)	
				Connective and other soft tissue (171)		6	1.23 (0.45–2.68)	
				Melanoma of skin (172)		15	0.74 (0.41–1.21)	
				Prostate (185)		82	0.85 (0.68–1.05)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Boffetta *et al.* (2001, 2003a) contd				Testis (186)		4	0.77 (0.21–1.98)	
				Bladder (188)		45	1.05 (0.77–1.41)	
				Kidney (189)		26	0.76 (0.50–1.11)	
				Nervous system (191–192)		22	0.63 (0.40–0.96)	
				lll-defined and unspecified sites (195, 199)		52	0.95 (0.71–1.24)	
				Non-Hodgkin lymphoma (200, 202)		23	0.78 (0.49–1.17)	
				Hodgkin disease (201)		8	1.24 (0.54–2.45)	
				Multiple myeloma (203)		12	0.70 (0.36–1.22)	
				Leukaemia (204–208)		28	0.78 (0.52–1.12)	
				Lymphoid leukaemia (204)		8	0.68 (0.30–1.35)	
				Myeloid leukaemia (205)		13	0.70 (0.37–1.20)	
					Road paver		*SMR*	
				All causes		2411	0.94 (0.90–0.98)	
				All cancers		623	0.96 (0.89–1.04)	
				Head and neck cancer		59	1.30 (0.99–1.68)	
				Lung cancer		189	1.17 (1.01–1.35)	
							RR	
				All causes			1.01 (0.95–1.07)	
				All cancers			1.01 (0.90–1.13)	
				Head and neck cancer			1.24 (0.91–1.68)	
				Lung cancer			1.08 (0.87–1.34)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Boffetta *et al.* (2001, 2003a) contd					*Roofer, waterproofer*		*SMR*	
				All causes		141	0.88 (0.74–1.04)	
				All cancers		44	1.21 (0.88–1.62)	
				Head and neck cancer		4	1.49 (0.40–3.81)	
				Lung cancer		14	1.33 (0.73–2.23)	
							RR	
				All causes			0.93 (0.77–1.14)	
				All cancers			1.27 (0.87–1.84)	
				Head and neck cancer			1.23 (0.45–3.37)	
				Lung cancer			1.34 (0.71–2.53)	
Boffetta *et al.* (2003b)				Lung cancer	Bitumen fume (JEM) (ever)		1.08 (0.92–1.19)	
Burstyn *et al.* (2003b)					*Lag 15 yr: average (mg/m^3)*			
					0	53	1.00 (-)	*P* for trend = 0.0005
					0.71– < 1.21	22	0.37 (0.21–0.66)	
					1.21– < 1.32	20	0.78 (0.42–1.47)	
					1.32– < 1.47	21	1.01 (0.54–1.90)	
					1.47–6.46	19	1.58 (0.79–3.13)	
					Lag 0 yr: average (mg/m^3)			
					0.31– < 1.03	32	1.00 (-)	*P* for trend = 0.0002
					1.03– < 1.23	37	2.72 (1.64–4.53)	
					1.23– < 1.37	33	2.22 (1.22–4.07)	
					1.37–5.38	33	3.02 (1.69–5.39)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Burstyn *et al.* (2003b) contd					*Lag 15 yr: cumulative (mg/m³.yr)*			*P* for trend = 0.2
					0	53	1.00 (-)	
					0.004– < 1.61	20	0.71 (0.40–1.26)	
					1.61– < 3.71	20	0.77 (0.43–1.37)	
					3.71– < 9.57	19	0.50 (0.27–0.91)	
					9.57– < 47.04	23	0.74 (0.38–1.45)	
					Lag 0 yr: cumulative (mg/m³.yr)			
					0.33– < 2.16	34	1.00 (-)	*P* for trend = 0.7
					2.16– < 4.61	31	1.14 (0.70–1.87)	
					4.61– < 9.66	33	0.97 (0.60–1.59)	
					9.66– < 71.96	37	0.55 (0.50–1.44)	
Behrens *et al.* (2009) Germany	7919 asphalt workers employed 1975–97	1975–2004	Exposure groups based on job titles	All causes		835	1.27 (1.19–1.36)	Age, calendar period No data on smoking or alcohol intake available
				All cancers		272	1.37 (1.22–1.55)	
				Oral/pharyngeal cancers		21	1.82 (1.19–2.79)	
				Oesophageal cancer		20	2.43 (1.57–3.76)	
				Laryngeal cancer		14	3.74 (2.21–6.31)	
				Lung cancer		101	1.77 (1.46–2.16)	
				Bladder cancer		14	3.29 (1.95–5.55)	
				Alcoholism		25	1.83 (1.23–2.70)	
				Liver cirrhosis		52	1.39 (1.06–1.83)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Hooiveld *et al.* (2003) the Netherlands	3714 asphalt workers		Semiquantitative exposure assessments of average exposure, exposure duration and cumulative exposure to bitumen	Lung	*Average bitumen exposure (units)*			Age, calendar period, smoking
					Non-exposed	3	1.00	Smoking habits for a subset (~ 1/3) of the cohort were used to assess smoking habits in the cohort
					> 0–1.29	13	0.99	
					≥ 1.29–3.21	52	1.36	
					≥ 3.21–4.76	4	1.57	
					≥ 4.76	0	0	
Burstyn *et al.* (2007) Denmark, Norway, Finland and Israel	7298 male asphalt-paving workers	Varied between countries	BaP was used as a marker of exposure to 4–6-ring PAHs	Bladder	*Average exposure level (ng BaP/m³) unlagged*			Age, country No smoking data available
					0 < – < 65	12	1	*P* for trend = 0.4
					65– < 126	11	1.01 (0.43–2.39)	
					126– < 198	12	1.41 (0.55–3.60)	
					≥ 198	13	1.36 (0.54–3.44)	
				Bladder	*Average exposure level (ng BaP/m³) lagged 15 yr*			
					0 < – < 99	10	1.00 (-)	*P* for trend = 0.15
					99– < 139	9	1.53 (0.54–4.38)	
					139– < 204	10	2.71 (1.01–7.27)	
					≥ 204	10	1.90 (0.66–5.47)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Olsson *et al.* (2010) Six European countries and Israel 1980–2005	433	1523	Semiquantitative using existing exposure data and expert judgement	Lung	Ever exposed to bitumen fume	303	1.1 (0.8–1.5)	Risk set, age, country, smoking, coal tar in analyses of bitumen fume Response rates: cases, 65%; controls, 58%; Interviews in person: cases, 2%; controls, 66%. Condensate indicates dermal exposure
					Ever exposed to bitumen condensate	309	1.2 (0.9–1.6)	
					Cumulative bitumen fume (unit-yr)			
					0.18–9.55	88	1.31 (0.93–1.85)	
					9.56–28.17	73	0.99 (0.68–1.45)	
					28.2–68.0	82	1.16 (0.78–1.72)	
					68.01–620.48	60	0.77 (0.50–1.19)	
					Duration of bitumen exposure (yr)			
					0.33–7.99	85	1.19 (0.84–1.69)	
					8.00–15.49	82	1.26 (0.87–1.83)	
					15.50–25.99	81	1.23 (0.84–1.79)	
					26.00–54.00	55	0.74 (0.49–1.11)	
					Average bitumen fume (units)			
					0.08–0.97	78	1.20 (0.84–1.71)	
					0.98–2.20	75	1.15 (0.78–1.70)	
					2.21–3.61	65	0.90 (0.60–1.34)	
					3.62–16.67	85	1.16 (0.78–1.73)	
					Cumulative bitumen condensate (unit-yr)			
					0.29–6.62	70	1.10 (0.77–1.57)	
					6.63–13.44	74	1.21 (0.83–1.76)	
					13.45–23.06	80	1.25 (0.84–1.87)	
					23.07–94.11	85	1.23 (0.81–1.88)	

BaP, benzo[*a*]pyrene; JEM, job-exposure matrix; PAH, polycyclic aromatic hydrocarbon; ref., reference; RR, relative risk; SMR, standardized mortality ratio; yr, year

season with a first year of employment between 1910 to 1930, except for Denmark (1953) and Germany (1965), and a last employment between 1992 (Sweden) and 1999 (Germany and the Netherlands). Based on job categories, workers were subdivided into bitumen workers (ever) n = 29820; building- and ground-construction workers (not exposed to bitumens), n = 32245; and other workers, not classifiable as to their exposure to bitumens n = 17757.

Information was retrieved from the companies regarding the calendar periods during which coal tar was used. This information was used to define a "coal-tar free" subcohort comprising 17 443 bitumen workers and 30 273 building- and ground-construction workers, to assess possible confounding from exposure to coal tar.

Start of follow-up for mortality ranged from 1953 (Norway) to 1979 (France) and end of follow-up was between 1995 and 2000. Only 0.7% of the cohort was lost to follow-up. Age, calendar period, country, and sex-specific expected numbers of deaths were calculated from the WHO mortality data bank. Standardized mortality ratios (SMRs) were used to estimate relative risks. Poisson regression was used for a cohort-internal analysis of risk per job title and quantitative and semi-quantitative measures of exposure.

The standardized mortality ratio for cancer of the lung was increased among the bitumen workers, based on 330 deaths (SMR, 1.17; 95% confidence intervals [CI], 1.04–1.30) (Boffetta *et al.*, 2003a). There were 189 deaths from cancer of the lung among road-paving workers (SMR, 1.17; 95% CI, 1.01–1.35). There were 14 deaths from cancer of the lung among roofers/waterproofers (SMR, 1.33; 95% CI, 0.73–2.23). The number of cancers observed was close to the number expected both among building- and ground-construction workers (SMR, 1.01; 95% CI, 0.89–1.15), and in the group of "other workers" (SMR, 1.01; 95% CI, 0.88–1.15). The number of cancers of the head and neck [defined as cancer of the mouth, pharynx, larynx and oesophagus] was significantly increased among the bitumen workers (SMR, 1.27; 95% CI, 1.02–1.56). There was no significant heterogeneity in standardized mortality ratios for cancer of the lung in asphalt workers between countries (P = 0.4), although a large variation in the standardized mortality ratios for cancer of the lung between countries was noted for building- and ground-construction workers who were used as unexposed referents in the internal analysis (P = 0.01). This heterogeneity was partly due to a marked deficit of deaths from lung cancer for this group found in Denmark. [The standardized mortality ratio was < 0.5, which raised concern about methodological problems possibly related to selection bias.] Standardized mortality ratios for cancer of the lung in other specific subgroups of bitumen workers (asphalt pavers, asphalt mixers, unspecified pavers/mixers, and unspecified bitumen workers) were essentially similar to that of the entire group of bitumen workers.

Thirty-three deaths occurred among mastic-asphalt workers, including five deaths from cancer of the lung (SMR, 2.39; 95% CI, 0.78–5.57) (Boffetta *et al.*, 2001). [The Working Group noted that this publication provided no information on a possible overlap with the Danish cohort of mastic-asphalt workers (Hansen, 1989b). However, the authors informed the Working Group during the meeting that there was no overlap, as two of the five deaths were from Germany while the other three were from Norway.]

A cohort-internal analysis based on Poisson regression and using building- and ground-construction workers as referents showed no statistically significantly elevated relative risks for cancer of the lung in any of the specific jobs mentioned above. The relative risk for bitumen workers was 1.09 (95% CI, 0.89–1.34), and was > 1.0 for most subgroups of bitumen workers. There was a marked heterogeneity in relative risk among bitumen workers between countries. [It was unclear to what extent the internal comparisons, based on job categories, reduced

confounding. While the internal comparison may have reduced the potential bias from smoking habits, it may not have provided an advantage over the external comparison group because in the internal group there may have been exposures to other substances, such as coal tar. While the study had an advantage in the large size and detailed data on work history, the lack of data on smoking habits limited the conclusions. The standardized mortality ratio of 1.17 observed with the external comparison group was of a magnitude that could be explained by confounding between the asphalt workers and the external comparison group. The internal analysis gave no firm support for an excess of lung cancer among the bitumen workers.]

Findings regarding non-cancer mortality in this cohort were presented in an IARC internal report (Boffetta *et al.*, 2001). The standardized mortality ratio for circulatory diseases among bitumen workers was 0.93 (95% CI, 0.89–0.98), and that for non-malignant respiratory diseases was 1.08 (95% CI, 0.96–1.22). [While this did not rule out confounding from tobacco smoking either in the internal (Poisson regression) or in the external comparisons (SMR analyses) of lung cancer in relation to bitumen exposure, it indicated that smoking habits in the cohort were not excessive when compared with those of the general population. See below for further data on mortality in the German subcohort.]

A job-exposure matrix was developed for a further analysis of risk of cancer of the lung associated with occupational exposures in the asphalt industry (Burstyn *et al.*, 2000a, 2003a). The matrix was based on questionnaires to the companies, a large database of 2007 industrial hygiene measurements from the asphalt industry, and expert evaluations. The hygiene measurements were mainly collected in the late 1970s and between 1985 and 1997. The matrix contained semiquantitative estimates of exposure to bitumen fume, organic vapours, PAHs, diesel exhaust, asbestos, silica and coal tar. In addition, quantitative assessments of exposure were performed for bitumen fume (mg/m^3), organic vapours (mg/m^3), and benzo[*a*]pyrene (ng/m^3), for jobs entailing exposure to bitumens in road paving. For benzo[*a*]pyrene, exposure levels were modelled to decrease from 322 ng/m^3 before the 1960s to 24 ng/m^3 in the early 1990s (Burstyn *et al.*, 2003a). The exposure assessments were applied to the work histories for all workers in the IARC multicentre cohort (Boffetta *et al.*, 2003b). For each worker, the following indices were derived for all agents assessed: never/ever exposed, duration of exposure, cumulative exposure, and average exposure level. Standardized mortality ratios and internally derived relative risks were estimated using the same methods as in Boffetta *et al.* (2003a).

The standardized mortality ratios for cancer of the lung were similar among workers ever exposed to bitumen fume ($n = 524$; SMR, 1.08; 95% CI, 0.99–1.18) and those unexposed to bitumen ($n = 232$; SMR, 1.05; 95% CI, 0.92–1.19) (Boffetta *et al.*, 2003b). The standardized mortality ratios for cancer of the lung and exposure to known occupational carcinogens (coal tar, asbestos, silica) were approximately equal to one, and no statistically significant trend was noted for cumulative exposure to any of those agents. A regression model simultaneously incorporating indices of all studied exposures showed that the relative risk of cancer of the lung associated with exposure to bitumen tended to be reduced by incorporation of exposure to coal tar in the model. In the "coal-tar free" subcohort, the standardized mortality ratio for cancer of the lung was significantly elevated (SMR, 1.23; 95% CI, 1.02–1.48), but no significant associations were noted with duration, cumulative exposure or average exposure to bitumen. For cancer of the head and neck, standardized mortality ratios were not significantly elevated in any of the exposure groups. No trend in relative risk with semiquantitatively assessed exposure indices (duration, cumulative exposure, average exposure) to bitumens

was found (Boffetta *et al.*, 2003b). [While it is possible that none of the investigated exposures were high enough to present an excess of cancer of the lung, a less intensive effort was made to quantify exposures other than bitumen and the lack of effect for known carcinogens underscored the challenges of developing retrospective exposure assessments.]

Quantitative estimates of exposure to bitumen fume were available for a subset of the cohort exposed during road paving. The subset included 135 cases of cancer of the lung, and a positive association was found between average exposure level of bitumen fume lagged 15 years (P = 0.0005) and average exposure with no lag (P = 0.0002). No association with cumulative exposure was found (P = 0.7) (Burstyn *et al.*, 2003b). [The Working Group noted that the reference categories were defined differently in the analyses lagged by 0 and 15 years. The exposure–effect and shape of the relationship was sensitive to introduction of lagging. Both analyses suggested a trend, but the lagged analysis showed a decline that the Working Group found difficult to interpret.]

A sensitivity analysis of the influence of assumptions made regarding exposure during periods for which no empirical data were available was based on the subset of the cohort for which there were quantitative exposure estimates (de Vocht *et al.*, 2009). This analysis showed that the conclusions presented in Boffetta *et al.* (2003b) were only marginally affected by various assumptions on time trends in exposure. The variability in exposure to bitumen fume between and within working crews was investigated based on the exposure data compiled for the IARC multicentre cohort study (Burstyn *et al.*, 2000a). A substantial variation in exposure between workers was found, while variation within workers was smaller. [The Working Group noted that variation in exposure between workers of the same job in the full cohort was not likely to have been fully accounted for in the exposure classification, which could lead to non-differential measurement error tending to attenuate observed risks.]

[This study had advantages in its very large size and the use of a detailed job-exposure matrix to assess exposures, but a lack of information on smoking habits limited conclusions. In addition, the quality of the data on job categories may have varied between countries. However, the subsequent case–control study nested within this cohort (Olsson *et al.*, 2010) addressed these issues. The applied job-exposure matrix indicated no clear excess of lung cancer in this cohort in association with established lung carcinogens such as asbestos, silica, and coal tar, and there was no effect of exposure to diesel exhaust. Possibly none of these exposures were high enough to cause an excess of lung cancer in the cohort, but it raised some concern for the validity of the exposure classification process for the agent under study, bitumens.]

(b) Additional analyses from the IARC multicentre cohort study

For some of the national cohorts contributing data to the IARC multicentre cohort, there were separate reports presenting additional data, e.g. extension of follow-up, or risk estimates adjusted for smoking. These are presented below.

The German part (n = 7919) of the IARC multicentre cohort was updated by extension of follow-up through 2004 (Behrens *et al.*, 2009). The study showed significantly increased standardized mortality ratios for cancer of the lung, larynx, oesophagus, and for oral/pharyngeal cancers and bladder cancer. However, the study also showed an excess of deaths from alcoholism, non-malignant respiratory diseases and liver cirrhosis, indicating that both alcohol and tobacco habits were in excess in this part of the cohort. [As has been commented above, this was not the case for the multicentre cohort.]

Randem *et al.* (2004) investigated the incidence of cancer in the Nordic part of the IARC

multicentre cohort study. [This study overlapped with studies by Bergdahl & Järvholm (2003), Kauppinen *et al.* (2003), and Randem *et al.* (2003).] The study included 22 362 male bitumen-exposed workers from Denmark, Finland, Norway and Sweden. Cancer cases were identified from the national cancer registries and expected numbers of cases were derived based on national cancer rates. There was a significant excess of lung cancer in the cohort (standardized incidence ratio, SIR, 1.21; 95% CI, 1.07–1.36), as well as among road pavers (SIR, 1.26; 95% CI, 1.08–1.47), but no trend was noted with time since first exposure. No overall excess of cancer of the bladder was noted, but there was a non-statistically significant positive trend of increasing risk with time since first exposure. [This study, based on cancer incidence, may be particularly informative regarding cancer sites with a good survival. There was no adjustment for smoking habits.]

Bergdahl & Järvholm (2003) reported the findings of the Swedish part of the IARC multicentre study, adding data on the incidence of cancer and individual data on smoking habits obtained from health surveys among Swedish construction workers. The incidence of cancer of the lung was not increased compared with the general population. The same findings were obtained using an internal reference group of construction workers not involved in the asphalt trade. Adjustment for smoking did not change the risk estimate.

Hooiveld *et al.* (2003) presented an analysis of the Dutch part of the IARC multicentre cohort study, comprising 3714 workers. Data on smoking habits were available from medical-evaluation records for about one third of the cohort, and were used to assess smoking habits for all workers, assuming that the smoking habits were representative for all workers within each job class. Workers exposed to bitumen were more likely than unexposed workers to have been current or former smokers. A positive trend was noted for cancer of the lung with average exposure to bitumen fume, but this trend was attenuated when adjusted for smoking. [The Working Group noted that the cut-off points for the categorical analysis were chosen to permit direct comparison with the IARC cohort results for the exposure–response analysis.]

Burstyn *et al.* (2007) presented an analysis of the incidence of cancer of the bladder in relation to exposure to PAHs in a subset of the IARC multicentre cohort study. The study included 7298 male asphalt-paving workers from Denmark, Norway, Finland and Israel. Occupational histories were extracted from personnel files. Cancer cases were identified from national cancer registries, follow-up time varied between countries. Benzo[*a*]pyrene was used as a marker of exposure to four- to six-ring PAHs. Relative risks were estimated in an internal analysis using Poisson regression. There were 48 cases of cancer of the bladder. There were indications of a positive trend in risk of cancer of the bladder with increasing average unlagged exposure to benzo[*a*]pyrene (Table 2.1), but the trend was not statistically significant ($P = 0.4$). Lagging of exposure by 15 years gave a P for trend of 0.15. [Risks were not adjusted for smoking or exposure to coal tar.]

(c) IARC multicentre nested case–control study

Olsson *et al.* (2010) conducted a case–control study nested in part of the previously described IARC cohort of 38 296 male asphalt workers in Denmark, Finland, France, Germany, the Netherlands, Norway and Israel, but excluding Sweden (Boffetta *et al.*, 2003a, b). Workers with at least two seasons of employment in an asphalt industry who were aged < 75 years and alive without cancer on 1 January 1980 were eligible. Cases ($n = 433$) were members of the cohort who died of or were diagnosed with cancer of the lung between 1980 and the end of follow-up, which ranged from 2002 to 2005, depending on the country, and was extended relative to the cohort study. Controls ($n = 1253$) were sampled randomly

from members of the cohort without respiratory or ill-defined cancer. Cases and controls, or their next-of-kin if the worker was deceased, were interviewed by telephone to obtain information about demographics, smoking and lifetime work history in asphalt industries and elsewhere. The overall response rate was 65% for cases and 58% for controls, with considerable variation among countries; 2% of cases and 66% of controls were interviewed in person. Further detailed information on asphalt industry jobs was obtained from living subjects and other workers identified from the cohort study or by industry representatives or workers' next-of-kin. Individual-level exposures to bitumen fume, organic vapours and PAHs (four- to six-ring) in air were estimated semiquantitatively from previous estimates and questionnaires from the case–control study. Algorithms were used to combine data on 85 jobs from an exposure database assembled for the cohort study (Agostini *et al.*, 2010) with information about determinants of time worked, such as the length of the working day and the duration of the working season from the case–control study. Dermal exposure to bitumen condensate was assessed based on a relative ranking of jobs. Previous semiquantitative estimates and measurement surveys were used when possible, and expert judgment was used when these were not available. Estimates of dermal exposure were adjusted for time worked and for hygienic behaviours that could reduce exposure. Potentially confounding exposures to asbestos, coal tar, crystalline silica and diesel exhaust both within and outside the asphalt industry were assessed by expert judgment using categories of "no", "low" and "high" exposure. The data were analysed by logistic regression with adjustment for risk set, age, country, and smoking pack-years. Analyses for bitumen fume also adjusted for exposure to coal tar. Results were reported for analyses by unconditional logistic regression with no exposure lag, but the authors reported that analyses using conditional logistic regression and a 15-year lag gave similar results. Fully adjusted odds ratios (OR) for lung cancer were 1.12 (95% CI, 0.84–1.49) for ever exposure to bitumen fume, 1.20 (95% CI, 0.93–1.55) for organic vapour, 1.20 (95% CI, 0.85–1.69) for PAHs in air and 1.17 for dermal exposure to bitumen condensate (95% CI, 0.88–1.56). Adjusted odds ratios for duration of exposure to bitumen fume, average exposure to bitumen fume and cumulative exposure to bitumen fume were generally elevated relative to never exposure (ORs, 1.1–1.3): all odds ratios for bitumen exposure and tests for trend were not statistically significant. Odds ratios with and without adjustment for smoking and coal tar were comparable. For dermal exposure to bitumen condensate classified by duration of exposure, cumulative exposure and average exposure, most adjusted odds ratios were of the order of 1.2 to 1.3 and none was statistically significant; tests for an exposure–effect trend were not statistically significant. The odds ratio for cumulative exposure to coal tar was 1.60 (95% CI, 1.09–2.36) in the highest exposure group and odds ratios for average exposure to coal tar were also elevated.

[This study was nested in a well characterized cohort of exposed workers and included details on smoking, full occupational histories and a more detailed exposure assessment than any other study. Further strengths were the efforts to estimate exposure separately for bitumen and coal tar and to examine inhalation and, for the first time, dermal exposures. Limitations included the relatively low rate of response and the extensive reliance on proxy interviews for cases, which could be a source of differential measurement error. Comparisons between this nested case–control study and the previous analyses of the cohort on which it is based were somewhat hampered by the different inclusion and exclusion criteria, non-response, and differences in the years of entry: subjects of the nested case–control study had typically been employed in later years when exposure was lower and the likelihood of exposure to coal tar was reduced.]

(d) Overall commentary on the results of the IARC multicentre cohort study

[The Working Group noted that the IARC multicentre cohort study (including the cohort study, subsequent analyses of the cohort, and the nested case–control study) was a significant improvement over all the previous studies in that it addressed the key limitations found in the previous reports. This study was large, multicentric, used quantitative exposure assessment of both inhalational and dermal exposure to bitumens, included data on both incidence and mortality, and adjusted for a broad range of confounders (e.g. smoking, coal-tar exposure, silica, and other occupational exposures). The Working Group noted that all the findings in the nested case–control study were based on a complex exposure-assessment model.]

2.2.2 Other cohort studies

See Table 2.2

Hammond *et al.* (1976) published a historical cohort study of roofers and waterproofers exposed to coal-tar pitch and asphalt [bitumen] in the USA. Members of the cohort were likely to have been exposed to both bitumen and coal tar because: "In former years, pitch was used more frequently than asphalt, but today asphalt is more commonly used. Most of the men work with both substances". Filter samples were collected from masks that workers were asked to wear for an entire working shift to analyse their content of benzo[*a*]pyrene. The amount of benzo[*a*]pyrene collected on the filters varied with the type of job, with average values ranging from 1.4 µg (felt layer) and 2.9 µg (mop man) to 51.8 µg (scraper), 53.0 µg (shovelman) and 31.0 µg (kettleman). It was uncommon to wear masks except for work in confined spaces. The authors stated that it was unusual for workers to specialize in a specific job, the custom being to take turns at various jobs. Workers were identified through membership of the United Slate, Tile and Composition Roofers, Damp and Waterproof Workers' Association, excluding locals confined to slate and tile work. The minimum duration of membership was 9 years before the start of the study in 1960. Since the union provided life insurance to both active and retired members, it was possible to obtain copies of the death certificates of all who died "while in good standing". Thus tracing of study subjects was done with assistance of the union. During the follow-up period from 1960 to 1971, 1798 men died. For 4.3% of these, no death certificate could be obtained. Thus the expected number of deaths was adjusted downward accordingly. Mortality of the cohort was compared with mortality of the total male population of the USA. No healthy-worker effect was observed, the standardized mortality ratio for total mortality was 1.02 and 1.09 for 9–19 years and ≥ 20 years since joining the union, respectively. Mortality from cancer of the lung in the cohort was increased after ≥ 20 years since joining the union: SMR for 20–29 years, 1.52 (66 deaths); SMR for 30–39 years, 1.50 (21 deaths); SMR for ≥ 40 years, 2.47 (12 deaths). Duration of membership was considered as a surrogate for exposure duration. Regarding other diseases related to smoking, mortality was elevated for non-malignant respiratory diseases, and was higher 9–19 years after joining the union (SMR, 1.96; 26 deaths; [95% CI, 1.28–2.87]) than ≥ 20 years after joining (SMR, 1.67; 71 deaths; [95% CI, 1.30–2.10]). [The Working Group noted that this study lacked information on tobacco smoking. However, as there was no trend for non-malignant respiratory disease with time since joining the union, it was not likely that confounding by smoking would explain the positive association between risk of cancer of the lung and years since joining the union. Overall, the informativeness of this study was limited since it did not assess bitumen exposure specifically.]

Menck & Henderson (1976) used mortality and morbidity data for white males aged 20–64 years from Los Angeles county (CA, USA) to

Table 2.2 Cohort studies of asphalt workers exposed to bitumen anterior to the IARC multicentre cohort study

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hammond *et al.* (1976) USA 1960–71	5939, men only	Membership of United Slate, Tile and Composition Roofers, Damp and Waterproof Workers Association, excluding locals confined to slate and tile work. Minimum membership duration, 9 yr before 1960	Codes NR	Time since joining the union calculated up to 1960		*SMR*	Age, calendar period Vital status and cause of death traced through death certificates provided by union life insurance. Expected numbers calculated from cause-specific proportions of age- and time period-specific total mortality. CI and *P* values, NR. Time since joining the union used as proxy for exposure duration.
				9–19 yr			
			All cancers		86	1.07 [0.87–1.32]	
			Lung		22	0.92 [0.57–1.39]	
			Oral cavity, pharynx, larynx, oesophagus		7	1.04 [0.42–2.15]	
			Bladder		2	0.82 [0.10–2.97]	
			Skin except melanoma		2	4.65 [0.56–16.80]	
			Stomach		3	0.54 [0.11–1.58]	
			Colon, rectum		14	1.46 [0.86–2.46]	
			Prostate		9	1.87 [0.85–3.54]	
			Leukaemia		5	1.67 [0.54–3.89]	
			Other		22	0.93 [0.61–1.41]	
				≥ 20 yr			
			All cancers		315	1.45 [1.30–1.62]	
			Lung		99	1.59 [1.29–1.94]	
			Oral cavity, pharynx, larynx, oesophagus		31	1.95 [1.32–2.77]	
			Bladder		13	1.68 [0.89–2.87]	
			Skin except melanoma		3	4.00 [0.82–11.69]	
			Stomach		24	1.67 [1.12–2.50]	
			Colon, rectum		37	1.32 [0.95–1.82]	
			Prostate		29	1.38 [0.96–1.99]	
			Leukaemia		13	1.68 [0.98–2.89]	
			Other		66	1.12 [0.88–1.43]	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Menck & Henderson (1976) Los Angeles County, USA 1968–70; 1972–73	1 560 800 (estimated from 1970 census); white males only; age 20–64 yr; 2161 death certificates citing cancer of the lung, trachea and bronchus (1968–70) pooled with 1777 incident cases of lung cancer reported to the Los Angeles CSP (1972–73)	Last occupation and industry as reported on death certificate (1968–70) or hospital admission sheets (1972–73)	Lung (code NR)	Roofers	11	4.96 ($P < 0.05$)	Age Expected numbers calculated for each occupation assuming that age-specific rates were identical for all occupations.
Povarov *et al.* (1988) Estonia 1974–84	1486 men only; age 20–74 yr	Employee in one of 11 plants producing hot-mix asphalt for ≥ 3 yr, in 1974–84	ICD version NR			[Presumably IRR]	Age Cancer incidence in cohort in 1974–84 compared with that in general population, Estonia, 1979–82
			All cancers		51	1.5 ($P < 0.05$)	
			Lung		17	1.5 ($P < 0.5$)	Narrative says NS.
			Stomach		7	1.1 (NS)	
			Skin		4	1.5 (NS)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Maizlish *et al.* (1988) California, USA 1970–83	27 162. Proportional mortality study inc. 1570 decedents (men, 88%; white, 90%), 327 in highway maintenance.	Last job classification of employees of the California Department of Transportation	ICD-8 (1968–78) ICD-9 (1979–83)	Highway maintenance		*PMR*	Age at death, sex, race, year of death
			All cancers		81	1.17 (0.93–1.46)	Proportional mortality for the USA population until 1980 used as reference to estimate standardized PMRs. Analysis for white men only
			Digestive organs		25	1.51 (0.97–2.23)	
			Stomach		6	2.27 (0.83–4.95)	
			Lung		25	0.98 (0.63–1.45)	
			Skin		2	1.22 (0.12–4.39)	
			Prostate		7	2.26 (0.91–4.66)	
			Lymphopoietic (all)		8	1.15 (0.50–2.26)	
Bender *et al.* (1989) Minnesota, USA 1945–84	4849	Highway maintenance workers employed by the Minnesota Department of Transportation for at least 1 yr	Study spanned 5 ICD revisions, transformed to ICD-9 equivalent codes	Highway maintenance		*SMR*	Age at death, year of death White men only; cause of death from death certificates. Expected numbers from white male Minnesota population, divided into urban/rural residence. Stratification by urban/rural may be considered rough adjustment for smoking
			Mouth, pharynx (140–149)		7	0.88 (0.35–1.81)	
			All GI tract (150–159)		90	0.82 (0.66–1.01)	
			Stomach (151)		23	0.91 (0.58–1.37)	
			Intestines (152–153)		30	0.86 (0.58–1.23)	
			Rectum (154)		8	0.66 (0.28–1.30)	
			All respiratory (160–165)		57	0.69 (0.52–0.90)	
			Lung (162)		54	0.69 (0.52–0.90)	
			Bladder (188)		12	1.09 (0.56–1.90)	
			Urinary tract (188–189)		19	0.92 (0.55–1.44)	
			Lymphoreticular (200–208)		34	0.95 (0.66–1.33)	
			Melanoma (172)		0	0 (-)	2.9 expected

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Bender *et al.* (1989) contd				*Highway maintenance* First employment 40–49 yr ago			
			Urinary tract (188–189)		7	2.92 (1.17–6.02)	
				30–39 yr duration			
			Leukaemia (204.0–208.9)		7	4.25 (1.71–8.76)	
Hansen (1989a) Denmark 1970–80	Men only; 1320 unskilled asphalt workers compared with cohort of 43 024 unskilled workers in other trades.	Census data: self-reported occupation and industry at day of census	ICD-8			*SMR*	Age, calendar period 10-yr mortality
			All cancers (140–209)	Mortality 1970–80	37	1.23 (0.87–1.70)	Data from one census
				Mortality 1975–80	29	1.59 (1.06–2.28)	Restricted to occupationally active census population
			Respiratory tract (160–163)	Mortality 1970–80	16	1.43 (0.82–2.32)	
				Mortality 1975–80	11	1.52 (0.76–2.71)	
			Bladder (188)	Mortality 1970–80	5	3.01 (0.98–7.03)	
				Mortality 1975–80	3	2.91 (0.60–8.51)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hansen (1989b) Denmark 1959–84	679 men only	Employment lists of mastic-asphalt plants (n = 400), union files of mastic-asphalt workers (n = 186), benefit society's membership files from one mastic-asphalt plant (n = 93).	ICD-7	Subcohort I (born 1893–1919): likely exposure to coal tar during World War II Subcohort II (born 1920–29): possible exposure to coal tar during World War II Subcohort III (born 1930–60): no exposure to coal tar during World War II			Age, calendar period Ascertainment of incident cases through Danish cancer register. Expected cancer incidence calculated from age-, period-, and site-specific rates in Danish men, 1958–82.
				Age 40–89 yr		*SIR*	
			Mouth (143–144)		2	11.11 (1.35–40.14)	
			Oesophagus (150)		3	6.98 (1.44–20.39)	
			Stomach (151)		4	1.90 (0.52–4.88)	
			Colon (153)		5	1.98 (0.64–4.63)	
			Rectum (154)		7	3.18 (1.28–6.56)	
			Liver (155)		2	4.76 (0.58–17.20)	
			Larynx (161)		3	4.35 (0.90–12.71)	
			Lung (162)		27	3.44 (2.27–5.01)	
			Prostate (177)		4	1.19 (0.32–3.05)	
			Bladder (181)		5	1.55 (0.50–3.61)	
			Skin (191)		3	0.67 (0.14–1.96)	
			Leukaemia		0	0.0 (0.0–4.01)	
			Other		9	1.01 (0.46–1.91)	
			Lung (162)	*Subcohort I*	18	3.02 (1.79–4.77)	Likely exposed to coal tar
			Lung (162)	*Subcohort II*	6	3.92 (1.44–8.54)	Possibly exposed to coal tar
			Lung (162)	*Subcohort III*	3	8.57 (1.77–25.05)	Not likely exposed to coal tar

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hansen (1991) Denmark 1959–86	679 men only	See Hansen (1989b).	ICD-7 and ICD-8	See Hansen (1989b)		*SMR*	Age, calendar period Assessment of vital status through national Danish register, ascertainment of cause of death from death certificates. Expected numbers calculated from national death rates, Danish men, 1960–85.
			Lung (162)		25	2.90 (1.88–4.29)	
			Non-pulmonary		37	2.00 (1.41–2.76)	
Engholm *et al.* (1991) Sweden 1971–85	226 000 construction workers; 2572 road-paving/ asphalt workers; 704 roofers	All workers registered with Bygghälsan, having undergone at least one medical check-up between 1971 and 1979.	ICD version, NR	*Roofers*		*SMR*	Age, calendar period Presumably men. Linkage with Swedish registers: (a) whole living population; (b) deaths; (c) migrants; (d) national cancer registry. Expected numbers calculated from national calendar year, age-, site-specific incidence rates, and from national calendar yr, age-, site- and cause-specific mortality rates. No information on other potentially confounding occupational exposures (coal tar). CI and *P* values NR
			Lung		3	2.79 [0.58–8.16]	
			Stomach		1	2.30 [0.06- 12.81]	
			Lymphatic/ haematopoietic		2	2.68 [0.32–9.68]	
						SIR	
			Lung		4	3.62 [0.99- 9.27]	
			Stomach		1	1.98 [0.05- 11.03]	
			Lymphatic/ haematopoietic		2	1.63 [0.20- 5.89]	
Engholm & Englund (1995) Sweden 1971–88	Same cohort as Engholm *et al.* (1991)	See Engholm *et al.* (1991)	[Probably ICD-8] (ICD version NR)	Asphalt paving workers		*SMR*	Age, calendar period see Engholm *et al.* (1991)
			All cancers (140–209)		42	0.88 (0.63–1.18)	
			Oesophagus (150)		1	0.85 (0.02–4.72)	
			Stomach (151)		6	1.62 (0.60–3.53)	
			Colon (153)		1	0.30 (0.01–1.69)	
			Rectum (154)		0	0.00 (0.00–1.91)	
			Liver (155.0)		1	0.96 (0.02–5.36)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Engholm & Englund (1995) contd			Lung (162.0–162.1)		8	0.79 (0.34–1.55)	
			Malignant melanoma (172)		3	2.08 (0.43–6.09)	
			Prostate (185)		3	0.81 (0.17–2.37)	
			Bladder (188)		0	0.00 (0.00–3.10)	
			Kidney (189)		5	1.98 (0.64–4.61)	
			Leukaemia (204–207)		2	0.98 (0.12–3.54)	
						SIR	
			All cancers (140–209)		72	0.82 (0.64–1.03)	
			Oesophagus (150)		0	0.00 (0.00–3.24)	
			Stomach (151)		8	1.80 (0.78–3.55)	
			Colon (153)		5	0.87 (0.28–2.04)	
			Rectum (154)		0	0.00 (0.00–0.90)	
			Liver (155.0)		1	0.91 (0.02–5.07)	
			Lung (162.0–162.1)		9	0.88 (0.40–1.68)	
			Prostate (185)		12	1.09 (0.56–1.91)	
			Bladder (188)		5	0.81 (0.26–1.90)	
			Kidney (189)		7	1.55 (0.62–3.18)	
			Lymphatic and haematopoietic (200–209)		7	0.77 (0.31–1.58)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hrubec *et al.* (1992) USA 1954–80	248 046	Mailed questionnaire about occupation, industry of employment and tobacco use (84% response). 170 occupational categories with 50+ respondents or 20+ deaths evaluated.	ICD-7	Roofers and slaters		90% CI	Age, calendar period, type and amount of smoking Mortality study of United States veterans of known smoking status, almost all white men. Death ascertainment through life-insurance claims (96% complete for World War I veterans). Internal analysis using all other occupations as reference (RR, 1) for a given occupation, Poisson regression. Of all roofers and slaters (n = 52) 28 had died.
			All cancers		8	1.2 (0.68–2.20)	Smoking; total group
			All cancers		3	3.7 (1.41–9.47)	Nonsmokers
			Stomach		1	3.3 (-)	Smoking; total group
			Rectum		1	4.3 (-)	Smoking; total group
			Respiratory tract		4	3.0 (1.30–6.75)	Smoking; total group
			Prostate		1	5.5 (1.05–28.37)	Nonsmokers
			Lymphoma		1	2.8 (-)	Smoking; total group
			Respiratory tract		4	3.0 (1.30–6.75)	Smoking; total group
Minder & Beer-Porizek (1992) Switzerland 1979–82	1 378 837	Occupation recorded on death certificate	ICD-8			*PMR*	Age Men aged ≥ 30 yr; death certificates from 1979–82. Census records from 1980 provided population at risk. Only statistically elevated or reduced risks reported.
			Mouth, pharynx (140–149)	Roofers	6	3.30 (1.21–7.20)	
Chiazze *et al.* (1993) Ohio, USA 1940–82	162 cases 363 controls	Personal interview linked with data from company documents, exposure assessment committee	Lung (162)	Asphalt fume (mg/m^3)			Place of birth, education, income, smoking status, year of hire, age at first hire, respirable fibres, asbestos, talc, formaldehyde, respirable silica Nested within a cohort of fibreglass manufacturers (industry sponsors).
				0	51	1.0 (ref.)	
				≥ 0.01	111	0.96 (0.65–1.42)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Pukkala (1992, 1995) Finland 1971–85	Economically active population of Finland, aged 35–64 yr	Occupation recorded on census in 1970, coded in 335 categories	ICD-7			*SIR*	Age and calendar period Census file linked to death records and cancer registry. Sex-, age- and period-specific incidence rates in the Finnish economically active population as referents. Additional adjustment for social class as proxy for smoking and other exposures.
			Lung (162.0–162.1)	"Asphalt roofers" (men only)	18	3.50 (2.07–5.53)	None The Working Group was informed that the term "asphalt roofers" given in the publication was wrong and that the risk estimate refers to "asphalt workers" [bitumen workers] in general.
						3.25 (1.92–5.13)	Social class
			Lung (162.0–162.1)	"Road building hands" (men only)	327	1.61 (1.44–1.79)	None
						1.13 (1.01–1.26)	Social class
Milham (1997) WA, USA 1950–89 (men); 1974–89 (women)	588 090 men; 88 071 women	219 and 68 occupational categories in men and women, respectively. Occupation statements abstracted from death records and manually coded until 1986, computer coded since 1987.	ICD-7 (ICD-8 and ICD-9 codes assigned during late observation period backtranslated to ICD-7)	Road graders, pavers, machine operators & excavators (code 425); roofers & slaters (code 514) *Code 425 (paving)*		*PMR*	Age, year of death Proportionate mortality study considering 161 causes of death incl. all WA resident deaths at age ≥ 20 yr. Female PMRs calculated without housewife category (134 569 deaths). Total deaths in white men, 7266
			All cancers (140–205)		1581	1.02	
			Buccal cavity & pharynx (140–148)		34	0.89	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Milham (1997) contd			Oesophagus (150)		32	0.89	
			Stomach (151)		87	1.12	
			Colon (153)		111	0.83	
			Rectum (154)		40	0.98	
			Liver (155)		19	1.26	
			Larynx (161)		18	1.06	
			Lung (162)		558	1.20 ($P < 0.01$)	
			Prostate (177)		161	1.03	
			Bladder (181)		43	0.83	
			Skin (191)		7	0.99	
			Lymphatic and haematopoietic (200–205)		152	1	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Milham (1997) contd				*Code 514 (roofing)*			Total white male deaths, 1057
			All cancers (140–205)		207	0.99	
			Buccal cavity & pharynx (140–148)		9	1.67	
			Oesophagus (150)		4	0.87	
			Stomach (151)		6	0.58	
			Colon (153)		15	0.87	
			Rectum (154)		6	1.11	
			Liver (155)		2	1	
			Larynx (161)		6	2.59 ($P < 0.05$)	
			Lung (162)		86	1.44 ($P < 0.01$)	
			Prostate (177)		12	0.68	
			Bladder (181)		3	0.49	
			Skin (191)		1	1	
			Lymphatic and haematopoietic (200–205)		11	0.47 ($P < 0.01$)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Stern *et al.* (2000) USA	Proportionate mortality analysis of 11 370 male deaths among unionized roofers and waterproofers.		Lung			*PMR*	Race, age, calendar year
				Entire cohort	1071	1.39 (1.31–1.48)	
				Decade of first membership in union:			
				Before 1935		1.41 (1.08–1.80)	
				1935–44		1.70 (1.49–1.93)	
				1945–54		1.39 (1.26–1.53)	
				1955–64		1.42 (1.24–1.62)	
				1965–74		1.53 (1.26–1.85)	
				After 1975		1.69 (1.16–2.39)	
			All malignant neoplasms (140–208)		2691	1.14 (1.10–1.19)	
			Buccal cavity and pharynx (140–149)		72	1.11 (0.87–1.40)	
			Oesophagus (150)		84	1.34 (1.07–1.16)	
			Stomach (151)		103	0.99 (0.81–1.20)	
			Biliary passages, liver, gall bladder (155.0, 155, 156)		53	1.34 (1.00–1.75)	
			Larynx (161)		46	1.45 (1.06–1.93)	
			Trachea, bronchus, lung (162)		1071	1.39 (1.31–1.48)	
			Bone (170)		15	1.64 (0.92–2.70)	
			Skin (172, 173)		33	0.69 (0.48–0.97)	
			Prostate (185)		181	0.91 (0.78–1.05)	
			Testis (186)		17	1.30 (0.76–2.08)	
			Bladder (188, 189.3–189.9)		89	1.38 (1.11–1.70)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Stern *et al.* (2000) USA contd			Kidney (189.0–189.2)		50	0.90 (0.67–1.19)	
			Other and unspecified sites (194–199)		195	1.30 (1.12–1.49)	
			Hodgkin disease (201)		19	0.82 (0.50–1.29)	
			Leukaemia (204–208)		79	0.85 (0.67–1.06)	

Bygghälsan, Swedish Construction Industry's Organization for Working Environment, Safety and Health; CI, confidence interval; CSP, Cancer Surveillance Program; GI, gastrointestinal; incl., including; IRR, incidence rate ratio; NR, not reported; NS, not statistically significant; PMR, proportional mortality ratio; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; yr, year; WA, Washington state

assess occupation- and industry-specific risks of cancer of the lung. Their database consisted of 2161 death certificates mentioning cancer of the lung, bronchus or trachea for 1968–1970, and 1777 incident cases that were reported to the Los Angeles County Cancer Surveillance Program (CSP) between 1972 and 1973. In addition, pathology records from nearby out-of-county hospitals were searched for inclusion of residents of Los Angeles County who were patients with cancer of the lung. California death certificates included the subject's last occupation, name of last employing firm and type of industry. CSP demographic data included the last occupation and industry of employment that the patient or the next-of-kin reported at admission to hospital. Of all 3938 cases of cancer of the lung, 689 had no reported occupation and 1222 had no reported industry of employment. The 1970 United States Census Industry and Occupation Classification System was used to code the occupation into one of 417 categories and the industry into one of 215 categories. The population at risk by industry and occupation was estimated from two Public Use Samples of the 1970 census, including respondent information on current occupation and industry for 31 216 white males, aged 20–64 years, providing a 2% sample of the corresponding population of Los Angeles county. No data were reported for road pavers. On this basis, the number of expected deaths and incident cases was calculated for each specific occupation. The population at risk was estimated to be 1 560 800, including 2000 roofers. Based on six roofers who died from lung cancer between 1968 and 1970, and five incident cases of lung cancer among roofers reported to the CSP between 1972 and 1973, an almost fivefold risk of lung cancer among roofers was estimated (SMR, 4.96; $P < 0.05$). Cancer risks in road-construction workers were not reported. [The Working Group could not evaluate whether the observed risk may be explained in part by exposure to coal-tar products or asbestos and possible confounding by smoking. The Working Group noted that the population at risk was estimated from a sample of the census and that occupational data were compiled from different sources for the exposed and unexposed populations.]

In 1988, Povarov *et al.* reported the analysis of cancer incidence between 1974 and 1984 in a cohort of male workers employed for at least 3 years during the same period in one of 11 hot-asphalt production plants ("asphalt concrete production") in Estonia. Concentrations of benzo[*a*]pyrene assessed by filtered samples of airborne dust varied between 0.2 and 0.7 μg/100 m^3 in major work areas based on two to three air samples in each of six work areas. The concentration of benzo[*a*]pyrene ranged from 2 to 21 μg/kg in sedimented dust. The cohort comprised 10 369 person-years of observation. The total Estonian population for 1979–82 served as the reference. Cancer cases were identified through the oncological centre of Estonia, which serves as the basis for the Estonian cancer registry. [The Working Group noted that the incidence rates were age-adjusted, but the method for comparison of incidence rates within the cohort to the reference population was not described with clear detail.] Overall, 51 incident cases of cancer were observed, of which 17 were cancer of the lung. The overall incidence of cancer between age 20 and 74 years was reported to be 471.0/100 000 in the cohort as compared with 309.1 in the reference population. The incidence of cancer of the lung in the age group 20–74 years (155.1/100 000) was 1.5 times higher than in the general population (100.8/100 000). [The Working Group noted that in the original manuscript the authors stated that this difference was not statistically significant, but reported a P value of < 0.05. No explanation was given for this discrepancy.] A statistically significant difference for lung cancer was observed in the age group 40–64 years (305.6/100 000 *versus* 144.4/100 000). The age at onset of lung cancer was lower in the cohort than in the general population. Only 44% of cases of cancer of the lung

in the cohort had worked for more than 5 years in the industry. The incidence of cancer of the stomach (7 men) was 77.9/100 000 in the cohort as compared with 69.7/100 000 in the Estonian population. The incidence of skin cancer in the cohort (35/100 000; 4 men) did not differ significantly from that in the general population (23.8/100 000). [The measurement data suggested that there was low exposure to benzo[*a*]pyrene, indicating that coal tar was not a major potential confounder in this cohort. No data on tobacco smoking were reported.]

Maizlish *et al.* (1988) conducted a proportional mortality study among California highway workers who had been employed by the California Department of Transportation (CalTRANS) and died in California between 1 January 1970 and 31 December 1983. Employees included maintenance workers, materials laboratory technicians, engineers, administrators, office workers and secretaries. Exposure to asphalt occurred during maintenance operations, including: paving, crack and joint filling, surface sealing, and repair of concrete roadways using asphalt. Exposure to coal tar was mentioned for surface sealing only. Vital status and cause of death were determined by computerized linkage of the CalTRANS files with the State California death certificate registry. Standardized proportional mortality ratios (PMRs) were calculated in strata of age at death, sex, race and year of death, using the proportional mortalities for the population of the USA through 1980 as the reference. Among men working in highway maintenance, the PMR for all cancers combined was 1.17 (95% CI, 0.93–1.46) based on 81 deaths. The PMR for lung cancer was not elevated (25 deaths; PMR, 0.98; 95% CI, 0.63–1.45). [The Working Group thought this study was not very informative because the confidence intervals were wide, risk estimates may have been confounded by coal tar, and there was no information on smoking.]

Bender *et al.* (1989) conducted a mortality study of 4849 male highway maintenance workers employed for a minimum of 1 year by the Minnesota Department of Transportation (MNDOT) between 1945 and 1984. Work histories were abstracted from personnel records to accommodate discontinuous work histories. No specific exposure data were collected. Expected numbers of deaths were obtained from the mortality experience of all white male Minnesota residents. Patterns of mortality were analysed for urban and rural populations and stratified by duration of employment and time since a person first started work. There were 96 567 person-years of follow-up and 1676 deaths were observed. The overall standardized mortality ratio of 0.91 (95% CI, 0.86–0.96) was due to deficits in heart disease (SMR, 0.93), cerebrovascular disease (SMR, 0.80) and cancer (274 deaths; SMR, 0.83; 95% CI, 0.73–0.94). Rates of cancer of the lung (54 deaths; SMR, 0.69; 95% CI, 0.52–0.90) and of the urinary tract (19 deaths; SMR, 0.92; 95% CI, 0.55–1.44) were not elevated. There were no clear trends in standardized mortality ratio for cancer of the urinary tract with time since first employment or length of employment overall, but an excess of cancers of the urinary tract was observed for workers who were first employed more than 40–49 years previously (seven deaths; SMR, 2.92; 95% CI, 1.17–6.02). The standardized mortality ratio for cancer of the lung did not increase with length of work or year started and did not differ between rural and urban dwellers. [The Working Group noted that this study was informative because it was an industry-based cohort of maintenance workers. However, this study was limited in that the presentation of the results appeared to be selective. The tables did not provide a comprehensive overview of risk in relation to start and duration of employment. It was unclear whether road pavers were included in the study. The Working Group noted that there was no information on smoking, but that no overall excess of lung cancer was observed in this cohort.]

Hansen (1989a) conducted a historical cohort study of men aged 15–74 years identified from the Danish census in 1970. A cohort of 1320 unskilled workers employed in the bitumen industry was selected on the basis of self-reported occupation and industry on the day of census. [The Working Group noted that this cohort may have included an unknown number of pavers.] These men, who had been employed at asphalt plants, roofing-felt plants or one tar plant, were followed for mortality until 1980 and compared with 43 024 men who reported having worked as unskilled workers in other industries, mainly agriculture and forestry. The only exposure information used to classify the cohort members was self-reported occupation at the day of census. Cause of death was ascertained from the Danish Death Certificate Register through an automatic record-linkage system. Mortality ratios standardized for age and calendar period were calculated for men aged 45 years or more. Cancer mortality was elevated overall, in particular for the second half of the observation period. No healthy-worker effect was observed: in total, 104 deaths were observed in men aged 45 years or more (SMR, 1.02; 95% CI, 0.80–1.31), 74 of them occurring in the last 5 years of the observation period (SMR, 1.16; 95% CI, 0.91–1.46). Rates were elevated for respiratory (SMR, 1.43; 95% CI, 0.82–2.32) and bladder cancer (SMR, 3.01; 95% CI, 0.98–7.03). In an analysis of the last 5 years of the observation period, there were elevated risks for cancer of the digestive system, based on six cases (ICD-8 150–159; SMR, 1.57; 95% CI, 0.58–3.43) and for cancer of the brain, based on three cases (SMR, 5.00; 95% CI, 1.03–14.61). [The Working Group noted that this was an indication of latency and a possible indicator of duration.] In addition to the reference cohort of 43 024 unskilled workers from other trades, mortality in the exposed cohort was compared with that in the total economically active, Danish census cohort, which gave similar standardized mortality ratios for cancer and total mortality. [The Working Group noted that a proportion of cohort members were probably exposed to coal-tar products, as workers from a tar plant were included in the exposed group. There were no data on smoking. This study may have overlapped with the IARC multicentre cohort study (see Section 2.1.2).]

In a historical cohort study, Hansen (1989b) analysed the incidence of cancer in 679 male mastic-asphalt workers between 1959 and 1984. Study subjects were identified through historical files covering the period 1959 to 1980 from various sources. Employment lists of four mastic-asphalt companies provided 400 workers; membership files of an organized group of mastic-asphalt workers within the National Union of General Workers provided another 186 men; and 93 subjects were identified from the membership files of a benefit society organized by the workers at one of the mastic-asphalt plants. A subject was enrolled into the study when first identified in one of these historical files. The cohort accumulated a total of 6692 person-years at risk. Incident cases of cancer were identified through linkage with the Danish Cancer Register. No individual exposure assessments were obtained for members of the cohort, but industrial hygiene data were collected by personal samplers during flooring. These data indicated that the median concentration of asphalt fume condensate was about four times higher than the TWA of 5 mg/m^3. Median PAH concentrations were reported to equal 0.183 mg/m^3 for total PAHs (mean, 0.195 mg/m^3) and 0.004 mg/m^3 for benzo[*a*]pyrene (mean, 0.0058 mg/m^3). Concentrations during manual road paving were lower. Hansen (1989b) stated that coal-tar products were not added to the mastic-asphalt mixture except during the Second World War, when a shortage of bitumen initiated the use of coal-tar pitch in the production of asphalt mixes. The author estimated that road paving had made up about two thirds and flooring operations about one third of the working hours of the cohort. In total, 75 new cases of cancer were observed, almost twice as many as expected in

the total male Danish population (SIR, 1.95; 95% CI, 1.53–2.44). The study cohort included men born between 1893 and 1960. As the older men had probably been exposed to tar-containing asphalt mixes during the Second World War, the cohort was divided into three subcohorts: subcohort I (born 1893–1919) with likely exposure to coal tar; subcohort II (born 1920–29) with possible exposure to coal tar; and subcohort III (born 1930–60) not exposed to coal tar. While the overall standardized incidence ratio of lung cancer among men aged 40–89 years was 3.44 (95% CI, 2.27–5.01; 27 cases), the stratified analysis of the incidence of cancer of the lung in the three subcohorts indicated higher risks in the younger cohorts, although based on only three deaths in subcohort III (SMR, 8.57; 95% CI, 1.77–25.05). The extent to which the observed excess of lung cancer could be explained by confounding by smoking was investigated using data from a survey of mastic-asphalt workers, which showed that 22% were non-smokers, 36% were medium smokers and 43% were heavy smokers in 1976, compared with 39%, 24% and 38%, respectively, in the general population of the same age in 1982. It was estimated that these differences in prevalence explained at most a 20% excess of cases of cancer of the lung in the cohort under study. [The Working Group noted that studies among mastic-asphalt workers may be particularly informative in the evaluation of the carcinogenic risk of bitumen fume, since mastic-asphalt work usually entails higher concentrations of fume due to the higher temperature of the asphalt mix compared with asphalt mixes for road paving. Consequently the composition of the fume in mastic-asphalt application differs from the composition of asphalt fume in road paving (see Section 1). Frequent manual working procedures, such as hand floating, and application within buildings such as multistory car parks may further increase exposure. Compared with the other cohort studies, the type of exposure was much more specific and homogeneous.]

The cohort presented in Hansen (1989b) was also followed for mortality until 1986 (Hansen, 1991). This analysis revealed an excess mortality for cancer (SMR, 2.29; 95% CI, 1.75–2.93) based on 62 cases. No healthy-worker effect was observed. Total mortality was elevated (169 deaths; SMR, 1.63; 95% CI, 1.41–1.90), while mortality due to cardiovascular diseases was about equal to that in the male Danish population (48 deaths; SMR, 1.00; 95% CI, 0.74–1.32). Among men aged 40–89 years at death, elevated standardized mortality ratios were observed for lung cancer (SMR, 2.90; 95% CI, 1.88–4.29), non-pulmonary cancer (SMR, 2.00; 95% CI, 1.41–2.76), liver cirrhosis (SMR, 4.67; 95% CI, 1.88–9.62), respiratory diseases (SMR for bronchitis, emphysema, asthma combined, 2.07; 95% CI, 0.95–3.93). Hansen discussed the potential confounding effects of smoking and urban residence; almost all asphalt workers lived in cities as opposed to 40% of the comparison population. Mortality due to the causes of death considered in this study was generally between 5% and 20% higher in urban municipalities in Denmark (137% higher for liver cirrhosis). Using correction factors for urbanization and smoking, Hansen (1991) calculated corrected standardized mortality ratios in a sensitivity analysis. Simultaneous correction for the difference in prevalence of smoking and urban residence gave a standardized mortality ratio of 2.24 for lung cancer in men aged 40–89 years (95% CI, 1.45–3.30). [This sensitivity analysis is an advantage in situations where individual-level smoking adjustment is not possible. However, there is some concern that the author's group-level correction for both smoking and urbanization may result in an over-adjusted estimate of the SMR for lung cancer.]

Wong *et al.* (1992) raised several concerns regarding the studies by Hansen, including: a possible "unhealthy" worker effect; inappropriate adjustment for smoking and urban residence; and possible confounding by exposure to coal-tar products used until 1975 in the Danish asphalt

industry. In response, Hansen (1992) presented further sensitivity analyses using more extreme correction factors to adjust for the confounding effect of smoking and concluded that even in the most extreme case not more than a 21% excess of lung cancer could be explained by differences in smoking habits. The possibility that exposure to coal tar after World War II could explain the observed excess was refuted by Hansen (1992) for three reasons: (1) the Danish asphalt industry always had denied the use of coal-tar products in mastic-asphalt work until the paper by Hansen appeared in 1989; (2) the risk of cancer of the lung observed in the studies by Hansen would require very high levels of exposure to tar, similar to those observed in British gas works until World War II; and (3) no excess risk of skin cancer –considered by Wong *et al.* as a marker of exposure to coal tar –was observed in the Danish mastic-asphalt workers cohort (SMR, 0.78; 95% CI, 0.21–1.99) (Hansen, 1992).

Engholm *et al.* (1991) assessed cancer incidence and mortality in workers registered with the Swedish construction industry's Organization for Working Environment, Safety and Health (Bygghälsan cohort). [The Working Group noted that this cohort was included in the IARC multicentre cohort study (see Section 2.1.2).] The cohort included all workers undergoing at least one medical check-up between 1971 and 1979. These workers were followed until 1984 for cancer incidence and until 1985 for cancer mortality by linkage with Swedish cancer and mortality registries. The average duration of follow-up was 11.5 years and the median age of workers was 42 years. The authors did not mention whether women were included in the cohort. The expected number of cases and of deaths was calculated on the basis of national calendar year, age, site-specific incidence rates, and national calendar year, age, site- and cause-specific mortality rates, respectively. A strong healthy-worker effect was observed, which diminished after 10–12 years of follow-up when the overall standardized mortality ratio was still < 1 (0.80). Based on a small number of cases, the authors observed in roofers an excess of cancer of the stomach (SMR, 2.30; SIR, 1.98), cancer of the lung (SMR, 2.79; SIR, 3.62), and of haematopoietic and lymphatic cancers (SMR, 2.68; SIR, 1.63). Confidence intervals and *P* values were not reported. As the information recorded during medical check-ups included current and previous smoking status, the authors conducted a nested case–control study of the cases of lung cancer with five controls individually matched for year of and age at first check-up. The relative risk of cancer of the lung among roofers, adjusted for smoking and population density of parish of residence, was in the order of six. [The Working Group noted that the adjustment for smoking was a strength of the study; however, the methods for adjustment for smoking habits were not described to usual standards and no confidence intervals were reported. Results for road pavers are not presented here because updated estimates were presented in a subsequent publication (Engholm & Englund, 1995).]

An extended follow-up of the Bygghälsan cohort until 1987 (incidence) and 1988 (mortality) was published by Engholm & Englund (1995). They reported non-statistically significantly elevated risks in asphalt-paving workers for cancer of the stomach (SMR, 1.62; 95% CI, 0.60–3.53; SIR, 1.80; 95% CI, 0.78–3.55) and kidney (SMR, 1.98; 95% CI, 0.64–4.61; SIR, 1.55; 95% CI, 0.62–3.18), but not for cancer of the lung (SMR, 0.79; 95% CI, 0.34–1.55; SIR, 0.88; 95% CI, 0.40–1.68).

Hrubec *et al.* (1992) published a mortality study of United States veterans of known smoking status. Of about 300 000 veterans who had served between 1917 and 1940, 248 046 responded to the mailed questionnaire on smoking. These accumulated 4 530 604 person-years and 164 785 deaths. Relative risks were calculated in an internal analysis by Poisson regression using all other occupations than the one in question as the reference group. Risk estimates were

adjusted for age, time period and smoking (type and amount smoked), and in addition stratified by smoking status at the time of questionnaire (1954–57). Among roofers and slaters, the overall mortality was reduced (smoking-adjusted relative risk, 0.8; [90% CI, 0.58–1.08]; 28 deaths). The healthy-worker effect was more pronounced for all cardiovascular diseases (smoking-adjusted relative risk, 0.6; 90% CI, 0.35–0.91; 12 deaths) and coronary heart disease (smoking-adjusted relative risk, 0.7; 90% CI, 0.39–1.18; nine deaths). Mortality from cancer overall was slightly elevated. Mortality from respiratory cancer was significantly elevated after adjustment for smoking, based on four deaths (RR, 3.0; 95% CI, 1.30–6.75). No cancers of the urinary tract were observed. [The Working Group had doubts that this study was informative regarding bitumen-associated risks of cancer because the proportion of workers potentially exposed to bitumen within the group including roofers may have been small. The information on smoking was a strength of the study. Although there were few deaths, excess risks remained after adjustment for smoking. However, even if a substantial proportion of the group were exposed to bitumen, there was presumably also possible exposure to coal tar and/or asbestos.]

Minder & Beer-Porizek (1992) published a study of mortality in Switzerland in which they screened all occupational groups for excess and deficit mortality from cancer. The database consisted of all death certificates of Swiss men aged 30 years or above who died between 1979 and 1982. The population at risk was obtained from census records for the year 1980. The expected number of deaths was calculated on the basis of these census records, which recorded the occupation of Swiss residents. For most occupations, both the standardized mortality ratios and the standardized proportional mortality ratios were estimated. Only those ratios that significantly differed from unity were reported. For roofers, an elevated proportional mortality ratio for tumours of the mouth and pharynx was observed (PMR, 3.30; 95% CI, 1.21–7.20; six deaths). [The Working Group noted that occupational data were compiled from different sources for the exposed and unexposed populations. There was also no adjustment for alcohol consumption, so it was possible that confounding by alcoholic beverages contributed to the elevated risk of cancer of the mouth and pharynx.]

Pukkala (1992) calculated standardized incidence ratios by social class and occupational group for Finland using the main occupation reported on the 1971 census. In 1995 an update of the original Finnish report was published in English (Pukkala, 1995). The classification of occupations was based on the Nordic Classification of Occupations from 1963 and the International Classification of Occupations published by the International Labour Office in 1958, allowing for 335 specific categories, of which 332 and 324 included men and women, respectively. Subjects were assigned to one of four social classes on the basis of their education, occupation, industrial status and industry socioeconomic grouping. The Population Census file was linked with the annual death files to obtain death certificate information and with the Finnish Cancer Registry to assess cancer incidence. Standardized incidence ratios were calculated using sex-, age- and period-specific incidence rates in the reference population, which was restricted to the economically active population of Finland. Standardized incidence ratios were also adjusted for social class. The cohort was restricted to economically active residents aged 35 to 64 years at the beginning of each follow-up period (1971–75, 1976–80, 1980–85). Overall, the study included 47 178 cases of cancer in men and 46 853 cases in women, of which 15 613 and 1868 were cancers of the lung in men and women, respectively. The social-class pattern of cancer of the lung parallels the corresponding pattern of smoking. Even after adjustment for social class, the risk of cancer of the lung in roofers was substantially elevated

(SIR, 3.25; 95% CI, 1.92–5.13; 18 deaths) and a slightly elevated risk remained in road-building hands [unskilled workers] (SIR, 1.13; 95% CI, 1.01–1.26; 327 deaths). Among women, standardized incidence ratios were reported for none of these occupational categories, either because the expected number of cases was < 5, or because the standardized incidence ratio did not differ statistically significantly from 1 ($P \geq 0.05$). [The Working Group noted that this cohort partially overlapped with the IARC multicentre cohort study (see Section 2.1.2). This study was included here because it adjusted for social class, which may have partly removed the confounding effect of smoking and because the reference population was restricted to the economically active population. The Working Group was aware that potential exposure to wood tar may have occurred in the Finnish cohort. It was unclear what proportion of road-building workers was exposed to bitumens. During the Meeting, information was forwarded to the Working Group from the author of the study that the category labelled as "asphalt roofers" in this publication actually may have referred to "asphalt workers" in general. Thus, the Working Group did not consider it informative for evaluating roofers. The adjustment, the restriction to the economically active reference population, and the good quality and completeness of cancer registration in Finland were considered as strengths of the study.]

Chiazze *et al.* (1993) carried out a nested case–control study of cancer of the lung among employees at a fibreglass manufacturing plant operated by Owens-Corning Fibreglass Corporation in Ohio, USA. The plant environment was described as "very complex", with little information provided about products or production processes. Cases (n = 162) and controls (n = 363) were drawn from a previously enumerated cohort of workers who were employed for at least 1 year between 1940 and 1962 and followed until the end of 1982. Cases and controls were matched on birth year and follow-up time. Information on demographics, occupational and residential history, smoking, hobbies and medical history was obtained by interview. Historical exposures to respirable fibres, TPM, asbestos, talc, formaldehyde, silica and bitumen fume were assessed by an expert committee, which estimated ranges of exposure and assigned numerical scores to the midpoints of one or more categories for each substance. The odds ratio for cancer of the lung among workers with any exposure to bitumen fume was 0.96 with only the matching variables and 1.13 (95% CI, 0.47–2.73) after adjustment for smoking, education, age and year at hire and other exposures. [The Working Group noted that this article provided relatively little information on how the exposure assessment of asphalt fumes was performed, despite an apparently lengthy process involving a wide range of historical documents. The extent to which quantitative data on industrial hygiene were used in the exposure assessment was not clear.]

Milham (1997) analysed the occupational proportionate mortality of residents of Washington State between 1950 and 1989 using occupation and industry data recorded on death records. In women, no deaths were observed in road graders, while six deaths were recorded in roofers. In white men, a significantly elevated risk of cancer of the lung was observed in roofers and slaters (PMR, 1.44; 86 deaths) and in the group of road graders, pavers, machine operators and excavators (PMR, 1.20; 558 deaths). In roofers and slaters, the standardized proportional mortality ratio for cancer of the larynx was also statistically significantly elevated (PMR, 2.59; six deaths). [The Working Group considered the occupational group of road graders, pavers, machine operators and excavators relatively unspecific for exposure to bitumen.]

Stern *et al.* (2000) carried out a proportionate mortality analysis of unionized roofers and waterproofers in the USA. A total of 11 144 men was available for analysis. Standardized

proportional mortality ratios were adjusted for race, age and calendar year. The standardized proportional mortality ratio for cancer of the lung was 1.39 (95% CI, 1.31–1.48; 1071 deaths). Other cancer sites with an excess of cancer risk were: larynx (PMR, 1.45; 95% CI, 1.06–1.93; 46 deaths); urinary bladder (PMR, 1.38; 95% CI, 1.11–1.70; 89 deaths); and oesophagus (PMR, 1.34; 95% CI, 1.07–1.66; 84 deaths). According to a survey among roofers in 1982 and 1983, the prevalence of exposures to various agents was estimated to be as follows: bitumen, 71.6%; asbestos, 47.9%; quartz, 26.4%; and coal-tar pitch, 13.4%. [The Working Group noted that exposure to asbestos, silica dust and coal-tar products may have contributed to the observed excess risks. No data on tobacco or alcohol consumption were available for cohort members.]

2.3 Case–control studies

2.3.1 Cancer of the lung

See Table 2.3

(a) Population- and hospital-based studies

A population-based case–control study by Schoenberg *et al.* (1987) examined associations between cancer of the lung and occupation in New Jersey, USA. The cases (n = 763) were white male residents of the study area, newly diagnosed with cancer of the lung between September 1980 and October 1981. The controls (n = 900) were sampled at random from drivers' licence records for living cases or death-certificate registers for deceased cases. Cases and controls were matched on age, race, area of residence, and date of death, if deceased. Subjects or their next-of-kin were interviewed in person to obtain information on occupational history and other risk factors. Additional questions were asked about specific occupational exposures, but exposure to bitumens or bitumen fume was not specifically assessed. A smoking-adjusted odds ratio of 1.7 (95% CI, 0.68–4.4) was reported for the occupational category of roofers and slaters. No data were reported for other occupation or industry categories with known exposures to bitumens. Employment duration and time since employment were examined, but no data were reported for roofers. [The main limitation of this study was the use of job titles as a surrogate indicator of exposure.]

Vineis *et al.* (1988) pooled data from five case–control studies of cancer of the lung conducted during the 1970s and 1980s in the states of Louisiana, Florida, Pennsylvania, Virginia, and New Jersey, USA. [The Working Group noted that this study included the study by Schoenberg *et al.* (1987), described above.] Information was collected on place and type of work and duration of employment for occupations held for 6 months or more. The pooled data set included 2973 cases in men and 3210 controls. Odds ratios adjusted for age, birth year and smoking were calculated for occupations and occupational exposures that were established or suspected to be associated with cancer. Jobs or exposures not classified as carcinogenic, probably carcinogenic, or possibly carcinogenic, were classified as unexposed. Risk from exposure to bitumen was not specifically assessed, but a smoking-adjusted odds ratio of 1.4 (95% CI, 0.9–2.3) was reported for employment as roofers or asphalt workers. [The Working Group noted that much of the exposure information was reported by next-of-kin.]

Zahm *et al.* (1989) studied associations of smoking and occupation with cancer of the lung of several histological types using data from a cancer registry in Missouri, USA. The cases were diagnosed with cancer of the lung between 1980 and 1985, and controls had diagnoses of other, non-smoking related cancers during the same period. All participants were white men. Occupation at the time of diagnosis and smoking information were abstracted from medical records. Odds ratios were adjusted for age and smoking. The odds ratio for the association of all

Table 2.3 Case–control studies of cancer of the lung and exposure to bitumens

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Schoenberg *et al.* (1987) New Jersey, USA, 1980–81	763 (334 next-of-kin)	900 (336 next-of-kin)	Population (drivers' license files; mortality files)	Face-to-face interview including detailed job history and questions on solvents, fumes, dust and asbestos. 42 job titles and 34 industry categories	Lung (162)	Roofers, slaters	13	1.7 (0.68–4.4)	Smoking (four strata) Response rate: cases, 70.4%; controls, 63.6%. Frequency-matched on race, age, area of residence and data on death (deceased controls). Possible confounding by asbestos and coal tar.
Vineis *et al.* (1988) Five areas in USA 1976–83	2973 men	3210	Hospital and death certificates	Interview (some with next-of-kin), coding of jobs for known and suspected carcinogens	Lung	Roofers or asphalt workers	45	1.4 (0.9–2.3)	Overlaps with Schoenberg *et al.* (1987)
Zahm *et al.* (1989) Missouri USA 1980–85	4431 men	11 326	Cancer registry	Job title from registry	Lung	Pavers, surfacers and material movers Roofers	32 6	0.9 (0.6–1.5) 2.1 (0.6–8.2)	Age, smoking White men only
Morabia *et al.* (1992) Nine areas, USA 1980–89	1793 men	3228	Hospital	Personal interview, checklist of 44 agents, usual occupation, ever employed	Lung (162)	Roofers, slaters	7	2.1 (0.7–6.2)	Age, race, smoking, region, questionnaire version Men only. Response rates not reported. Controls: any non-lung cancer patient; matched on race, age, hospital and smoking. Possible confounding by asbestos and coal tar.

Table 2.3 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Jöckel *et al.* (1992) Western Germany Recruitment period not stated	194 men and women	388	Hospital and population	Job title, JEM and expert assessment	Lung	Road-construction workers	29	2.6 (1.38–4.99)	Age, sex, smoking Hospital and population controls combined for analysis. OR for roofers said to be "not high" but not shown. OR = 1.4 for highest level of PAH exposure
Jöckel *et al.* (1998) Western Germany 1988–93	1004 men and women	1004	Population	Job title, agent checklist, job-specific questionnaire and list of "risk occupations"	Lung	Road-construction, pipe-laying, well-digging and unskilled construction	155	1.02 (0.76–1.36)	Region, sex, age, smoking, asbestos
Brüske-Hohlfeld *et al.* (2000) Germany 1988–96	3498 men	3541	Population	Job title, checklist, job-specific questionnaire, expert assessment of BaP	Lung	Road construction workers and pipe-layers	492	1.2 (1.0–1.5)	Age, region, smoking, asbestos Includes cases and controls from Jöckel *et al.* (1998), men only
Bovenzi *et al.* (1993) Northern Italy 1979–86	756 men	756	Autopsy records	Job title from next-of-kin interview, expert assessment	Lung	Asphalt workers	7	2.3 (0.5–10.3)	Age, death, year, smoking All cases and controls deceased.

Table 2.3 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Watkins *et al.* (2002) USA, 28 plants 1977–97	39	133	Employer records, matched on age, birth year, race	Expert, committee, using historical records	Lung	Ever exposed to bitumen fume	12	1.6 (0.6–4.6)	Matching variables only All cases deceased. Controls deceased or retired. Exposures imputed for year before 1977; no coal tar after 1977; unclear if controls arose from the same study base as cases Sensitivity analyses reflected in different scenarios: *Scenario* 1 based on rate of decline; *Scenario* 2 assumed doubling of exposure
						< 20 yr	7	2.3 (0.7–7.7)	
						≥ 20 yr	5	1.1 (0.3–3.7)	
						Bitumen fume mg/m^3-day			
						Scenario 1	1	2.0 (0.1–17.5)	
						> 0– < 1 000			
						1000–9999	7	1.9 (0.6–6.4)	
						≥ 10 000	4	1.3 (0.4–4.1)	
						Scenario 2			
						> 0– < 1 000	2	2.8 (0.4–18.4)	
						1000–9999	5	1.1 (0.3–4.0)	
						≥ 10 000	5	1.8 (0.6–6.0)	
Richiardi *et al.* (2004) Northern Italy 1990–92	1171 men and woamen	1553	Population	Job title from interview	Lung	Roofers, asphalt workers, insulators and pipe-coverers	9	2.0 (0.6–6.5)	Sex, age, region, smoking, number of jobs Broad definition of exposed group. Confounding by asbestos likely.

Table 2.3 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
McClean *et al.* (2011) USA 1998–2003	422	894	African American and Latin American men and women in San Francisco Bay area	Interview, self-assessed exposure to 21 substances	Lung	Ever exposed to tar and asphalt Cumulative exposure to asphalt and tar	32	1.2 (0.7–2.1) 1.11 (1.01–1.22)	Age, calendar period, race, smoking habits, asbestos, automobile exhaust Broad definition of exposure combining tar and asphalt; OR per exposure year

BaP; benzo[*a*]pyrene; d, day; OR, odds ratio; PAH, polyaromatic hydrocarbon; RR, relative risk; yr, year

types of cancer of the lung with the combined occupational category of pavers, surfacers and material-moving operators was 0.9 (95% CI, 0.6–1.5), based on 32 cases and 64 controls. For roofers, the odds ratio was 2.1 (95% CI, 0.6–8.2), based on six exposed cases and seven controls, and this effect did not differ significantly by histological subtype. Data were not reported for other occupations with known exposure to bitumen. The numbers of subjects with specific combinations of cell type and occupation were small, and no odds ratio for any combination was significantly elevated. [Only white men were included and the use of other cases of cancer as controls may have lead to bias if those cancers were associated (positively or negatively) with exposure. The main limitations of this study were the use of job titles alone as indicators of exposures and the reporting of results for selected occupations only.]

Morabia *et al.* (1992) conducted a case–control study of cancer of the lung and occupation in 24 cities across the USA between 1980 and 1989. The cases were 1793 men with diagnoses of cancer of the lung while controls were 3228 men with other diagnoses, who were matched to cases on age, race, hospital, and smoking history. Information was obtained by interview on smoking, usual occupation and exposure to 44 specific substances on the job or in a hobby. A complete list of the 44 substances was not provided, however, and results were shown only for asbestos and coal dust. Odds ratios were adjusted for the matching factors. An adjusted odds ratio of 2.1 (95% CI, 0.7–6.2) was reported for roofers and slaters. Data were not reported for other occupations known to involve exposure to bitumen or bitumen fume. [The utility of this study was limited by the selective reporting of results for certain occupations and substances.]

Occupational and environmental risk factors for cancer of the lung were analysed by Jöckel *et al.* (1992) in a case–control study in an industrial area of western Germany. Cases were 194 men and women with incident histologically confirmed cancer of the lung recruited from seven hospitals in five cities. Each case was matched by age and sex to one hospital control with a non-smoking related diagnosis and one control selected at random from population registries. Information including job history, occupational exposure and smoking was obtained by structured interview. Occupational exposures were assessed by job-specific questionnaires or responses to a checklist of specific exposures. Exposures to substances regarded as known lung carcinogens, including soot and tars, were assigned numerical weights. Road-construction workers and roofers were given a supplemental questionnaire due to their potential exposure to PAHs. The hospital and population controls were combined for analysis. In analyses restricted to men, the smoking-adjusted odds ratio for road-construction workers was 2.6 (95% CI, 1.38–4.99), while the odds ratio for roofers was reported not to be high, but was not shown. Semiquantitative scores for exposure to PAHs, including fume, coal tar and coal tar products, were not related to risk of cancer of the lung: only the odds ratio for the highest category was greater than unity (OR, 1.40; 95% CI, 0.48–4.20) and a test for trend was not statistically significant. No results were reported for other specific exposures or for exposed occupations among women because of small numbers. [The exposure-assessment approach used in this study was an improvement over the use of job titles alone in earlier studies, but the focus was on PAHs in general, exposure to bitumens were not distinguished specifically, and an effect of coal tar could not be ruled out. There was also some selective reporting, with data not shown for all relevant occupations.]

A subsequent case–control study in the same part of Germany also evaluated occupational risk factors for cancer of the lung (Jöckel *et al.*, 1998). A total of 1004 incident cases of cancer of the lung were recruited between 1988 and 1993 from hospitals; controls matched by region, age

and sex were selected from population registers. Data were collected by interview with similar rates of response (68–69%) for cases and controls. Occupational exposures were assessed by job history, exposure checklist and job-specific questionnaire, including specific questionnaires for roofing and road construction. A subset of occupations including road construction was designated "risk occupations" using lists of jobs with known and suspected exposures (Simonato & Saracci, 1988). Results were tabulated only for men. An odds ratio of 1.02 (95% CI, 0.76–1.36), adjusted for asbestos and smoking, was reported for the occupational category including road construction, pipe-laying, well-digging and unskilled construction work. Odds ratios were not reported for roofing or for exposure to bitumens. [While this study used improved methods of exposure assessment relative to job titles alone, it contributed relatively little to the assessment of bitumen because only results for a single broad job category with potential exposure were reported. The Working Group noted that this study did not replicate the results of the earlier study (Jöckel *et al.*, 1992), which used similar methods.]

Data from the preceding study and another case–control study covering a larger area in Germany were pooled in a study by Brüske-Hohlfeld *et al.* (2000). The pooled study included 3498 cases of cancer of the lung and 3541 matched population controls. Only men were included. The exposure assessment was similar to that used in Jöckel *et al.* (1998), with the addition of a measure of cumulative exposure to benzo[*a*] pyrene that incorporated external exposure data via expert assessment. Matched odds ratios were estimated by conditional logistic regression with adjustment for smoking and exposure to asbestos. Men ever employed as road-construction workers or pipe-layers had an odds ratio of 1.24 (95% CI, 1.04–1.47) with adjustment for smoking and asbestos. Increased risks were seen for roofers and asphalt workers exposed to PAHs, but odds ratios for those occupations were not shown. [This study was comparatively large, but information on specific exposures remained limited, as in the earlier study using similar methods (Jöckel *et al.*, 1998). Notably, exposures to bitumens were combined with exposure to PAHs from sources including coal tar.]

Associations of cancer of the lung with occupational exposure were characterized by Bovenzi *et al.* (1993) in a case–control study of mortality in an industrial area in northern Italy. The cases included 756 men who had died of primary cancer of the lung between 1979 and 1986, identified from a provincial cancer registry. The controls were 756 men who had died of causes other than lung disease or cancers of the aerodigestive, urinary or gastrointestinal tracts, or of the pancreas or liver, sampled at random from autopsy records and matched to cases on time of death and age. Assessment of occupational exposure was based on occupation and industry titles, obtained through interviews with next-of-kin, which were classified as involving exposure to known or suspected carcinogens identified from a review of *IARC Monographs* (Simonato & Saracci, 1988). Odds ratios were adjusted for smoking using information obtained in the interviews. The odds ratio for asphalt workers was 2.27 (95% CI, 0.50–10.3). [It was not clear whether this represented ever being employed in the occupation, the usual occupation or the last occupation.] Data were not reported for other indicators of bitumen exposure.

Associations of cancer of the lung with occupation were also examined in a population-based case–control study in two areas of northern Italy (Richiardi *et al.*, 2004). Cases and controls were enrolled between 1990 and 1992: 1171 men and women with incident, confirmed cancer of the lung were matched by region, sex and age to 1553 controls from population registries. Information on exposure to risk factors for cancer of the lung and a lifetime occupational history were collected by personal interview. Jobs were grouped according to whether exposure to occupational

carcinogens was previously known (list A) or suspected (list B) using an approach similar to that used by Jöckel *et al.* (1998) and Bovenzi *et al.* (1993). Data were analysed by unconditional logistic regression with adjustment for age, region, tobacco use, and total number of jobs. Detailed results were reported only for men. A category of construction occupations on list A that included roofers, asphalt workers, insulators and pipe-coverers had an odds ratio of 2.0 (95% CI, 0.6–6.5), which reduced to 1.5 after further adjustment for education. [The study did not specifically seek to address exposures to bitumen, and the category including potentially bitumen-exposed workers also included occupations with known exposure to asbestos.]

McClean *et al.* (2011) reported a population-based case–control study including 422 incident cases of cancer of the lung and 894 controls identified between 1998 and 2003 among African Americans and Latin Americans in the San Francisco Bay area, California, USA. Both men and women were included. Information on occupational exposure to a list of 21 substances and on smoking habits was obtained in a personal interview. Exposures were self-assessed. Study subjects also provided blood or buccal samples to investigate potential effect modification by cytochrome P450 (CYP) 1A1 type. Refusal rates were about 7% among controls and 14% among referents. The study found an odds ratio of 1.2 (95% CI, 0.7–2.1) for ever having worked with asphalt and tar. Cumulative exposure was investigated and an odds ratio of 1.11 (95% CI, 1.01–1.22) per year of exposure to asphalt and tar was reported. This risk estimate was adjusted for age, sex, race, smoking habits, asbestos and automobile exhaust. In Latin Americans, a higher risk was noted with CYP1A1 wildtype than with the variant type. [The Working Group noted that a broad exposure category combining exposure to asphalt and tar was investigated, and separate effects could not be estimated.]

(b) Industry-based studies

The association of cancer of the lung with exposure to bitumen fume among workers engaged in bitumen-roofing manufacture and bitumen production was examined specifically in a case–control study by Watkins *et al.* (2002). There was no exposure to coal tar in this population from 1977 onward, and there was no information about the presence or absence of exposures to coal tar before that year. The cases were 39 men who had been employed at 28 roofing-manufacture and bitumen-production facilities and had died of cancer of the lung between 1977 and 1997. Twenty-three of the cases had worked at roofing-manufacturing plants. The controls were 133 men employed at the plants who had retired or died and were not cases, matched to cases on age, race and year of birth. Exposures to bitumen fume and respirable crystalline silica were assessed using the same information as in the earlier study by Chiazze *et al.* (1993), but in greater detail. Exposure to bitumen fume was classified in categories of ever/never exposed, duration < 20 years, duration ≥ 20 years, and cumulative exposure of 0, < 1000, 1000–9999 or > 10 000 mg/m^3-days. Since data on industrial hygiene were not available for years (not specified) before 1977, two scenarios were used to extrapolate exposures measured between 1977 and 1989 to the earlier period. Scenario 1 assumed that the average level of exposure had declined at the same rate in the years before 1977 to 1977–82 as in the years from 1977–82 to 1983–89, while scenario 2 assumed that average exposures before 1977 were double those in 1977–82. Only matched, unadjusted odds ratios for the association between cancer of the lung and bitumen fume were reported. The odds ratios for cancer of the lung associated with exposure to bitumen fume were: ever exposure, 1.59 (95% CI, 0.60–4.57); exposure for < 20 years, 2.27 (95% CI, 0.74–7.73); and exposure for ≥ 20 years, 1.06 (95% CI, 0.30–3.65). The highest

odds ratios for quantitative estimates of exposure were observed for workers with exposures between 0 and 1000 mg/m^3-days: scenario 1, 1.99 (95% CI, 0.14–17.52); and scenario 2, 2.84 (95% CI, 0.41–18.42). Odds ratios in the highest exposure category of > 10 000 mg/m^3-days were lower (1.30 and 1.84 for scenarios 1 and 2, respectively). [Exposure assessment in this study focused on bitumen and was more detailed than in any population-based study. The data on exposure duration and estimated cumulative exposure allowed exposure–response relationships to be considered. However, the lack of exposure information for the period before 1977, when exposures were thought to have been higher, is a significant limitation that the authors attempted to address in sensitivity analyses. The number of years without exposure data was not specified, but 85% of workers had worked before 1977. Other notable limitations were the small number of cases and doubts about whether the controls were representative of the base population for the cases.]

2.3.2 Cancer of the urinary bladder

See Table 2.4

Mommsen *et al.* (1983) studied risk factors for cancer of the bladder in a mostly rural region of Denmark. Cases (n = 212) were patients seen between 1977 and 1980 at an oncology service, while controls (n = 259) were matched on sex, age, geographic area and urbanization; the crude odds ratio for work with petroleum or asphalt was 3.78 (95% CI, 1.12–12.81). This was reduced to 2.36 (confidence intervals not given) after adjustment for tobacco, alcohol and other exposures. No other indicators of potential exposure to bitumen were reported. [Although this study suggested a relatively strong association between cancer of the bladder and exposure to petroleum and asphalt, there were several weakness that may compromise validity, including the unknown source of controls, different interviewing methods for cases (face-to-face interview) and controls (telephone interview or mailed questionnaire), and details of the occupational questions and the substances that were queried were not given. In addition, exposures to asphalt and petroleum were aggregated, making the effect of bitumen difficult to gauge.]

Occupational risk factors for cancer of the bladder, including tars and asphalt, were investigated in a population-based case–control study by Risch *et al.* (1988). Cases (n = 826) were residents of two Canadian provinces that were newly diagnosed with histologically confirmed cancer of the bladder during 1979–82. Controls (n = 792) were selected at random from provincial population registers and matched to cases on birth year, sex and area of residence. Information on occupation, exposure to fume, dust, smoke and chemicals, smoking and other risk factors was obtained by face-to-face interview. The method of assessing specific occupational exposures was not reported. The response proportion was 67% for cases and 53% for controls. Associations between incidence of cancer of the bladder and exposure to tars and asphalt were observed for men, but not for women. The smoking-adjusted odds ratio was 1.44 (95% CI, 0.78–2.74) for ever having been exposed to tar and asphalt, and 2.02 (95% CI, 1.08–4.97) for 10 years of exposure. For men exposed 8–28 years in the past, the odds ratio was 3.11 (95% CI, 1.19–9.68). Associations of cancer of the bladder with occupational titles were also reported, but data were not provided for pavers, roofers or other occupations with known exposure to bitumen. [While the effort to identify specific occupational exposures was useful, the grouping of tar and asphalt in the same category prevented their effects from being separated and the lack of information about the exposure assessment methods further hampered interpretation of the results. The low response proportion for controls was also of some concern.]

Some information potentially relevant to the effects of bitumen was reported in

Table 2.4 Case–control studies of cancer of the bladder and exposure to bitumens

Reference, study location and period	Total cases	Total controls	Control source (hospital, Population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Mommsen *et al.* (1983) Denmark 1977–80	212	259	Not specified. Matched on age, sex, area and urbanization	Occupation and chemical exposure in-person interview (cases) and phone interview or mail questionnaire (controls)	Petroleum or asphalt	9	2.36 (95% CI not given)	Tobacco, alcohol, other substances Unspecified source of controls, different interview methods for cases and controls and lack of detail about exposure assessment methods. Crude OR, 3.78 (95% CI, 1.12–12.81)
Risch *et al.* (1988) Canada 1979–82	826	792	Population register. Matched on age, sex, birth year, residence	Personal interview	Tars and asphalt			Smoking Method of assessing exposure to specific substances not specified. Response rate: cases, 67%; controls, 53%. ORs for men only. Exposure to tar and asphalt combined.
					Ever exposed	NR	1.44 (0.78–2.74)	
					Exposed for 10 yr	NR	2.02 (1.08–4.97)	
					Exposed 8–28 yr ago	NR	3.11 (1.19–9.68)	
Bonassi *et al.* (1989) Italy 1972–82	121	342	Population registers. Matched for age, sex and time at risk	Interview and classification of occupations as definitely, possibly or not exposed.	Road menders	2	1.4 (0.3–7.3)	Smoking, aromatic amines Men only

Table 2.4 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, Population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Kogevinas *et al.* (2003) Pooled data from 11 studies in 6 countries in Europe 1976–96	3346	6840	Hospital (7 studies), population (3 studies) or both (1 study)	Job title, list of high-risk occupational and JEM.	Roofers	13	0.72 (0.36–1.43)	Age, smoking, study centre Relevant data presented only by job title. Men only.
Geller *et al.* (2008) Germany	156 men	336	Prostate cancer cases in same registry as cases.	Postal questionnaire	Frequent exposure to bitumen.	NR	2.92 (1.32–6.48)	Age, smoking, multiple occupational exposures Controls had prostate cancer.

NR, not reported; OR, odds ratio; yr, year

a population-based study of cancer of the bladder and exposure to PAHs in Italy (Bonassi *et al.*, 1989). Cases (n = 121) were identified from hospital records for the years 1972–82 and controls (n = 342) matched by age, sex, and time of case occurrence were sampled at random from population registries. Only men were included in the analysis. Information about risk factors for cancer of the bladder, including occupation, was obtained by interview with subjects or next-of-kin. Occupations were classified as definitely, possibly or not exposed to PAHs using a job-exposure matrix constructed from a review of the literature. Road menders were among the occupations classified as definitely exposed. Odds ratios were adjusted for smoking and exposure to aromatic amines. An odds ratio of 1.4 (95% CI, 0.27–7.28) based on two cases and six controls was reported for work as a road mender. The remaining analyses focused on PAHs in general and were not informative about exposure to bitumens. [The very small numbers of exposed cases and controls limited the information contributed by this study.]

Associations of cancer of the bladder with occupational exposures were considered in a large study (Kogevinas *et al.*, 2003) pooling 11 previous case–control studies of cancer of the bladder in men in six European countries. The pooled analysis included data for 3346 cases and 6840 controls. Occupational exposures were estimated from work histories taken in the original studies (lifetime histories in ten studies and usual occupation in one). Occupations were aggregated according to prior information about risk factors for cancer of the bladder and results were reported only for those with at least 10 subjects. Odds ratios were adjusted for age, smoking and study centre. Roofers were considered to have a high-risk occupation, but had an odds ratio of 0.72 (95% CI, 0.36–1.43) based on 13 cases and 39 controls. No other information relevant to exposure to bitumens was reported. [The sample size for this study was larger than for the other case–control studies of cancer of the urinary bladder and provided a risk estimate with a relatively narrow confidence interval. The exposure assessment was crude, hampering the ability to distinguish specific exposures in roofers.]

Occupational risk factors for cancer of the bladder were also examined in a registry-based case–control study in Germany (Geller *et al.*, 2008). The cases were 156 men with bladder cancer who had applied for cancer treatment, and controls were 336 men with prostate cancer identified from the same database. Information on occupational history, potential exposure to carcinogens, smoking and other factors was obtained by mailed questionnaire. Questions specifically addressed exposure to bitumens, tar and pitch, which could be classified as seldom, often or "permanent." Odds ratios were adjusted for age, smoking and multiple occupational exposures. For frequent exposure to bitumens, the smoking-adjusted odds ratio was 2.92 (95% CI, 1.32–6.48). [This study sought specifically to identify exposure to bitumens, but the Working Group questioned whether it were possible for the workers to differentiate exposures to coal tar, pitch and bitumens. There may be collinearity between these three exposures, but the authors did not attempt to adjust one factor for the other factors in the model.]

2.3.3 Cancer of the kidney

A hospital-based case–control study of cancers of the renal pelvis and ureter in Denmark (Jensen *et al.*, 1988), which was directed primarily towards the effects of smoking, included some information about occupational risk factors. Cases (n = 96) and controls (n = 288) were matched on hospital, sex and age; patients with other diseases of the urinary tract or smoking-related diseases were excluded from the control group. A structured questionnaire was used to obtain information on occupations and occupational exposures. Odds ratios were adjusted for age, sex

and smoking history. Findings for occupational exposure to asphalt and tar were reported only for men: the smoking-adjusted relative risk based on nine exposed cases and six exposed controls was 5.5 (95% CI, 1.6–19.6) [apparently for ever *versus* never exposure.] [The utility of this study for evaluating risks due to bitumens was limited by the lack of detail about the methods and the classification of exposures to asphalt and tar in the same category.]

The relationship between renal cell carcinoma and occupational exposure to chemicals was examined by Hu *et al.* (2002) using data from a national cancer-surveillance system in Canada. Cases were 1279 men and women with incident kidney cancer reported between 1994 and 1997. Controls were 5370 individuals without cancer selected at random from the population of each province, frequency-matched on age and sex. Data on employment, smoking, alcohol use, and other risk factors was collected by mailed questionnaire. Subjects were also asked if they had ever been exposed for > 1 year at work to 17 substances, and, if so, the duration of the exposure. Exposures to coal tar, soot, pitch, creosote and asphalt were combined in the analysis: the odds ratio for ever exposure to this group of substances was 1.4 (95% CI, 1.1–1.8) among men and 1.3 (95% CI, 0.7–2.3) among women with adjustment for age, province, education, body-mass index, smoking, alcohol use and meat consumption. Associations with duration of exposure were reported only for selected substances, and coal tar, soot, pitch, creosote and asphalt were not included. [The large nationally representative sample was a strength of this study. Exposures were assessed by a self-administered checklist of substances, providing more detail than job titles alone. However, the exposure classification did not allow exposures to bitumen, coal tar and other substances to be separated. Selective reporting of results also limited the inferences that could be made.]

2.3.4 Cancer at other sites

The relationship between hepatocellular carcinoma and occupational exposure to chemicals was evaluated as part of a multisite case–control study in the USA that was targeted primarily on the effects of cigarettes, alcohol and hepatitis B virus (Austin *et al.*, 1987). Eighty cases with histologically confirmed cancer and 146 hospital controls were matched on sex, age, race and study centre. Patients with smoking-related diseases, including cancer of the lung, and other liver diseases were not eligible to be controls. Information on all jobs held for 6 months and on ever having been exposed to 26 substances, including tar and asphalt separately, was obtained by interview. Results were tabulated only for jobs and substances reported by at least 10 subjects. Seven cases and five controls reported exposure to asphalt, giving an odds ratio of 3.2 (95% CI, 0.9–11). Data for exposure to tar were not reported. Five cases and two controls had been employed in highway and street construction (OR, 5.0; 95% CI, 1.0–26). [The Working Group observed that this study was noteworthy in that it attempted to differentiate between exposures to tar and asphalt.]

Associations of occupational exposure and cancer of the brain in Canada were investigated by Pan *et al.* (2005) using data sources and methods similar those used by Hu *et al.* (2002) to study cancer of the kidney. The cases were 1009 individuals with incident primary malignant tumours of the brain, including glioblastoma, astrocytoma, oligodendroglioma, ependymoma, and others not specified. The controls were 5039 people selected at random from population registries. Data were obtained by a mailed questionnaire, which had questions about employment history and exposure to 18 substances, including bitumen. The rate of participation was 62% for cases and 67% for controls. Analysis was by logistic regression with adjustment for age, province, sex, education, alcohol, smoking and

energy intake. The odds ratio for ever exposure to bitumen was 1.29 (95% CI, 1.02–1.62) after adjustment. Elevated odds ratios for exposure to bitumen were observed for men (OR, 1.20; 95% CI, 0.93–1.54) and women (OR, 1.85; 95% CI, 1.03–3.34). Monotonically increasing odds ratios and statistically significant (P = 0.33) trends were observed with increasing duration of exposure. The odds ratio for > 10 years exposure was 1.39 (95% CI, 0.97–1.99) with full adjustment as described above. In analyses based on job titles, odds ratios were elevated for ever (OR, 1.16; 95% CI, 0.73–1.85) or usually (OR, 1.22; 95% CI, 0.47–2.19) working in excavating, grading, paving and related occupations. An odds ratio of 1.73 (95% CI, 0.52–5.81) was observed for four individuals who had ever worked as roofers; no result was reported for usual occupation as a roofer. [The large size of this study facilitated a range of analyses. Reasonable precision and the use of an exposure checklist afforded more details about exposure than job titles alone. The study also assessed the trend with duration of exposure.]

Exposure to bitumen and related materials was considered in a study of the relationship between skin cancer and occupational exposure to PAHs among men in Poland (Kubasiewicz *et al.*, 1991). Cases (n = 376) were men with skin cancer enrolled in a cancer registry between 1983 and 1988. Population- and hospital-control groups of 752 men each were randomly sampled from the population at large and hospital services. Information on occupational history was obtained by interview. The analysis of occupational exposure considered substances believed to be risk factors for skin cancer, including pitch, tar, "asphalt", "soft asphalt", and "bituminous mass", but details of how exposure was assessed were not given. The odds ratios for tar, pitch and bituminous mass were 1.09, 0.93 and 2.03, respectively with population controls and 1.00, 0.86 and 2.02, respectively, with hospital controls. No confidence intervals or P values were reported. Results were not reported for asphalt or soft asphalt. [This study was considered to be minimally informative because of its broad focus on PAHs, the lack of detail on the exposure-assessment methods, unclear definitions of the substances of interest, and weak statistical methods that were poorly described.]

2.3.5 Multiple cancer sites

Associations between 11 cancers (oesophagus, stomach, colon, rectum, pancreas, lung, prostate, bladder, kidney, skin melanoma and non-Hodgkin lymphoma) and occupational exposure to bitumen were evaluated in a large, hospital-based case–control study in Canada (Siemiatycki, 1991). A total of 3505 individuals with the cancers of interest were enrolled during 1979–85. Subjects served both as cases and as controls in analyses of other cancers. Occupational exposures were assessed by combining expert assessment with detailed interviews with subjects. Odds ratios were adjusted for potential confounders selected *a priori* for each cancer site. Adjusted odds ratios for any exposure to bitumen were 1.0 or less for all of the cancers evaluated, with the exception of cancer of the colon (OR, 1.6; 90% CI, 1.1–2.5) and non-Hodgkin lymphoma (OR, 1.4; 90% CI, 0.8–2.7). For substantial exposure to bitumen, odds ratios were elevated for cancers of the stomach (OR, 2.0; 90% CI, 1.0–4.1) and prostate (OR, 1.8; 90% CI, 0.8–4.0), but not for other sites. In subgroup analyses, substantial exposure to bitumen was associated with cancer of the prostate (OR, 3.0; 90% CI, 1.0–9.0), bladder (OR, 2.2; 90% CI, 1.0–4.9) and non-Hodgkin lymphoma (OR, 1.5; 90% CI, 0.4–5.1) among French Canadians only. [The Working Group considered the detailed exposure assessment to be a noteworthy strength of this study. Small numbers reduced precision in analyses of some combinations of cancer site and exposure, and this combined with the unusually large number of comparisons made it likely that some associations occurred by chance.]

2.4 Meta-analyses

Several articles have reviewed the epidemiological literature on risk of cancer among bitumen workers or associated with exposure to bitumens and/or bitumen fume; however, only meta-analyses are presented here (Table 2.5).

Partanen & Boffetta (1994) conducted a study of cancer risks among asphalt workers and roofers in a meta-analysis of 19 epidemiological studies (11 cohort, eight case–control) from both Europe and North America. [The authors' text stated that 20 studies were reviewed, but the Working Group counted only 19. All studies are described in Sections 2.1.1 and 2.2.] The timeframe for case ascertainment in these studies ranged from 1945 to 1989. Risk ratios for all bitumen workers were: cancer of the lung, 1.19 (95% CI, 1.08–1.30) (1.21 for cohort and 1.12 for case–control studies); cancer of the stomach, 1.28 (95% CI, 1.03–1.59) (1.33 for cohort and 1.00 for case–control studies); cancer of the bladder, 1.22 (95% CI, 0.95–1.53) (1.38 for cohort and 0.80 for case–control studies); non-melanoma skin cancer, 1.74 (95% CI, 1.07–2.65) (four cohort studies); and leukaemia, 1.41 (95% CI, 1.05–1.85) (four cohort studies). In a subsequent analysis, studies of roofers and pavers/highway-maintenance workers were considered separately for easier understanding of patterns. Risk ratios for cancer of the lung were 0.87 (95% CI, 0.76–1.08) among pavers and highway-maintenance workers and 1.78 (95% CI, 1.50–2.10) among roofers. Some studies included adjustment for smoking. Only one such study was available for pavers and highway-maintenance workers and had a risk ratio of 0.9 (95% CI, 0.6–1.50); among studies of roofers, the smoking-adjusted risk ratio for cancer of the lung was 2.0 (95% CI, 1.3–2.8) based on four studies. [This was one of the most informative reviews because of the details provided by the meta-analysis. Smoking did not appear to confound the risk ratios in these studies. It was not possible to separate the effects of coal tar from those of bitumen.]

Fayerweather (2007) conducted a meta-analysis of the epidemiological literature on bitumen exposures to update the previous meta-analysis (Partanen & Boffetta, 1994) and adjust for possible confounding from exposure to coal tar. Only peer-reviewed, published reports were included. When multiple reports were available on the same study, the most recent version was selected. Also relative risks based on internal referents were selected over those based on external referents. Reported relative risks from exposure to bitumen were adjusted for potential confounding from exposure to coal tar using information from the literature on coal-tar exposure among asphalt workers and referents, and the relative risk for cancer of the lung associated with coal tar. Adjustments were based on concentrations of benzo[*a*]pyrene of 20 μg/m^3 for coal-tar roofing and 10 μg/m^3 for coal-tar paving. Sixteen country-specific epidemiological studies of roofers and eleven studies of pavers were entered into the meta-analysis. The meta-relative risk dropped from 1.67 (95% CI, 1.39–2.02) to 1.10 (95% CI, 0.91–1.33) with adjustment for coal tar in roofing studies, and from 0.98 (95% CI, 0.81–1.18) to 0.96 (95% CI, 0.80–1.16) with adjustment in paving studies. Studies were also stratified by design, with cohort (SMR, SIR, RR) and case–control studies considered to be stronger designs than proportionate mortality, public census, and cross-sectional designs. The point estimates were similar for roofing and paving studies combined: 1.03 (95% CI, 0.85–1.25) for the studies with weaker study designs, *versus* 1.01 (95% CI, 0.88–1.17) for studies with stronger designs. [The Working Group found this meta-analysis noteworthy in its efforts to disentangle exposures to coal tar and bitumen. Group-level adjustments are useful when information is not directly available at the individual level, as in group-level adjustment for smoking differences by occupational category. The interpretation

Table 2.5 Meta-analyses of risk of cancer and exposure to bitumens

Reference	Years covered	Type of analysis	Organ site	Exposure categories	Meta relative risks (95% CI)	Covariates	Comments
Partanen & Boffetta (1994)	1976–93	Meta-analysis of 20 studies		Roofers			Not possible to separate effects of tar and bitumen. Smoking did not appear to confound ORs.
			Lung		1.8		
			Stomach		1.7		
			Non-melanoma skin		4.0		
			Leukaemia		1.7		
				Road pavers			
			Lung		0.9		
			Stomach		1.1		
			Non-melanoma skin		2.2		
			Leukaemia		1.3		
Fayerweather (2007)	Up to 2005	Meta-analysis of 27 studies with adjustment for coal-tar exposure	Lung	Roofers	1.67 (1.39–2.02)	Unadjusted	External adjustment for coal tar. Potential for over-adjustment.
					1.10 (0.91–1.33)	Adjusted for coal-tar exposure	
				Pavers	0.98 (0.81–1.18)	Unadjusted	
					0.96 (0.80–1.16)	Adjusted for coal-tar exposure	

OR, odds ratio

of these group-level adjustments relies on the extent to which the external data, such as historical levels of exposure, prevalence of exposure in the current population, and the risk associated with a given level of exposure, correspond to the experience of the study population. The Working Group noted that some of the studies included in this meta-analysis were already adjusted for coal tar (i.e. the IARC study) and this analysis was therefore over-adjusted for coal tar. Adjusting for coal tar using benzo[*a*]pyrene as a marker could also result in an over-adjustment, because benzo[*a*]pyrene can also be generated by bitumen emissions.]

3. Cancer in Experimental Animals

Several independent studies in mice, rats, guinea-pigs or rabbits, using skin application, subcutaneous injection, intramuscular injection and inhalation were evaluated as inadequate by the Working Group and were not taken into consideration for the evaluation of the carcinogenicity of bitumens in experimental animals (reported in Simmers *et al.*, 1959; Simmers, 1964, 1965a, b, 1966; Hueper & Payne, 1960; Kireeva, 1968). Limitations of these included poor reporting of the study, the small number of animals tested, lack of information on dose and duration of treatment, no information of distribution of "fumes", lack of concurrent vehicle-control group, the use of a carcinogenic agent as vehicle, poor survival, no information on survival, animals lost and replaced in the middle of the experiment, and pathology not provided. These independent studies are not presented in the tables. This section provides a brief summary of each and a more detailed review of the relevant studies.

[To avoid confusion in the nomenclature, the agent administered was always first reported using the name given in the original publication, in quotation marks, followed by the bitumen class, as classified by the Working Group, in square brackets.]

3.1 Mouse

See Table 3.1

3.1.1 Skin application

A group of 32 male and 36 female C57 Black mice (age not reported) were treated with a pooled sample of six "steam-refined and air-blown (oxidized) petroleum asphalts" [bitumens class 1 and 2] dissolved in benzene. A group of 31 male and 32 female C57 Black mice served as controls and were treated with benzene alone. The treated mice received an unspecified dose, applied with a glass rod onto the interscapular skin, twice per week. Twelve epidermoid carcinomas [$P = 0.0002$] appeared at the site of application, with the first tumour appearing during week 54 of the study. No skin tumours were reported in the control mice. [The study was poorly reported, with no indication of the duration of treatment, or the amount of compound applied, or survival. The vehicle used is an IARC Group 1 carcinogen (Simmers *et al.*, 1959).]

Simmers (1965a) treated a group of 25 male and 25 female C57 Black mice (age, approximately 6 weeks) with 75–100 mg of a pooled sample of three "steam-refined petroleum asphalts" [bitumen class 1] heated in a boiling-water bath and applied to the skin with a glass rod, three times per week, for up to 23 months. Because only 15 males and 12 females survived an epidemic of pneumonitis that occurred after 7 weeks, and only 1 male and 5 females were alive after 1 year, 8 males and 5 females of unknown age were added to the group. The total number of applications ranged from 16 to 240. Topical squamous cell carcinomas were found in 3 of the 21 autopsied mice. [The Working Group noted the high mortality in the early part of the study.]

Table 3.1 Studies of carcinogenicity in mice exposed to bitumens

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Skin application				
Mouse, C57 Black (M, F) 92–96 wk Simmers (1965a)	0, 20–30 mg of a 90% solution of an "air-refined (oxidized) petroleum asphalt" (bitumen class 2) in toluene; 3 ×/wk to skin with glass rod 20 mice; 15 toluene controls.	Topical squamous-cell carcinoma: 0/15, 9/20 (45%)	[$P = 0.002$, Fischer's exact test]	No information on survival.
Mouse, Swiss albino (M, F) Duration NR Wallcave *et al.* (1971)	2.5 mg of eight different "road-paving-grade asphalts" (bitumen class 1) produced by vacuum distillation from well-defined crude sources dissolved in benzene (10% solutions); 2 ×/wk to the skin of the back 24–32 treated; 30 control mice treated with benzene only.	Skin tumours: Carcinoma: 1/218, 0/26 Papilloma: 5/218 (2%), 1/26 (4%) Combined: 6/218 (3%), 1/26 (4%)	[NS] [NS] [NS]	No indication of duration of treatment, vehicle used is an IARC Group 1 carcinogen.
Mouse, C3H/HeJ (M) 80 wk Emmett *et al.* (1981)	0, 50 mg of a solution of a standard "roofing petroleum asphalt" (bitumen class 2) dissolved in toluene (1:1 w/w); 2 ×/wk on the intrascapular skin 50 mice/group.	No skin tumours	[NS]	Skin tumours were observed in 31/39 (79%) of a benzo[*a*]pyrene (0.1% toluene solution, 50 mg of solution/application) positive control.
Mouse, Sencar (F) 52 wk Robinson *et al.* (1984), Bull *et al.* (1985)	0%, 89% (asphalt A), 98% (asphalt B) 97% (asphalt C), 97% (asphalt D) "asphalt cutbacks" (class 3 bitumen, a solid petroleum asphalt material cut back to 64% solid with mineral spirits) diluted with xylene and/or mineral spirits to give a dosing volume of 0.2 mL; a single application of 200 µL applied to the shaved dorsal surface followed in 2 wk by topical application of 1.0 µg of TPA in 0.2 mL acetone 3 ×/wk for 20 wk 30 or 40 mice/group.	Squamous cell carcinoma: 2/36 (6%; asphalt A), 0/31 (asphalt B), 5/31 (16%; asphalt C), 6/33 (18%; asphalt D) Acetone control: 0/23	$P \leq 0.05$ for asphalts C and D	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Mouse, Sencar (F) 52 wk Robinson *et al.* (1984), Bull *et al.* (1985) contd	0%, 89% (asphalt A), 98% (asphalt B) 97% (asphalt C), 97% (asphalt D) "asphalt cutbacks" (class 3 bitumen, solid petroleum bitumen material cut back to 64% solid with mineral spirits) diluted with xylene and/or mineral spirits to give a dosing volume of 0.2 mL; 3 weekly applications of 200 µL applied to the shaved dorsal surface followed in 2 wk by topical application of 1.0 µg of TPA in 0.2 mL acetone 3 ×/wk for 20 wk 30 or 40 mice/group.	Squamous cell tumours: 10/38 (26%; asphalt A), 6/35 (17%; asphalt B), 13/36 (36%; asphalt C), 4/35 (11%; asphalt D) Mineral-spirits control: 1/37	$P \leq 0.05$ for asphalts A, B, and C	
Mouse, CD1 & C3H/HeJ (M) 78 wk Niemeier *et al.* (1988), NIOSH (2001a)	Fumes generated by heating "type I or type III asphalts" (produced by distillation and air blowing of Arabian crude) or type I or type III coal tar pitch at either 232 °C or 316 °C were collected and diluted in 1/1 cyclohexane/acetone to an unspecified concentration and then applied to the clipped interscapular area. Thirty-two groups of mice for the primary factorial experiment, i.e. 2 strains × 4 materials × 2 generation temperatures × 2 light exposure conditions (presence or absence of simulated sunlight); each animal was dosed 2×/wk with 50 µL of the appropriate test material; four groups for a combination treatment of bitumen and coal-tar pitch fume condensate; eight groups for controls; and four groups for positive control with benz[*a*]pyrene 50 mice/group.	Specific tumour data not provided. The average latent period in the groups treated with the condensed fume of the roofing materials ranged from 39.5 to 56.1 wk among the C3H/HeJ groups, and from 47.4 to 76.5 wk among the CD-l. Skin tumours – see Tables 3.2 and 3.3.		

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Mouse, C3H/HeJ and Sencar (M) 96 wk Sivak *et al.* (1997)	Exposure to a 'type III "steep" asphalt' (class 2), produced by distillation and air-blowing Arabian crude, heated at 316 °C and the fume condensates collected, fractionated, and then applied dermally at doses of 1.6–25 mg bitumen, bitumen + fume or fume alone in 0.05 mL cyclohexane/acetone (1/1); 2 ×/wk for 104 wk 39 treated and 2 control groups of 30.	Skin tumours – see Table 3.4	NR	
Mouse, C3H/HeNCrl (M) 104 wk Clark *et al.* (2011)	Exposed to a "field-matched" BURA [class 2 bitumen] fume condensate (collected at 199 °C) or a "lab-generated" BURA fume condensate (collected at 232 °C) applied 2×/wk in a volume of 37.5 µL (25 mg) mineral oil for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and benzo[*a*]pyrene (BaP 0.05% in 37.5 µL toluene, applied 2 ×/wk) were used as negative and positive controls 2 ×/wk for 104 wk 80 treated and 2 control groups of 50 and 80 mice.	*Skin tumours* Squamous cell carcinoma: 0/80 (control), 8/62 (13%), 35/64 (55%), 34/49 (69%) (BaP) Squamous cell papilloma: 0/80 (control), 4/62 (6%), 3/64 (5%), 2/49 (4%) (BaP)	$P < 0.0001$, Fisher's exact test $P < 0.0001$ for BaP	
	Exposed to a "field-matched" paving [class 1 bitumen] fume condensate (collected at 148 °C) applied daily in a volume of 37.5 µL (7.14 mg) mineral oil for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and BaP (0.05% in 37.5 µL toluene, applied 2×/wk) were used as negative and positive controls 2×/wk for 104 wk 80 treated and 2 control groups of 50 and 80 mice	*Skin tumours* Squamous cell carcinoma: 0/80 (control), 0/80, 37/50 (74%) (BaP) Squamous cell papilloma: 0/80 (control), 1/80 (1%), 1/50 (2%) (BaP)	$P < 0.0001$ for BaP	
Mouse, C_3H/HeNCr1BR (M) 96 wk Goyak *et al.* (2011)	An "asphalt cement 20" and a "coastal residuum" (both class 1) were diluted with mineral oil and applied at the limits of solubility in mineral oil, 30% and 75% ([w/w]), respectively, as 37.5 µL doses. BaP was applied at a 0.05% (w/v) dilution in toluene; 2×/wk to clipped back. 50 mice/group	*Skin tumours* Carcinoma: 0/50 (oil), 0/50 (toluene), 46/50 (92%) (BaP), 0/50, 0/50.	[NS]	Mineral oil should have been used as vehicle for the positive control group. Significant for BaP.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Mouse, Crl:CD1 (M) 28 wk Freeman *et al.* (2011)	Initiation/promotion A "field-matched" roofing (BURA) fume condensate (collected at 148 °C) was tested as an initiator by applying it in a volume of 37.5 µL (25 mg) mineral oil 2 ×/wk (total weekly dose, 50 mg) for 2 wk followed by administration of TPA (5 µg, 0.01% in acetone) 2×/wk for 25 wk and as a promoter by applying it in a volume of 37.5 µL (25 mg) mineral oil 2 ×/wk for 28 wk after a single 50 µg dose of DMBA. Mineral oil, TPA and DMBA controls were included. 30 mice/group	*Skin tumours* Squamous cell papilloma: 0/30 (control), 0/30 (BURA), 1/30 (3%) (TPA) 5/30 (17%) (BURA/TPA), 0/30 (DMBA), 2/30 (7%) (DMBA/BURA), 27/30 (90%) (DMBA/TPA).	$P < 0.01$	
Subcutaneous or intramuscular injection				
Mouse, C57 Black (M, F) 54 wk Simmers *et al.* (1959)	Subcutaneous injection 0, unspecified dose of a pooled sample of six steam- and air-blown (oxidized) petroleum asphalts (bitumens class 1 and 2) suspended in olive oil (1%). 0.2 mL of 2 ×/wk for 41 wk and then 1×/wk for unspecified time in the interscapular region 63 or 68 mice/group	Injection site sarcoma: 0/63, 8/68 (12%)	$P = 0.0035$, one-tailed Fisher's exact test	Study poorly reported, no indication of duration of treatment, author indicates "Thus far distant metastasis has not been seen …" implying this is a preliminary report of an unfinished study, no indication of the number of tumour bearing animals, no statistical analysis applied.

BaP: benzo[*a*]pyrene; BURA, built-up roofing asphalt; d, day; DMBA, 7,12-dimethylbenz[*a*]anthracene; h, hour; mo, month; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; wk, week; yr, year.

In a second experiment, a group of 25 male and 25 female C57 Black mice (age, approximately 6 weeks) was treated with 75–100 mg of "air-refined (oxidized) petroleum asphalt" [bitumen class 2] heated in a boiling-water bath and applied to the skin with a glass rod, one to three times per week for up to 7 weeks. No carcinomas were observed at the site of treatment in the 10 mice autopsied. [The Working Group noted the absence of concurrent controls for both experiments.]

In a third experiment, a group of 10 male and 10 female mice were treated with the same "air-refined asphalt" [bitumen class 2], diluted in toluene (10% toluene : 90% asphalt) that was applied to the skin with a glass rod, three times per week for up to 2 years. Squamous cell carcinoma of the skin developed in 9 of the 20 mice autopsied [$P = 0.002$]. No squamous cell carcinomas were observed in the 15 control mice treated with toluene only; one mouse developed a skin papilloma (Simmers, 1965a).

A group of 25 male and 25 female C57 Black mice (age, 20–22 weeks) was treated with a mixture of "aromatics" and "saturates" [a fraction of a class 1 bitumen], isolated by fractionation of a "steam-refined asphalt" [bitumen class 1] from a California crude petroleum. The steam-refined asphalt had been separated into four fractions: asphaltenes, aromatics, saturates and resins. The thick oily liquid was applied with a glass rod three times per week (about 33.4 mg per application) to the intrascapular non-shaved skin (duration of study not given). The number of applications ranged from 72 to 242 because of differential survival. Forty mice (18 males, 22 females) survived to be autopsied. Thirty of the autopsied mice had gross evidence of neoplastic pathology and were studied microscopically: 13 skin papillomas, 7 epidermoid skin carcinomas, 5 baso-squamous cell cancers and 1 sebaceous-gland carcinoma were observed. Other tumours found included one epidermoid carcinoma of the anus and two leiomyosarcomas (one subcutaneous and one intestinal) (Simmers, 1965b). [The study was poorly reported, with no indication of the duration of treatment, and no controls were used.]

Groups of 25 male and 25 female C57 Black mice were exposed to "road petroleum asphalts" [bitumen class 1] obtained by steam distillation of crudes from Mississippi and California, USA, Venezuela, or by steam-vacuum distillation of one Oklahoma crude, respectively. Each mouse received one drop of an unspecified dose of bitumen, liquefied with acetone, applied to the neck skin, twice per week for up to 2 years. One skin carcinoma was observed in the group treated with the Mississippi sample, and one skin papilloma was observed in the groups treated with the Oklahoma and the Mississippi samples. No skin tumours were found in the groups treated with the samples from Venezuela or California or in 100 male and 100 female untreated mice (Hueper & Payne, 1960). [The study was poorly reported, and there were no vehicle controls.]

A group of 25 male and 25 female C57 Black mice received an unspecified dose of a sample (heated to liquefy) of an "air-blown asphalt" [bitumen class 2] used for roofing purposes, applied to the skin of the nape of the neck, twice weekly, for up to 2 years. One skin carcinoma was reported (Hueper & Payne, 1960). [The study was very poorly reported, no controls were used, and survival data were not provided.]

Different sized groups of SS-57 white mice (sex and age unspecified) were exposed to two cracking-residue [destructive thermal distillation] bitumens (BN-5 and BN-4) (bitumen class 6) and four residual bitumens [straight distillation] (BN-5, BN-4, BN-3 and BN-2) (class 1). Carcinogenicity was tested by skin painting with each bitumen in a 40% solution in benzene, once per week for 19 months (70 applications) (Kireeva, 1968).

The cracking-residue bitumen BN-5 study started with 52 mice, and 49 survived to the time of appearance of the first tumour (month 9). Nine

animals developed skin tumours at the treatment site [$P < 0.05$, *versus* untreated controls]: five cornified squamous cell carcinomas, one fibrosarcoma and three papillomas. In addition, seven mice developed pulmonary adenoma and adenocarcinoma, and one developed a squamous cell carcinoma of the forestomach.

The cracking-residue bitumen BN-4 study started with 47 mice, and 42 survived to the time of appearance of the first tumour (month 10). Four mice had skin tumours (one cornified carcinoma, one noncornified carcinoma, and two papillomas), and all four also had pulmonary adenoma.

There were initially 50 animals in the residual bitumen BN-5 study, 37 animals in the BN-4 study, 50 animals in the BN-3 study, and 40 animals in the BN-2 study. Skin tumours were reported in two (one cornified squamous cell carcinoma and one sebaceous carcinoma) of 43 (BN-5), none of 30 (BN-4), two (one fibrosarcoma and one papilloma) of 43 (BN-3) and none of 30 (BN-2) mice surviving 9 months, respectively. In addition, tumours of the lung were observed in 5 out of 43 (12%), 1 out of 30 (3%), 1 out of 43 (2%) and 1 out of 30 (3%) mice, respectively. In 23 control mice painted with benzene only, no skin tumours were seen; one mouse developed lung adenomas (Kireeva, 1968). [The study was poorly reported, survival data were lacking, and the vehicle used is an IARC Group 1 carcinogen.]

Groups of 24–32 male and female random-bred Swiss albino mice (age, 7–11 weeks) were exposed to samples of eight "road-paving-grade asphalts" [bitumen class 1] produced by vacuum distillation from well-defined crude sources. The different bitumens were dissolved in benzene (10% solution) and applied twice per week to the skin of the back with a calibrated dropper delivering 2.5 mg of bitumen per application. An additional group of 15 males and 15 females were painted with benzene only and served as controls. Mean survival times were 81 weeks for bitumen-treated mice and 82 weeks for benzene-treated mice. At the end of the experiment, 6 out of 218 animals treated with the different bitumens developed skin tumours: one was a carcinoma and there were five papillomas. In 26 control mice treated with benzene only, one papilloma was observed (Wallcave *et al.*, 1971). [There was no indication of duration of treatment. The vehicle used is an IARC Group 1 carcinogen.]

A group of 50 male C3H/HeJ mice (age, 6 weeks) were treated with "standard roofing petroleum asphalt" [bitumen class 2] dissolved in toluene (1:1 w/w). Each mouse received 50 mg of the solution on the intrascapular skin, twice per week for 80 weeks. A group of 50 mice were treated with toluene alone and served as controls. No skin tumours were observed in 26 treated mice that survived 60 or more weeks, or in 37 mice of the control group. An additional group of 50 mice served as positive controls and were treated with benzo[*a*]pyrene (0.1% toluene solution, 50 mg of solution per application). Skin tumours were observed in 31 out of 39 (79%) mice surviving at the time of appearance of the first skin tumour (24 malignant, 7 papillomas; average latent period of papilloma, 32 weeks) (Emmett *et al.*, 1981).

Groups of 40 female Sencar mice (age, 6 weeks) were exposed to an "asphalt cutback"[class 3 bitumen], a solid petroleum asphalt material, cut back to 64% solid with mineral spirits), designated "asphalt D", diluted with xylene to 97% asphalt D, 3% xylene, and 200 µL of the resultant solution was applied to the shaved dorsal surface of the mice, once per week for 30 weeks. An additional group of 40 mice were treated with mineral spirits alone and served as controls. All surviving mice were euthanized at week 52. There was one papilloma observed in the treated group and three papillomas in the controls. No carcinomas were observed in either the treated or control group (Robinson *et al.*, 1984).

A study of initiation-promotion was also conducted in groups of 40 Sencar mice given a single initiation dose of 200 µL of four "asphalt

cutbacks" [class 3 bitumen], solid petroleum asphalt materials cut back to 64% solid with mineral spirits): 89% asphalt A, 1% xylene, and 10% mineral spirits; 98% asphalt B and 2% xylene; 97% asphalt C and 3% xylene; and 97% asphalt D and 3% xylene. This was followed 2 weeks later by topical application of 1.0 µg of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 mL of acetone, three times per week for 20 weeks. All surviving mice were euthanized at week 52. Squamous cell tumours were observed in 4 out of 23 (17%; acetone control), 6 out of 36 (17%; asphalt A), 5 out of 31 (16%; asphalt B), 8 out of 31 (26%; asphalt C), and 9 out of 33 (27%; asphalt D) mice. Squamous cell carcinomas were observed in 0 out of 23 (acetone control), 2 out of 36 (asphalt A), 0 out of 31 (asphalt B), 5 out of 31 (16%; asphalt C), and 6 out of 33 (18%; asphalt D) mice. The incidence of squamous cell carcinoma in the groups receiving asphalt C and asphalt D was significantly different from the control group [$P \leq 0.05$; one-tailed Fisher exact test] (Robinson *et al.*, 1984; Bull *et al.*, 1985).

An additional study of initiation–promotion was conducted in four groups of 40 mice given 200 µL of the four "asphalt cutbacks" [class 3 bitumen] used in the previous experiment, once per week for 3 weeks. Mice in the control group received 600 µL of mineral spirit. This was followed 2 weeks later by topical application of 1.0 µg of TPA in 0.2 mL of acetone, three times per week for 20 weeks. All surviving mice were euthanized at week 40. Squamous cell tumours (papilloma and/or carcinoma) were observed in 1 out of 37 (3%; acetone control), 10 out of 38 (26%; asphalt A), 6 out of 35 (17%; asphalt B), 13 out of 36 (36%; asphalt C), and 4 out of 35 (11%; asphalt D) mice. The incidence of squamous cell tumours in mice treated with asphalts A, B, and C was significantly different from that in the control group [$P \leq 0.05$; one-tailed Fisher exact test]. Squamous cell carcinoma was only observed in mice treated with the asphalt preparations (Robinson *et al.*, 1984; Bull *et al.*, 1985).

Groups of 50 CD1 and C3H/HeJ male mice (age, 12–15 weeks) were exposed to condensed fumes from "type I or type III asphalts" [class 2 bitumen], produced by distillation and air blowing of Arabian crude) generated by heating at either 232 °C or 316 °C. Fumes were collected and diluted in 1/1 cyclohexane/acetone and applied to the clipped interscapular area [final dose could not be calculated from reported data]. For the primary factorial experiment, there were two strains × two materials × two generation temperatures × two light-exposure conditions (presence or absence of simulated sunlight); each mouse was dosed twice per week with 50 µL of the appropriate test material. Four groups of 50 mice of each strain served as vehicle controls, while two groups of 50 mice of each strain treated with benzo[*a*]pyrene served as positive controls. Tumours were induced in both strains given condensed fumes from both types of bitumen. Tumour incidence and histology are provided in Table 3.2 for CD-1 mice and in Table 3.3 for C3H/HeJ mice. Condensed neat bitumen fume produced similar and statistically increased tumour yields of papilloma and carcinoma in both strains compared with respective vehicle controls. Recombination of all fractions resulted in a tumour response similar to neat bitumen fume. Raw unheated bitumen produced few tumours in C3H mice, but no tumours were seen when raw bitumen heated to 316 °C, with the fume permitted to escape, was applied. In the C3H/HeJ mice, there was a significant increase in the incidence of malignant and benign tumours with all of the bitumen samples at all temperatures, both with and without simulated sunlight. In the CD1 mice, the response was lower but there was a statistically significant increase of benign tumours in all samples and at all temperatures in the absence of simulated sunlight (Niemeier *et al.*, 1988; NIOSH, 2001a).

Groups of 30 male C3H/HeJ and Sencar mice (age, 8 weeks) were exposed dermally to 'type III "steep" asphalt' [class 2 bitumen], produced

Table 3.2 Histopathology of tumours induced in CD-1 mice treated dermally with roofing bitumen-fume condensates, with or without the presence of sunlight

Material tested	Sunlight	Number of tumour-bearing animals	Number of tumours			
		Benign	Malignant	Papilloma	Squamous cell carcinoma	Total[a]
Type I bitumen @ 232 °C[b]	–	6[c]	0	12	0	12
	+	2	0	3	0	3
Type I bitumen @ 316 °C[b]	–	13[c]	1	18	0	19 [a]
	+	3	0	3	0	3
Type III bitumen @ 232 °C[b]	–	9[c]	1	11	1	13 [a]
	+	5[c]	2	5	1	7 [a]
Type III bitumen @ 316 °C[b]	–	13[c]	3	17	1	20 [a]
	+	4	1	5	1	6
Benzo[*a*]pyrene[d]	–	24[c]	11[c]	43	10	58[a]
	+	9[c]	3	11	1	18[a]
Cyclohexane/acetone[e]	–	0	0	0	0	0
	+	0	0	0	0	0

[a] Other tumours observed included fibrosarcoma, keratoacanthoma, fibroma, and unclassified benign epithelioma
[b] 25 mg of total solid per application
[c] Significantly different ($P \leq 0.05$; one-tailed Fisher's exact test) from the appropriate cyclohexane/acetone control group
[d] 5 µg per application
[e] 50 µL of a 1:1 solution
Adapted from NIOSH (2001a)

Table 3.3 Histopathology of tumours induced in C_3H/HeJ mice treated dermally with roofing bitumen-fume condensates, with or without the presence of sunlight

Material tested	Sunlight	Number of tumour-bearing animals		Number of tumours		
		Benign	Malignant	Papilloma	Squamous cell carcinoma	Total[a]
Type I bitumen at 232 °C[b]	–	24[c]	22[d]	34	26	76
	+	14[c]	27[d]	22	25	62
Type I bitumen at 316 °C[b]	–	13[c]	31[d]	27	31	78
	+	18[c]	26[d]	36	26	73
Type III bitumen at 232 °C[b]	–	15[c]	25[d]	32	19	66
	+	11[c]	20[d]	14	19	54
Type III bitumen at 316 °C[b]	–	12[c]	28[d]	24	36	82
	+	20[c]	18[d]	34	20	65
Benzo[*a*]pyrene[e]	–	11[c]	27[d]	12	29	53
	+	7[c]	27[d]	11	22	43
Cyclohexane/acetone[f]	–	0	0	0	0	0
	+	1	0	2	2	4

[a] Other tumours observed included fibrosarcoma, keratoacanthoma, fibroma, and unclassified benign epithelioma
[b] 25 mg of total solid per application
[c] Significantly different ($P \leq 0.03$; one-tailed Fisher's exact test) from the appropriate cyclohexane/acetone control group
[d] Significantly different ($P \leq 0.001$; one-tailed Fisher's exact test) from the appropriate cyclohexane/acetone control group
[e] 5 µg per application
[f] 50 µL of a 1:1 solution
Adapted from NIOSH (2001a)

by distillation and air-blowing Arabian crude, heated at 316 °C, with the fume condensate collected and fractionated, for up to 24 months. The aims of this study were: (a) to examine the co-carcinogenic and tumour-promoting activities of three bitumen-fume fractions with benzo[*a*]pyrene; (b) to evaluate the direct tumorigenic activity of the five fractions individually and in a variety of combinations; (c) to assess the proportion of tumorigenic activity in the fume and heated residue; and (d) to compare the tumorigenic responses of neat bitumen fume in male C3H/HeJ and Sencar mice. The C3H/HeJ mice were given the test materials in a 50 µL volume of cyclohexane/acetone (1 : 1), applied to the shaved dorsal skin, twice per week. The Sencar mice were treated with the neat bitumen fume only. The tumorigenic responses are presented in Table 3.4. The composition of the bitumen-fume fractions by chemical class determined by GC-MS is presented in Table 3.5. Condensed neat bitumen fume produced similar and statistically significant increased incidence of papilloma in both strains [$P < 0.05$ for Sencar and $P < 0.002$ for C3H/HeJ mice] and of carcinoma in the C3H/HeJ mice [$P < 0.0001$] compared with respective vehicle controls. Among individual fractions, fraction C was most potent, followed by B. The other single fractions were without significant tumorigenic activity. Raw unheated bitumen produced a few tumours in C3H/HeJ mice, but no tumours were seen when raw bitumen heated to 316 °C, with the fumes permitted to escape, was applied (Sivak *et al.*, 1997).

Groups of 80 male C3H/HeNCrl mice (age, 8 weeks) were exposed to a "field-matched" built-up roofing asphalt (BURA) (CASRN 64742-93-4) type III [class 2 bitumen] fume condensate (collected at 199 °C in tank and matched to the chemistry of field fumes collected at higher

Table 3.4 Mortality analysis and tumorigenic response in mice exposed dermally to 'type III "steep" asphalt' and its fume, and fractions thereof

Group	Treatment	Bitumen dose[a]	Mean survival (days)	Median survival (days)[b]	No. of deaths[c]	Total no. of tumours per group[d]		No. of tumour-bearing mice	Multiplicity
						Papilloma	Carcinoma		
C3H/HeJ mice									
1	Raw bitumen	25	610	698	15	1	3	4	1.0
2	Heated bitumen (less fume)	25	655	–	12				
3	Heated bitumen (plus fume)	25	692	–	9				
4	Neat bitumen fume	25	526	573	28	12[e]	25[e]	21	1.8
5	Solvent control	0	607	629	19				
6	Fraction A	16	690	–	11				
7	Fraction B	2.3	643	678	18	2	10[e]	11	1.1
8	Fraction C	2.6	610	659	24	4	18[e]	20	1.1
9	Fraction D	2.3	572	588	23				
10	Fraction E	1.6	629	675	17				
11	Fractions ABCDE	24.8	555	533	29	30[e]	23[e]	25	2.1
24	0.01% BaP [f]	0	449	464	30	1	28[e]	27	1.1
25	0.001% BaP [f]	0	666	732	15	2	3	5	1.0
26	0.0001% BaP [f]	0	630	727	15				
Sencar mice									
41	Neat bitumen fume	25	571	592	25	21[e]	18[e]	20	2.0
42	Solvent control	0	672	–	12				

[a] mg bitumen, bitumen plus fume, or bitumen fume alone/50µL application
[b] For certain groups with low mortality, this percentile could not be estimated
[c] Number of mice that died before the final euthanasia
[d] Only histologically confirmed skin tumours are presented.
[e] Significantly more tumours or earlier onset or both in this group compared with the respective control
[f] 5, 0.5 and 0.05 µg BaP/50 µL application/group, respectively
From Sivak *et al.* (1997)

Table 3.5 Composition of bitumen-fume fractions by chemical class, as determined by gas chromatography-mass spectrometry

Fraction	Composition
A	Alkanes, alkenes and/or cycloalkanes, alkylated benzenes, naphthalenes, benzothiophenes, biphenyls, fluorenes, indanes and indenes
B	Alkylated benzothiophenes, dibenzo- and/or naphthothiophenes, anthracenes and/or phenanthrenes, fluorenes, pyrenes and/or fluoranthenes, benzo- and dibenzofurans and fluorenones
C	Cycloalkenones and/or alkadienones, alkylated phenylethanones, dihydrofuranones, dihydroindenones, isobenzofuranones, hydroxybenzenethiols, pyrenes and/or fluoranthenes, chrysenes and tricarbocyclic fused-ring thiophenes
D	Alkanones, cycloalkenones and/or alkadienones, alkanoic acids, alkylated carbazoles, dihydrofuranones, furanones, isobenzofuranones, naphthols and phenols
E	Alkanones, alkanoic acids and alkylated benzoic acids

From Sivak *et al.* (1997)

temperatures in the kettle and on the rooftop) or a "laboratory-generated" BURA fume condensate (collected at 232 °C) applied twice per week in a volume of 37.5 µL (25 mg) of mineral oil for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and benzo[*a*]pyrene (0.05% in 37.5 µL of toluene, applied twice per week) were used as negative and positive controls, respectively. The incidence of squamous cell carcinoma was significantly increased (35 out of 64; 55%; $P < 0.0001$) in the mice treated with "laboratory-generated" BURA fume condensate. Squamous cell carcinoma was also observed in "field-matched" BURA fume condensate (8 out of 62; 13%; $P = 0.0063$) and in the positive-control group receiving benzo[*a*]pyrene (34 out of 49; 69%; $P < 0.0001$). No tumours were observed in the negative-control group receiving mineral oil (Clark *et al.*, 2011).

In a parallel study, groups of 80 male C3H/HeNCrl mice (age, 8 weeks) were exposed to a "field-matched" paving fume condensate (CASRN 8052-42-4) [class 1 bitumen] (collected at 148 °C), applied daily in a volume of 37.5 µL (7.14 mg) of mineral oil, for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and benzo[*a*]pyrene (0.05% in 37.5 µL of toluene, applied twice per week) were used as negative and positive controls, respectively. No squamous cell carcinomas were observed in either the treated or vehicle-control mice and a single squamous cell papilloma was observed in the treated group. The incidence of squamous cell carcinoma was significantly increased in the group receiving benzo[*a*]pyrene (37 out of 50; 74%; $P < 0.0001$) (Clark *et al.*, 2011).

Groups of 50 male C3H/HeNCr1BR mice (age, 8–10 weeks) were treated with an "asphalt cement 20" (CASRN 8052-42-4) and a "coastal residuum" (CASRN 64741-56-6), both produced from a naphthenic crude [both class 1 bitumens], which were diluted with mineral oil (USP grade) and applied at the limits of solubility in mineral oil, 30% and 75% (w/w), respectively. A dose of 37.5 µL was applied twice per week to the clipped back of the mice. Benzo[*a*]pyrene was used as a positive control and was applied at a 0.05% (w/v) dilution in toluene. Control groups received mineral oil or toluene. No skin tumours were observed in mice treated with asphalt cement 20, the coastal residuum or the vehicle controls (toluene and mineral oil). Treatment with benzo[*a*]pyrene produced histopathologically confirmed tumours (all but one being carcinoma) in 92% of the mice (Goyak *et al.*, 2011).

Groups of 30 male Crl:CD1 mice (age, 8 weeks) were exposed to a "field-matched" "BURA type III" (CASRN 64742-93-4) [class 2

bitumen] fume condensate (collected at 199 °C) in an initiation-promotion study. The BURA condensate was tested as an initiator by applying it in a volume of 37.5 µL (25 mg) of mineral oil twice per week (total weekly dose, 50 mg) for 2 weeks, followed by 5 µg of TPA (0.01% in acetone), twice per week for 25 weeks. Squamous cell skin papillomas were observed in 5 out of 30 (17%; $P < 0.01$) mice in the group treated with BURA/TPA compared with 1 out of 30 (3%) in the group receiving TPA. The BURA condensate was also tested as a promoter by applying it in a volume of 37.5 µL (25 mg) mineral oil, twice per week for 28 weeks after a single treatment with 7,12-dimethylbenz[*a*]anthracene (DMBA) at a dose of 50 µg. The BURA condensate did not act as a promoter when tested with DMBA (Freeman *et al.*, 2011).

3.1.2 Subcutaneous and/or intramuscular injection

A group of 33 male and 29 female C57 Black mice (age not reported) were treated with a pooled sample of six "steam-refined and air-blown (oxidized) petroleum asphalts" [bitumens class 1 and 2] suspended in olive oil (1%). A group of 32 male and 28 female mice served as controls and were treated with olive oil only. The treated mice were injected subcutaneously in the interscapular region with 0.2 mL of the suspension twice per week for 41 weeks and then once per week. Eight sarcomas ($P = 0.0035$) appeared at the site of injection, with the first tumour appearing during week 36 of the study. No tumours were reported in mice in the control group (Simmers *et al.*, 1959). [The study was poorly reported, the age of the mice was not given, and there was no indication of duration of treatment.]

Two groups of 25 male and 25 female C57 Black mice received a single subcutaneous injection of 200 µg of "steam-refined asphalt" [bitumen class 1] heated to 70 °C, or to "air-refined (oxidized) asphalt" [bitumen class 2] heated to 100 °C in the interscapular region. After 111 days, all mice without palpable deposits of steam-refined bitumen (9 males and 4 females) were re-injected with an additional 200 µg of bitumen. After 4 months, those mice without palpable deposits of air-refined bitumen (11 males and 7 females) were re-injected with an additional 200 µg of bitumen . The mice were maintained for a total of up to 23 months. No skin tumours were observed in 32 autopsied mice from the group receiving steam-refined bitumen. Five malignant tumours (two rhabdomyosarcomas, one sebaceous-gland carcinoma, two not described) were found in the 38 autopsied mice treated with air-refined asphalt (Simmers, 1965a). [There was no information on survival or use of controls in this study. The author reported that study material was found at the intended site of injection in only a small percentage of the treated mice.]

Groups of 12–26 male and 16–27 female C57 Black mice (age, 9–12 weeks) were exposed to a mixture of "aromatics" and "saturates" [a fraction of a class 1 bitumen] isolated by fractionation of a steam-refined bitumen from California crude petroleum by injecting subcutaneously either a single injection of 0.5 µL, 8 injections of 0.25 µL over a 16-week period or 9–11 injections of 1.0 µL over their lifetime. Tumours of the lung and "skin accessory organs" were observed in all groups (Simmers, 1966). [The study was poorly reported, mice were lost and replaced in the middle of the experiment, and no controls were used.]

Groups of 50 C57 Black mice (sex not reported) were exposed to one of four "road petroleum asphalts" [bitumen class 1] obtained by steam distillation of crudes from Mississippi or California, USA, or Venezuela, and by steam-vacuum distillation of one Oklahoma crude. The mice received six injections (twice per week) of 0.1 mL of the respective bitumens, diluted with equal parts of tricaprylin, into the right thigh muscle, and were maintained for 2 years. Another group of 144 mice similarly received six injections

Table 3.6 Study of carcinogenicity in rats exposed to bitumens by inhalation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance
Rat, SPF-Wistar (M, F) 96 wk Fuhst *et al.* (2007)	0, 4, 20, or 100 mg/m^3 bitumen-fume condensate collected at 175 °C comprising a majority (70% mass) of air-rectified bitumen (CAS 64 742-93-4, class 2 bitumen) with the remainder being straight-run vacuum residue (CAS 64 741-56-6, class 1 bitumen) (representative of exposure of workers during road paving) Nose-only exposure, 6 h/d for up to 24 mo 50 males and 50 females/group	See Table 3.7	[NS]

d, day; F, female; h, hour; M, male; mo, month; NS, not significant; wk, week

(twice per week) of 0.1 mL of tricaprylin alone and served as controls. After 2 years, sarcoma at the injection site were noted in one mouse in each of the groups treated with samples from crudes from Mississippi, California and Venezuela. No sarcomas were observed in the group treated with the sample from Oklahoma crude, or in the controls treated with tricaprylin (Hueper & Payne, 1960). [The study was poorly reported and there was no information on survival.]

3.1.3 Inhalation

Groups of 10 male and 10 female C57 Black mice (age not reported) were exposed in a nose-only apparatus to an aqueous aerosol of "petroleum asphalt" droplets suspended in moist air for 30 minutes per day, 5 days per week, for 72 weeks. The aerosol was a pooled sample from six different California refineries and contained both steam- and air-blown samples [class 1 and 2 bitumens]. Seventeen mice were autopsied and the tracheo-bronchial tree and lungs were examined microscopically. One papillary adenoma was observed.

In another experiment, a group of 30 C57 Black mice (sex and age not reported) was exposed to "smoke" generated by heating the pooled petroleum-bitumen sample at ~250 °F [121 °C] for 6–7.5 hours per day, 5 days per week, for 21 months. Twenty-one mice were autopsied and the tracheo-bronchial tree and lungs were examined microscopically. No tumours of the respiratory tract were observed. The author reported epithelial hyperplasia occurred occasionally (Simmers, 1964). [The study was poorly reported, especially the information about aerosol and "smoke" generation and dose. There was limited histopathology, a small number of animals, and no controls were used.]

3.2 Rat

See Table 3.6

3.2.1 Intramuscular injection

Groups of 30 Bethesda Black rats (sex not reported) were exposed to one of four "road petroleum asphalts" [bitumens class 1] obtained by steam distillation of crudes from Mississippi or California, USA, or Venezuela, and by steam-vacuum distillation of one Oklahoma crude. The rats received 12 biweekly injections of 0.2 mL of the respective bitumens, diluted with equal parts of tricaprylin, into the right thigh muscle and held for 2 years. Another group of 60 rats served as untreated controls. After 2 years of observation, injection-site sarcomas were noted in two rats in the Venezuela group, 2 rats in the Mississippi group, 4 rats in the Oklahoma group [P = 0.01] and 6 rats in the California

group [P = 0.001]. No sarcomas were observed in the untreated control group (Hueper & Payne, 1960). [The study was poorly reported. While a significant increase in the incidence of injection-site sarcoma was observed in treated rats compared with the untreated controls, the study was considered inadequate because of the lack of vehicle controls.]

3.2.2 Inhalation

A group of 65 female Bethesda black rats (age, 2 months) were exposed to "fumes" of a "blown-petroleum roofing asphalt" [bitumen class 2] for 5 hours per day, 4 days per week, for 2 years. The "fumes" were generated inside the exposure chamber by placing the bitumen in a large evaporating dish that was heated to ~250–275 °F [121–135 °C]. The authors reported that there were no cancers of the lung observed (Hueper & Payne, 1960). [The study was poorly designed and reported. No controls were used.]

Using a rat model that had been demonstrated to be sensitive to PAH-mediated effects on the respiratory tract, Fuhst *et al.* (2007) exposed groups of 50 male and 50 female SPF-Wistar [WU] rats (age, 8 weeks) to atmospheres containing bitumen-fume condensate collected at 175 °C comprising a majority (~70% mass) of air-rectified bitumen [class 2] (CAS 64742-93-4) with the remainder (~30% mass) being straight-run vacuum residue (class 1) (CAS 64741-56-6). The study material was representative of that to which workers are exposed to during road paving The rats were exposed to concentrations of 0, 4, 20, or 100 mg/m^3 by nose-only exposure, 6 hours per day, 5 days per week for up to 24 months. Target concentrations using Berufsgenossenschaftliches Institut für Arbeitssicherheit (BIA) guidelines were 4, 20, and 100 mg/m^3. Actual mean concentrations were 4.1, 20.7, and 103.9 mg/m^3. Taking into account the conversion factor (1.66) between the absolute concentration of bitumen fume determined using the BIA method, the concentrations were 6.8, 34.4, and 172.5 mg/m^3. Mortality was comparable in all groups, but slightly higher in females than in males. Both males and females exposed to 100 mg/m^3 had a statistically significant increase in bronchiolo-alveolar hyperplasia. However, there was no increase in the number of tumour-bearing animals in any of the bitumen-exposed groups compared with the clean-air control group after an exposure of 24 months (see Table 3.7). One of the males at the highest dose had a nasal adenocarcinoma. There were no statistically significant increases in total or organ-specific tumour incidence observed between the clean-air control and the bitumen exposure groups (Fuhst *et al.*, 2007).

3.3 Rabbit

Skin application

Groups of six New Zealand rabbits (sex not reported) were exposed to one of four "road petroleum asphalts" [bitumen class 1] obtained by steam distillation of crudes from Mississippi and California, USA, and Venezuela, and by steam-vacuum distillation of one Oklahoma crude, respectively. Each rabbit received a skin application of undiluted, heated test material painted on the inside of both ears and on a shaved (2 cm^2) area of the back twice per week for up to 2 years. No results were reported (Hueper & Payne, 1960). [The study was very poorly reported, the age of the rabbits was not reported, the number of treated animals was small, and there were no controls.]

A group of six New Zealand rabbits (sex not reported) received an unspecified dose of heated sample of an "air-blown asphalt" [bitumen class 2] used for roofing purposes twice per week on the inside of both ears and on a shaved area (2 cm^2) of the back for up to 2 years. No tumours were observed (Hueper & Payne, 1960). [The study was very poorly reported, the age of the

Table 3.7 Incidence of tumours in rats exposed to bitumens by inhalation

Number of rats	Concentration of bitumen (mg/m³)							
	Males				Females			
	0 (control)	4	20	100	0 (control)	4	20	100
Total number of rats	50	50	50	50	50	50	50	50
With tumours	28	30	27	30	42	34	39	33
With single tumours	19	21	15	23	24	21	25	18
With multiple tumours	9	9	12	7	18	13	14	15
With benign tumours	25	27	27	25	39	32	36	32
With malignant tumours	7	5	5	6	6	8	5	4
With metastasizing tumours	0	0	0	0	1	0	0	2

From Fuhst *et al.* (2007)

rabbits was not reported, the number of treated animals was small, no controls were used, and survival data were not provided.]

3.4 Guinea-pig

Inhalation

A group of 30 Strain 13 guinea-pigs (age, 2 months; sex not reported) were exposed to "fumes" of a "blown petroleum roofing asphalt" [bitumen class 2] for 5 hours per day, 4 days per week, for 2 years. The "fumes" were generated inside the exposure chamber by placing the bitumen in a large evaporating dish that was heated to ~250–275 °F [121–135 °C]. No tumours were observed (Hueper & Payne, 1960). [The study was poorly designed and reported. No controls were used. No dose concentration was provided.]

4. Mechanistic and Other Relevant Data

4.1 Overview of the mechanisms of carcinogenesis of PAHs

This chapter is a short summary of data relevant to the mechanisms of carcinogenesis of PAHs from *IARC Monograph* 92 (IARC, 2010), with a focus on the PAHs detected in bitumens and bitumen emissions.

4.1.1 Introduction

The toxicokinetics of PAHs have been reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR, 1995) and the International Programme on Chemical Safety (IPCS, 1998), while Conney (1982), Cooper *et al.* (1983), Shaw & Connell (1994), Penning *et al.* (1999), the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) and Xue & Warshawsky (2005) have also reviewed the metabolism and bioactivation of PAHs. Little is known about the toxicokinetics of individual PAHs, or mixtures of PAHs, in humans. Multiple studies have been conducted to monitor urinary metabolites of PAHs and PAH–DNA adducts in

the lymphocytes of workers exposed to mixtures of PAHs. However, most of the available data on toxicokinetic parameters for PAHs are derived from studies of benzo[*a*]pyrene in animals.

Because of their lipophilicity, PAHs dissolve into and are transported by diffusion across lipid/lipoprotein membranes of mammalian cells, thus facilitating their absorption by the respiratory tract, gastrointestinal tract and skin. PAHs with two or three rings can be absorbed more rapidly and extensively than those with five or six rings. Once absorbed, PAHs are widely distributed throughout the body, with some preferential distribution to or retention in fatty tissues. They are rapidly metabolized to more soluble metabolites (epoxides, phenols, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, quinones and tetrols), and conjugates of these metabolites are formed with sulfate, glutathione (GSH) or glucuronic acid. The covalent binding of reactive PAH metabolites to form DNA adducts may represent a key molecular event in the development of mutations and the initiation of cancer. From the structures of the DNA adducts that are formed, the precursor metabolites may be inferred. PAHs are eliminated from the body principally as conjugated metabolites in the faeces, via biliary excretion, and in the urine.

Most PAHs with potential biological activity range in size from two to six fused aromatic rings. Because of this vast range in relative molecular mass, several of the physicochemical properties that are critical to their biological activity vary greatly. Five properties in particular have a decisive influence on the bioavailablilty of PAHs: vapour pressure; adsorption onto surfaces of solid carrier particles; absorption into liquid carriers; lipid/aqueous partition coefficient in tissues; and limits of solubility in the lipid and aqueous phases of tissues. These properties are intrinsically linked to the metabolic activation of the most toxic PAHs, and an understanding of the nature of this interaction may facilitate the interpretation of studies on their deposition and disposition that are occasionally conflicting.

4.1.2 Absorption

PAHs can be absorbed via the respiratory tract, the gastrointestinal tract and the skin.

(a) Absorption via the respiratory tract

Respiratory absorption depends on the vapour pressure of the PAH between the particulate and gaseous phase of the aerosol by which the substance is emitted into the atmosphere. The vapour pressure of PAHs decreases drastically with increasing molecular mass (Lohmann & Lammel, 2004), so that two-ring naphthalenes are mostly found in the gas phase, whereas five-ring PAHs such as benzo[*a*]pyrene are mostly adsorbed on airborne particles at room temperature (Lane & Gundel, 1996). Strong sorption of a PAH onto particles can further increase the particle-bound fraction of that substance (Lohmann & Lammel, 2004). Gas/particle partitioning is also of great importance during exposure by inhalation, to determine the probable sites of deposition within the respiratory tract. The smaller gaseous PAHs are deposited mostly as soluble vapours, whereas five- to six-ring aromatic compounds are mostly particle-associated at ambient temperatures and can be expected to be deposited with the carrier particles. The rate and extent of absorption by the respiratory tract of PAHs from PAH-containing particles are dependent on particle size (i.e. aerodynamic diameter, which influences regional deposition in the respiratory tract) and the rate of release of PAHs from the particle. Because the release of PAHs is extraneous in exposure to vapours, the rate and extent of absorption of inhaled vapour-phase PAHs are different from those of particle-bound PAHs.

After deposition in the respiratory tract, the sorptive properties of PAHs are a major determinant of the bioavailability of the substance in the organism. For solid particles, the major

determinant for the release is the rate of desorption of the hydrocarbons from the surface, whereas for liquid aerosols, either the dissolution of the entire particle or desorption from insoluble carrier particles is a decisive factor. Substantial fractions of inhaled PAHs deposited in the tracheobronchial region and upper airways can be redistributed by the mucociliary escalator to the gastrointestinal tract, which thereby changes the exposure route from inhalation to ingestion (Sun *et al.*, 1982).

After deposition and desorption from their carrier particles, PAHs are absorbed through the epithelial barriers onto which they are deposited. Highly lipophilic PAHs that are released from particles deposited in the conducting and bronchial airways are retained for several hours and absorbed slowly by a diffusion-limited process, whereas PAHs that are released from particles in alveolar airways are absorbed within minutes (Gerde *et al.*, 1991a, b; Gerde & Scott, 2001). A major effect of the metabolic conversion of PAHs of lower molecular mass is to decrease their lipophilicity and thus accelerate their mobility in tissues (Gerde *et al.*, 1997). Phase I metabolites are slightly more mobile and phase II metabolites are considerably more mobile than the parent compound. As a result, the overall effect of metabolism in the epithelium at the site of entry is to accelerate transport of a lipophilic substrate into the circulation and thereby directly decrease high, acute exposures to this particular epithelial cell population. This local metabolism in airway epithelium probably explains the high levels of benzo[*a*]pyrene-related DNA adducts that have been measured in pure preparations of bronchial epithelial cells from patients with cancer of the lung (Rojas *et al.*, 2004).

(b) Absorption via the gastrointestinal tract

PAHs are absorbed via the gastrointestinal tract through diffusion across cellular membranes, based on their lipophilicity, and through normal absorption of dietary lipids (O'Neill *et al.*, 1991). Absorption of specific PAHs, such as benzo[*a*]pyrene, has been demonstrated after oral administration of radiolabelled compounds to laboratory animals (for review, see ATSDR, 1995; IPCS, 1998). Results from studies in animals have indicated that absorption is rapid (Rees *et al.*, 1971; Modica *et al.*, 1983), that fractional absorption of PAHs of lower relative molecular mass, such as two-ring naphthalene, may be more complete than that of PAHs of higher relative molecular mass, such as five-ring benzo[*a*]pyrene (Chang, 1943; Modica *et al.*, 1983), and that the presence of other materials, such as bile salts or components of the diet, can influence the rate or extent of absorption of PAHs from the intestine (Rahman *et al.*, 1986).

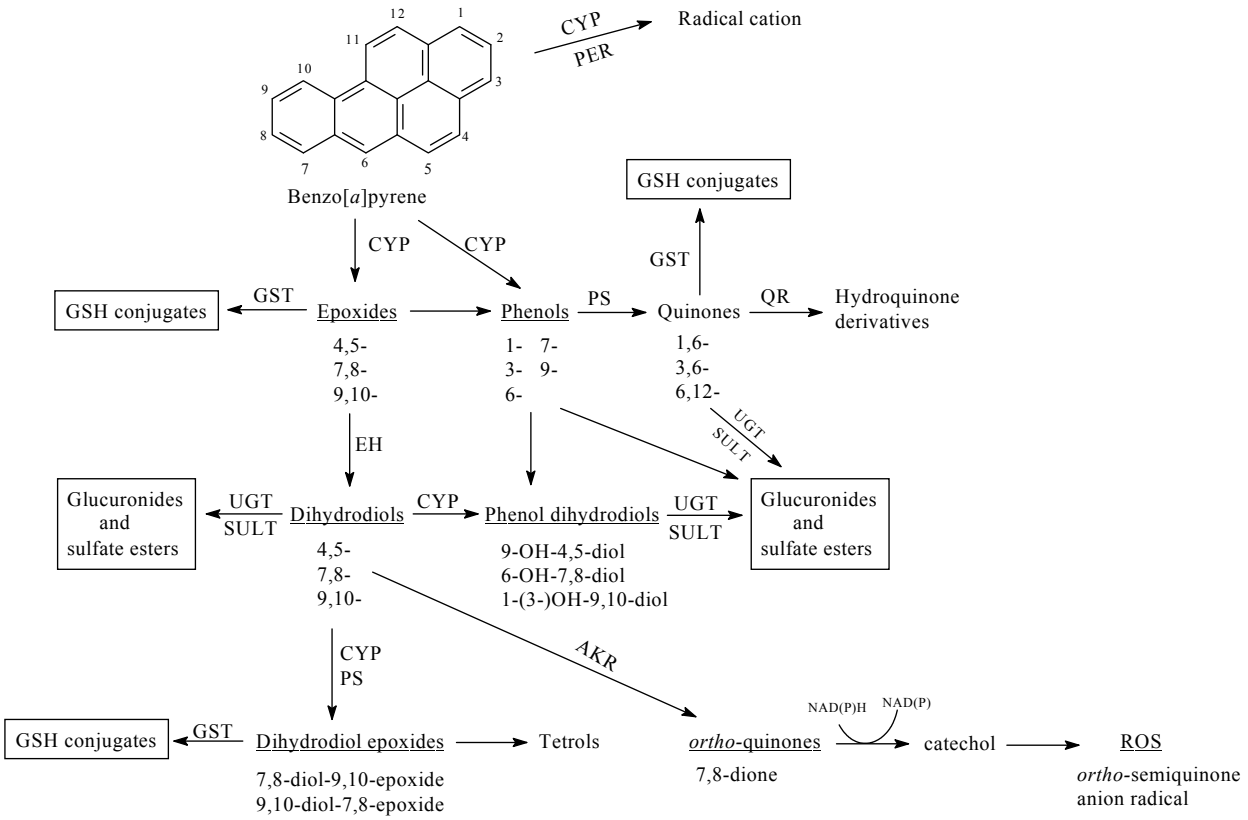
(c) Absorption via the skin

Evidence for the dermal absorption of PAHs includes the detection of elevated levels of PAH metabolites, such as 1-hydroxypyrene, in the urine of humans exposed dermally to complex mixtures of PAHs, such as coke-oven emissions or creosote mixtures in the workplace (Van Rooij *et al.*, 1993a, b), or coal-tar ointments (Godschalk *et al.*, 1998). Results from studies in animals have indicated that dermal absorption of PAHs can be rapid and extensive (Withey *et al.*, 1993a).

4.1.3 Distribution

The data on distribution of PAHs are based mainly on studies in rats, and indicate that: (i) absorbed PAHs are widely distributed to most organs and tissues; (ii) fatty tissues can serve as storage sites to which PAHs may be gradually absorbed and from which they are then released; and (iii) the gastrointestinal tract can contain high concentrations of PAHs and their metabolites after exposure (by any route), due to mucociliary clearance from the respiratory tract and hepatobiliary excretion of metabolites (Mitchell & Tu, 1979; Mitchell, 1982, 1983; Sun *et al.*, 1984; Withey *et al.*, 1991, 1993b). The results from

Fig. 4.1 Metabolic schema for benzo[*a*]pyrene



AKR, aldo-keto reductase; CYP, cytochrome P450; EH, epoxide hydrolase; GSH, glutathione; GST, glutathione *S*-transferase; NAD(P)H, nicotinamide adenine dinucleotide (with or without phosphate); PER, peroxidases; PS, prostaglandin H synthase; QR, quinone reductase; ROS, reactive oxygen species; SULT, sulfotransferase; UGT, Uridine 5′-diphosphate-glucuronosyltransferase
Adapted from Cooper *et al.* (1983), ATSDR (1995), IPCS (1998).

these studies are consistent with the concept that PAHs are, in general, cleared rapidly from the initial sites of deposition in the respiratory tract and distributed to a significant extent in the gastrointestinal tract, liver and kidney; the kinetics and patterns of distribution, however, can be influenced by size and compositional characteristics of the particulate matter, as well as by the chemical properties of the PAHs themselves (IARC, 2010).

4.1.4 Metabolism

The metabolism of benzo[*a*]pyrene has been studied extensively in human and animal tissues, and generally serves as a model for the metabolism of other PAHs (for review, see ATSDR, 1995; IPCS, 1998). A metabolic scheme for benzo[*a*]pyrene is presented in Fig. 4.1, which shows pathways to the formation of epoxides, phenols, quinones, hydroquinones, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, tetrols and other potentially reactive intermediates.

Benzo[*a*]pyrene is initially metabolized by cytochrome P450 (CYP) monooxygenases to several epoxides. CYP1A1 can metabolize a wide range of PAHs, but other CYPs, including CYP1A2 and members of the CYP1B, CYP2B, CYP2C and CYP3A families of enzymes, have been demonstrated to catalyse the initial oxidation of benzo[*a*]pyrene and other PAHs to varying extents (for review, see IPCS, 1998; Xue

& Warshawsky, 2005). PAHs are recognized inducers or inhibitors (Shimada & Guengerich, 2006) of CYP enzymes, and exposure to PAHs can therefore influence the balance of phase I and phase II enzymes, which can determine whether or not a toxic cellular response occurs. The mammalian CYP genes that encode CYP1A1, 1A2 and 1B1 are regulated in part by the aryl hydrocarbon receptor (AhR). Differences in AhR affinities in inbred mice correlate with variations in the inducibility of CYP and may be associated with differences in the risk for cancer from PAHs (Nebert *et al.*, 2004). A correlation between the variability in AhR affinity in humans and differences in cancer risk remains unproven. Therefore, the role of CYP in activation *versus* detoxification probably depends on multiple factors such as the subcellular content and location, the degree of phase II metabolism and the pharmacokinetics of the chemical.

Epoxides may rearrange spontaneously to phenols, be hydrated via epoxide hydrolase catalysis to dihydrodiols or be conjugated with GSH, either spontaneously or via glutathione-*S*-transferase (GST) catalysis. It has been proposed that the formation of 1-, 3- and 6-hydroxybenzo[*a*]pyrene from benzo[*a*]pyrene and their subsequent conversion to quinones involve CYP isoforms (Cavalieri *et al.*, 1988) and 6-hydroxybenzo[*a*]pyrene can also be formed by prostaglandin H synthase (Cooper *et al.*, 1983; for a review, see IPCS, 1998). Quinones can be converted to hydroquinone derivatives by quinone reductase or be conjugated with GSH, sulfate or glucuronic acid.

Dihydrodiol derivatives can be further oxidized by CYPs to form phenol dihydrodiols or dihydrodiol epoxides. Phenols, phenol dihydrodiols and dihydrodiols can be conjugated with glucuronic acid or sulfate. Dihydrodiol epoxides may also be formed from dihydrodiols by reaction with peroxyl radicals generated from the oxidative biosynthesis of prostaglandins from fatty acids via prostaglandin H synthase (Marnett, 1981, 1987; Reed *et al.*, 1988; Eling *et al.*, 1990). The metabolic fate of dihydrodiol epoxides includes conjugation with GSH or covalent modification of cellular macromolecules that possibly lead to mutagenic and carcinogenic responses.

Dihydrodiols may also be metabolized to *ortho*-quinones by aldo-keto reductases (AKR1C1–AKR1C4, AKR1A1). *ortho*-Quinone derivatives have been demonstrated *in vitro* to produce, via redox cycling with nicotinamide adenine dinucleotide (with or without phosphate) (NAD(P)H) and copper, reactive oxygen species that cause DNA fragmentation and mutation of *TP53* (Flowers *et al.*, 1996, 1997; Penning *et al.*, 1999; Yu *et al.*, 2002). PAH *ortho*-quinones produced by this pathway are also ligands for AhR (Burczynski & Penning, 2000). This effect of *ortho*-quinones may play a role in the mutagenicity and carcinogenicity of benzo[*a*]pyrene and other PAHs.

The stereochemistry of the dihydrodiol epoxide derivatives of benzo[*a*]pyrene is important in the toxicity of benzo[*a*]pyrene and other PAHs (Conney, 1982; Shaw & Connell, 1994; for a review, see IPCS, 1998). Of the four possible stereoisomers of the 7,8-dihydrodiol-9,10-epoxide benzo[*a*]pyrene derivative, the predominant one formed in mammaliam systems, (+)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, has been shown to have the highest tumour-initiation activity and to be the predominant metabolite that forms DNA adducts in mammalian tissues exposed to benzo[*a*]pyrene. The formation of DNA adducts may be a first step in the initiation of carcinogenesis by PAHs.

4.1.5 Elimination

Results from studies of animals exposed to PAHs indicate that their metabolites are largely excreted as conjugates of GSH, glucuronic acid or sulfate in the faeces, via biliary excretion and in the urine (for review, see ATSDR, 1995; IPCS, 1998).

4.1.6 Mechanisms of metabolic activation and carcinogenesis

(a) Bay- and fjord-region PAH diol epoxide

In benzo[*a*]pyrene, the bay region encompasses four carbons (carbons 10, 10a, 10b and 11) and three carbon–carbon bonds. In the case of benzo[*a*]pyrene, metabolism by CYP isozymes at the C7–C8 aromatic double bond creates an arene oxide, benzo[*a*]pyrene-7,8-oxide. Benzo[*a*]pyrene-7,8-oxide is hydrated by epoxide hydrolase to form a dihydrodiol (diol), benzo[*a*]pyrene-7,8-diol. Benzo[*a*]pyrene-7,8-diol is further metabolized (epoxidized) by the CYP isozymes at the C9–C10 double bond to give the bay-region diol epoxide, benzo[*a*]pyrene-7,8-diol-9,10-oxide. This diol epoxide possesses the inherent ability to undergo carbon–oxygen bond scission or ring opening, to form a carbonium ion (i.e. a positively charged carbon atom) on carbon 10. Carbonium ions are highly reactive species that react with nucleophiles, such as DNA and proteins, to form covalent adducts. One of the postulated quantitative measures of the reactivity of diol epoxides is carbonium ion delocalization energy ($\Delta E_{deloc}/\beta$), which is based on perturbational molecular orbital calculations that predict the ease of carbonium-ion formation. The greater the $\Delta E_{deloc}/\beta$ value, the more reactive the carbonium ion; greater values were associated with PAHs that exhibited higher tumorigenic activities (Jerina *et al.*, 1976). This theory was expanded to include PAH structures with deeper peripheral indentations in their structure – those that contain a fjord region (e.g. dibenzo[*a*,*l*]pyrene). The fjord region encompasses five carbons and four carbon–carbon bonds; in some cases, the steric interactions between atoms within the fjord region of the PAH forces the PAH ring system out of planarity (Katz *et al.*, 1998). Some PAH fjord-region diol epoxides are non-planar (Lewis-Bevan *et al.*, 1995), and these non-planar PAH diol epoxides possess even higher reactivities than those predicted by $\Delta E_{deloc}/\beta$ alone. The enzymes primarily responsible for phase I metabolism of PAHs are CYP1A1, CYP1A2 and CYP1B1 and NADPH CYP reductase, which convert PAHs to different arene oxides, and epoxide hydrolase that catalyses the addition of water to the arene oxides to form *trans*-diols. PAH phenols are also formed either by rearrangement of arene oxides or by direct oxygen insertion into a carbon–hydrogen bond. Quinones are formed by further oxidation of phenols or by the enzymatic action of aldo-keto reductases (AKRs) on PAH diols. The phase II enzymes, uridine 5′-diphosphate (UDP)-glucuronosyltransferase (UGT), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), sulfotransferase (SULT) and GST, conjugate PAH diols, phenols and epoxides to glucuronic acid, sulfate and GSH, respectively.

The stereochemistry of the metabolic transformation of PAHs to diols and diol epoxides is an important component of this mechanism of action and affects the biological activities of these metabolites. CYPs can be regio- and stereospecific in their action. The stereospecific metabolizing activity of each CYP, in combination with the capacity of many PAH carbons to form chiral centres through metabolism, can create multiple forms of many PAH metabolites. For example, benz[*a*]anthracene is metabolized in a stereospecific manner at the C3–C4 bond to give two benz[*a*]anthracene-3,4-oxides (benz[*a*]anthracene-3*S*,4*R*-oxide and benzo[*a*]anthracene-3*R*,4*S*-oxide) in different amounts (Yang, 1988), which are then hydrated in a stereospecific manner by epoxide hydrolase to give two benzo[*a*]anthracene-3,4-diols (benzo[*a*]anthracene-3*R*,4*R*-diol and benzo[*a*]anthracene-3*S*,4*S*-diol) in different amounts (Yang, 1988). Each diol can form two diol epoxides that vary depending on the relative position of epoxide function in relation to one of the diol hydroxyls – a *syn*-benzo[*a*]anthracene diol epoxide and an *anti*-benz[*a*]anthracene diol epoxide – for a total of four benz[*a*]anthracene diol epoxides. While diol epoxides are not subject to enzymatic hydrolysis

by epoxide hydrolase (Thakker *et al.*, 1976; Wood *et al.*, 1976), they are non-enzymatically hydrolysed to tetrols (Jankowiak *et al.*, 1997) and are enzymatically detoxified by GSTs (Dreij *et al.*, 2002). Therefore, the formation and degradation of stereochemically specific diol epoxides is dependent on species, strain, sex, organ, tissue, type of CYP and phase II enzymes.

Bay-region and fjord-region diol epoxides possess many biological activities; one of the most important of these is the formation of stable covalent adducts with DNA. The nature and sequence specificity of these DNA adducts is based, in part, on the absolute configuration, molecular conformation and stereochemistry of the diol epoxide, the specific purine (or pyrimidine base) that is adducted, the site of adduction and the nature and sequence of the DNA that is adducted (Jerina *et al.*, 1976). As described previously, each PAH diol can form four diastereomeric *syn*- and *anti*-diol epoxides. When diol epoxides react with DNA (mainly at the purines, i.e. deoxyguanosine and deoxyadenosine), each can form both *cis* and *trans* adducts, thus giving a total of 16 possible DNA adducts. However, in most cases, far fewer DNA adducts are actually observed. While PAH–DNA adducts represent a type of DNA damage, they can be converted into heritable mutations by misrepair or faulty DNA synthesis (Watanabe *et al.*, 1985; Rodriguez & Loechler, 1995). Bay- or fjord-region diol epoxide–DNA adducts are repaired by nucleotide-excision repair (Geacintov *et al.*, 2002). Numerous examples have shown that bay- and fjord-region diol epoxides of PAHs are mutagenic in bacteria, cause damage to DNA or induce chromosomal damage in human and mammalian cells in culture, and induce skin, lung or liver tumours in mice, similarly to the parent PAH. Furthermore, PAHs or their bay- or fjord-region diol epoxides induced mutations in critical genes associated with chemical carcinogenesis such as proto-oncogenes (Prahalad *et al.*, 1997; Chakravarti *et al.*, 1998) and tumour-suppressor genes (Ruggeri *et al.*, 1993; Rämet *et al.*, 1995). A strong relationship exists between the nature of the DNA adducts of the diol epoxide and the type of *ras* proto-oncogene mutations observed in DNA from tumours induced by PAHs. In general, PAHs that form DNA adducts at deoxyguanosine primarily induce mutations in the *ras* gene at codons 12 or 13, while those that form DNA adducts at deoxyadenosine induce mutations in the *ras* gene at codon 61. PAHs that induce adducts at both purine bases induced both types of mutations (Ross & Nesnow, 1999). In addition to their genotoxic effects, some bay- or fjord-region diol epoxides are reported to induce apoptosis and cell-cycle arrest in mammalian cells (Chramostová *et al.*, 2004).

The diol epoxide–DNA adducts of PAHs have also been identified in populations exposed to complex mixtures that contain PAHs, i.e. foundry workers (Hemminki *et al.*, 1988; Perera *et al.*, 1988), coke-oven workers (Rojas *et al.*, 1995; Pavanello *et al.*, 1999), cigarette smokers (Rojas *et al.*, 1995; Lodovici *et al.*, 1998), chimney sweeps (Pavanello *et al.*, 1999) and people exposed to mixtures in smoke emissions from coal combustion (Mumford *et al.*, 1993). Some bay- or fjord-region diol epoxides form DNA adducts in the human *TP53* tumour-suppressor gene at sites that are hotspots for cancer of the lung (Smith *et al.*, 2000).

(b) Cyclopenta-ring oxidation

The cyclopenta-ring oxidation mechanism involves the formation of the arene oxide at a highly electron-rich isolated double bond that is located at a five-membered ring within a PAH. The cyclopenta ring is an external five-membered carbocyclic ring that is situated on a carbocyclic hexameric fused-ring system. For example, a cyclopenta-ring derivative of pyrene is cyclopenta[*cd*]pyrene. Since the cyclopenta ring is usually the region of highest electron density, it is a major site of oxidation by the CYP isozymes (Nesnow *et al.*, 1984, 1988). Preparations of rat

and mouse liver, human and rodent cells in culture, human CYP1A1, CYP1A2 and CYP3A4, human liver microsomes and rats *in vivo*, metabolize cyclopenta-fused PAHs at the cyclopenta-ring double bond to give cyclopenta-ring oxides and diols (Gold & Eisenstadt, 1980; Mohapatra *et al.*, 1987; Kwon *et al.*, 1992; Nyholm *et al.*, 1996; Johnsen *et al.*, 1998a, b; Hegstad *et al.*, 1999). Cyclopenta-ring oxides are reactive intermediates and bind to DNA to form DNA adducts *in vitro* and *in vivo* mainly at deoxyguanosine (Surh *et al.*, 1993; Beach & Gupta, 1994; Hsu *et al.*, 1997, 1999). Cyclopenta-ring oxides are hydrated by epoxide hydrolase to diols. Some cyclopenta-ring diols are conjugated to sulfate esters by PAPS SULT. Cyclopenta-ring oxides, like their parent cyclopenta-PAHs, are mutagenic in bacteria and mammalian cells and can morphologically transform immortalized cells in culture (Bartczak *et al.*, 1987; Nesnow *et al.*, 1991). In general, cyclopenta-ring derivatives of PAHs are more mutagenic and more carcinogenic than their unsubstituted counterparts (e.g. pyrene is not carcinogenic, while cyclopenta[*cd*]pyrene has been shown to induce mutations at the K_i-*Ras* proto-oncogene in lung tumours of treated mice and is highly carcinogenic) (Nesnow *et al.*, 1994, 1998).

(c) *Formation of radical cations*

Removal of one electron from the π system of a PAH generates a radical cation, in which the positive charge is usually localized at an unsubstituted carbon atom or adjacent to a methyl group. Nucleophilic attack at the position of highest charge density at an unsubstituted carbon atom produces an intermediate radical that is further oxidized to an arenium ion to complete the substitution reaction. Development of the chemistry of PAH radical cations has provided evidence that these intermediates can play a role in the process of tumour initiation by several potent PAHs (Cavalieri & Rogan, 1985, 1992; for a review see IARC, 2010).

(d) *Formation of* ortho*-quinones and generation of reactive oxygen species*

As seen above and in Fig. 4.1, $NAD(P)^+$-dependent dehydrogenation of PAH dihydrodiols, which is catalysed by monomeric cytosolic oxidoreductases of the AKR superfamily, yield ketols, which spontaneously rearrange to catechols. Catechols are extremely air-sensitive and undergo two sequential one-electron auto-oxidation steps to yield the corresponding reactive PAH *ortho*-quinones (Smithgall *et al.*, 1986, 1988). An intermediate in this auto-oxidation is the corresponding *ortho*-semiquinone anion radical. Each one-electron oxidation event (either catechol → *ortho*-semiquinone anion radical or *ortho*-semiquinone anion radical → *ortho*-quinone) yields reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxyl radical). This leads to oxidative stress and a pro-oxidant state. For benzo[*a*]pyrene, this reaction sequence would comprise dehydrogenation of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to form 7,8-dihydroxybenzo[*a*]pyrene (catechol) and auto-oxidation to yield benzo[*a*]pyrene-7,8-dione (Penning *et al.*, 1996, 1999).

The resulting PAH *ortho*-quinone is a highly reactive Michael acceptor that can undergo 1,4- or 1,6-Michael addition reactions with cellular nucleophiles (e.g. L-cysteine, GSH) to yield conjugates (Murty & Penning, 1992a, b; Sridhar *et al.*, 2001) or with macromolecules (e.g. protein, RNA and DNA) to yield adducts (Shou *et al.*, 1993; McCoull *et al.*, 1999; Balu *et al.*, 2004). The PAH *ortho*-quinones and the reactive oxygen species that they generate may form mutagenic lesions in DNA (initiation) or act as electrophilic and pro-oxidant signals that may affect cell growth. In this manner, the pathway may contribute to the complete carcinogenicity of the parent PAH (for a review, see IARC, 2010).

4.1.7 Activation of AhR and carcinogenesis

Several of the biological effects of PAHs, such as induction of xenobiotic metabolizing enzymes, immunosuppression, teratogenesis and carcinogenicity, are thought to be mediated by activation of AhR signalling. This receptor is widely distributed and has been detected in most cells and tissues.

AhR is a ligand-activated transcription factor that mediates responses to a variety of toxins; PAHs and halogenated aromatic toxins such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD) are among the best characterized, high-affinity, exogenous AhR ligands (Stejskalova *et al.*, 2011).

This receptor plays an essential role in the regulation of the metabolism of xenobiotics (phase I/phase II enzymes; also termed AhR signalling in the adaptive-response pathway) and in the initiation of homeostatic responses (also termed AhR signalling in endogenous pathways) upon exposure to xenobiotics.

There is also evidence that AhR signals act through a variety of pathways, and more recently cross-talk with other nuclear receptors has been demonstrated to enable cell-type- and tissue-specific control of gene expression (Puga *et al.*, 2009; Stevens *et al.*, 2009).

Different high-affinity ligands for AhR have been shown to differ in their biological responses. Furthermore, translocation of activated AhR may require threshold concentrations of the ligand and involves a variety of cellular response. Altered AhR-signalling responses may therefore be designated as adaptive or toxic, and/or as perturbations of endogenous pathways. Among these effects, alteration of xenobiotic enzymes and alteration of immunological mechanisms are most relevant to PAH-induced carcinogenesis.

(a) Alteration of xenobiotic enzymes and carcinogenicity

AhR-induced expression of CYP1 enzymes impacts the metabolism of PAHs and results in genotoxicity, mutations and tumour initiation (Nebert *et al.*, 2000). Individual risk for cancer may be attributed to metabolic activation of PAHs, but the balance between detoxification and metabolic potentiation depends on many factors (Nebert *et al.*, 2004), and loosely or tightly coupled phase I and phase II metabolic reactions may be influential factors for risk of toxicity and cancer (see Sections 4.1.6 and 4.3 in this *Monograph*).

(b) Alteration of immunological mechanisms

Carcinogenic PAHs have been found to suppress the immune system of animals (White & Holsapple, 1984; Wojdani & Alfred, 1984; Wojdani *et al.*, 1984).

Generally, a positive correlation is seen between the carcinogenicity and immunotoxicity of a PAH. This correlation probably exists because both carcinogenicity and immunotoxicity are largely dependent on AhR binding, increased CYP expression and the formation of bioactive metabolites (White *et al.*, 1985; Burchiel & Luster, 2001).

PAHs exert many important effects on the immune system of many species. The dose and route of exposure determine the nature of the effect on specific and adaptive immune responses. Studies with pure PAHs suggest that AhRs play a critical role in the activation of immunotoxic PAHs, such as benzo[*a*]pyrene, via the diol epoxide mechanism that leads to DNA interactions that cause genotoxicity and suppress immunity by TP53-dependent pathways. Benzo[*a*]pyrene diol epoxide may also affect protein targets and modulate lymphocyte signalling pathways via non-genotoxic (epigenetic) mechanisms. Certain oxidative PAHs, such as benzo[*a*]pyrene quinones, may be formed

via CYP-dependent and -independent (peroxidase) pathways. Redox-cycling PAHs quinones may exert oxidative stress in lymphoid cells.

4.2 Absorption, distribution, metabolism, and excretion of bitumens and bitumen fume

4.2.1 Introduction

Bitumen fume comprises a complex mixture of constituents, which is strongly dependent on how the fume is generated. The pharmacokinetics of these individual components will depend on their physicochemical properties and on the biological interactions with different tissues and organs. Notably, to become available systemically, components of bitumen fume and condensate need to cross barriers such as the alveolar barrier in the case of inhalation, or the intestinal epithelial barrier after ingestion, or the skin after dermal exposure. Upon entry into the systemic circulation many of the constituents of bitumen are subject to extensive and tissue-specific metabolism that modifies highly lipophilic molecules to facilitate their clearance from the body through excretion into urine and faeces (see the Overview in Section 4.1).

Several studies in humans and animals *in vitro* have demonstrated that PAHs present in condensates of bitumen fume are subject to extensive metabolism. As summarized in Section 4.1, PAHs undergo metabolic activation by cytochrome P450 (CYP450) enzymes, primarily CYP1A1, CYP1A2 and CYP1B1. However, there are considerable differences in the activity of CYP monooxygenase between animal species and between animals and humans. An interpretation of animal data for assessing human absorption, distribution, metabolism and excretion is therefore confounded by several factors, most notably the significant interspecies differences in metabolism and metabolic clearance. While there is overwhelming evidence that aliphatic, aromatic and/or polycyclic aromatic hydrocarbons within bitumen are substrates for CYP monooxygenases, the carcinogenic potential of hydrocarbons is primarily caused by their metabolic activation to proximate and ultimate carcinogens. In an initial step, aromatic hydrocarbons are metabolically activated to epoxides that either isomerize to phenols or upon addition of water form *trans*-dihydrodiols. Further oxidative metabolism leads to the production of potentially genotoxic, DNA-reactive metabolites (ultimate carcinogens).

Naphthalene, phenanthrene and pyrene are PAHs of low relative molecular mass that are found in bitumen fume at various concentrations, depending on the temperature at which the fume is generated (see Section 1).

Naphthalene is metabolized in humans and rodents to reactive intermediates by hepatic and extrahepatic CYP enzymes; these reactive metabolites deplete GSH and bind covalently to proteins to cause necrosis in Clara cells of the lung. Naphthalene is metabolized by CYP1A1, 1A2, 2A1, 2E1, 2F (Waidyanatha & Rappaport, 2008) and 2S1 (Karlgren *et al.*, 2005) to its 1,2-epoxide, which may undergo non-enzymatic isomerization to 1- and 2-hydroxynaphthalene (1- and 2-naphthol), and is further metabolized by microsomal epoxide hydrolase EPXH1 to 1,2-dihydro-1,2-dihydroxy-naphthalene (*trans*-1,2-dihydrodiol). Both 1- and 2-naphthol are further oxidized to 1,2- and 1,4-naphthoquinone (Waidyanatha & Rappaport, 2008). The main route for excretion of naphthalene metabolites in humans and rodents is via the urine (Buckpitt *et al.*, 2002).

Another significant component of bitumen fume is phenanthrene, which is initially converted to three different isomeric epoxides. This non-enzymatic isomerization process results in five different isomeric phenols, whereas three different *trans*-dihydrodiols are formed by the action of epoxide hydrolase. The regio-selective oxidation differs among species and according

to the CYP enzyme involved, as was demonstrated by Jacob & Grimmer (1996). In rats, the 9,10-position of phenanthrene is mainly oxidized by CYP1A1, 1A2, and 2B1 (84–100%), whereas in humans the positions 1,2-, 3,4-, and 9,10- of phenanthrene are oxidized by the enzymes CYP1A1, 1A2, 3A4, 2A6 and 2E1 (Jacob *et al.*, 1996a, b). In rats, phenanthrene-9,10-epoxide is further conjugated by GST and excreted predominantly via the mercapturic-acid pathway (Boyland & Sims, 1962a, b, c; Lertratanangkoon *et al.*, 1982). In humans, phenanthrene is metabolized at the 9,10-position to 9-phenanthrol and *trans*-9,10-dihydrodiol.

The phase-II enzymes catalyse conjugation reactions, resulting in conversion of xenobiotic substances into water-soluble metabolites that can be excreted via the urine or bile. Such conjugation reactions include glucuronidation, sulfation, and GSH and amino-acid conjugation.

A fine balance exists between activation and detoxification of constituents of bitumen fume and condensate, whereby inadequate or saturated detoxification pathways may result in accumulation of DNA-reactive metabolites. Information on profiles of absorption, distribution, metabolism and excretion associated with exposure to bitumen is therefore of critical importance for understanding its toxicological properties. Moreover, while metabolic activation of constituents of bitumen fume and condensate may lead to DNA damage, this damage is also subject to DNA repair, which is not always error-free. As a consequence, single cells bearing DNA adducts may undergo mutation during replication, which can result in malignant transformation leading to tumour formation by clonal expansion.

It is therefore important to study the capacity of target tissues to metabolically activate individual constituents of bitumen fume. In addition, a wide range of PAHs are capable of activating nuclear transcription factors, including the AhR, which control transcriptional activation of genes encoding metabolic enzymes. This may also influence pharmacokinetics and toxicity of the constituents of bitumen fume and condensate.

It should be noted that many of the laboratory-generated bitumen fumes or condensates used in experimental studies do not necessarily represent field-generated fumes. Due to the complexity of the problem, models specific for bitumen-fume exposures have not yet been proposed. Nonetheless, urinary 1-hydroxypyrene (1-OHP) and other PAH metabolites have been used as markers to monitor exposure to bitumen fume and PAHs (see Section 4.2.3).

4.2.2 Toxicokinetics of bitumens and bitumen emissions

(a) Humans

Toxicokinetic information in humans was obtained from a study designed to investigate the potential for percutaneous absorption of aerosols and vapours of bitumen (Walter & Knecht, 2007). Ten male non-smoking volunteers were exposed in an experimental chamber to bitumen emissions generated from commercial bitumen B65 for 8 hours. To ensure that there was no exposure by inhalation, the subjects used a powered air-purifying respirator (PAPR) for the entire period of exposure and wore only shorts and shoes. Under the same conditions, two other volunteers did not use a PAPR, so that a comparison could be made between inhalation of PAH from bitumen emissions and dermal uptake. Urinary PAH metabolites of phenanthrene, pyrene and chrysene were determined.

The bitumen emissions in the chamber were measured to be ~20 mg/m^3 with a vapour content of about 88%. The components of higher relative molecular mass were present predominantly in the aerosol phase, whereas lighter polycyclics remained in the vapour phase. The proportion of PAHs absorbed via the skin was between 50% and 60% of the total amount incorporated for pyrene, chrysene and phenanthrene.

Table 4.1 Half-lives of urinary metabolites of polycylic aromatic hydrocarbons (PAHs) after exposure of male non-smoker volunteers to bitumen emissions

PAH metabolites	With PAPR (n = 10 volunteers)[a]	Without PAPR (n = 2 volunteers)[a]
1-OH-phenanthrene	5.96 ± 1.70	5.43 ± 2.70
4-OH-phenanthrene	8.49 ± 1.65	4.83 ± 1.55
Total phenanthrene	8.63 ± 2.01	5.22 ± 3.07
6-OH-chrysene	7.61 ± 1.49	7.74 ± 3.34
1-OH-pyrene	8.16 ± 1.54	5.65 ± 0.99

[a] Values after percutaneous only or combined inhaled/percutaneous exposure were obtained from subjects with or without powered air-purifying respirator (PAPR), respectively. Values are given in hours
Adapted from Walter & Knecht (2007)

Biomonitoring of the main urinary metabolites of phenanthrene, pyrene and chrysene was shown to be an objective measure of the PAH contribution to exposure to bitumen emissions. Under this experiment setting, the concentration of 6-OH-chrysene in urine was markedly higher than concentration of other PAH metabolites examined, e.g. 1-OHP, the most often used marker of exposure to PAHs (Walter & Knecht, 2007).

The values for the biological half-life for PAH metabolites examined varied only slightly and were about 5–8 hours. Detailed results after percutaneous only or combined inhaled/percutaneous exposure to bitumen emission are shown in Table 4.1.

(b) Rodents

The animal pharmacokinetic profile of individual components of bitumen and bitumen emissions after inhalation, ingestion or dermal uptake was studied in considerable detail by Syracuse Research Corporation (1985). Data indicated that, after inhalation, hydrocarbons with 9–16 carbons were distributed into the blood, brain, liver, kidneys and fat of rats (ATSDR, 1999). Aliphatic hydrocarbons may be oxidized to alcohols, ketones and carboxylic as well as fatty acid derivatives, and some of these compounds are slowly eliminated in the urine and faeces. Detectable concentrations of PAHs occured in almost all internal organs and accumulate in fatty tissues to be eliminated only via urinary or biliary excretion of metabolites.

To determine the bioavailability of genotoxic compounds in bitumen and viscous oils, samples were spiked with radiolabelled benzo[*a*]pyrene and were then applied to mouse skin *in vivo* and to human skin biopsies in short-term culture (Potter *et al.*, 1999). High-viscosity oils and bitumens caused 10 times less binding of benzo[*a*]pyrene to skin DNA, relative to a low-viscosity oil.

The levels of representative metabolites of benzo[*a*]pyrene were measured by mass spectrometry in the urine of rats (16 female Sprague-Dawley) exposed to fume from hot-performance grade bitumen (PG 64–22) in a whole-body inhalation chamber (4 hours per day for 10 days) (Wang *et al.*, 2003a). Eight other rats were controls. The fume was generated by heating the bitumen to 170 °C, then blowing hot air (150 °C) over it. The fume had a concentration of 76–117 mg/m^3. Benzo[*a*]pyrene was detected in the urine of the exposed rats at 21.9 ± 4.9 ng/L, 3-hydroxybenzo[*a*]pyrene at 161.7 ± 3 ng/L, benzo[*a*]pyrene-7,8-dihydrodiol at 62.8 ± 3.6 ng/L and (±)benzo[*a*]pyrene-7,8,9,10-tetrahydrotetrol at 293.5 ± 2.6 ng/L. Only the tetrol metabolite was detectable in the urine of the control rats, at the significantly lower concentration of 1.9 ng/L.

The urinary excretion of metabolites of PAHs was determined after exposure of SPF-Wistar rats to bitumen fume (Halter *et al.*, 2007).

Here, an exposure atmosphere was generated using an evaporation condensation generator. The hot vapour issued through a nozzle into a slowly flowing cool air stream surrounding the jet. The fume, diluted with clean air to achieve the intended concentration, was directed to the nose-only inhalation chambers. The rats were exposed for 4 hours per day, 5 days per week, for the required period. Target exposure concentrations were 0, 4, 20, or 100 mg/m^3 total hydrocarbon (THC). Upon exposure to bitumen fume at 4 mg/m^3, urinary excretion of naphthols, phenanthrene, phenanthrene-premercapturic acid and phenanthrols was similar to that in air-exposed controls. A clear time-dependent increase in excretion of naphthols, phenanthrene, phenanthrene-premercapturic acid, 1-hydroxy-phenanthrene and phenanthrene-1,2-dihydrodiol was observed in male and female rats in the groups receiving the intermediate and highest dose after 12 months of exposure. In this study, CYP1A1 gene and protein expression was dose-dependently induced in lung tissue and nasal epithelium, as confirmed by RT-PCR and Western blotting, and this enzyme induction agreed well with the observed production of 1-OH-phenanthrene. In the study, Halter *et al.* (2007) noted that diet was an unexpected source of exposure to naphthalene and phenanthrene. It is well known that diet can be a source of PAHs (Phillips, 1999).

4.2.3 Urinary PAH metabolites in workers exposed to bitumen emissions

Hydroxylated PAHs (OH-PAHs) have been measured in the urine of bitumen-exposed workers (roofers, pavers and mastic workers). Metabolites typically found were 1-OHP, naphthalene, phenanthrene and fluorene metabolites.

(a) 1-Hydroxypyrene (1-OHP)

Urinary levels of 1-OHP measured in road-pavers (0.20 ng/mL) were shown to be significantly ($P < 0.05$) higher than in controls (0.11 ng/mL). Although there was no difference between the road-pavers samples post- and pre-shift (0.21 ng/mL *versus* 0.20 ng/mL), urine samples collected on Monday morning had significantly ($P < 0.05$) lower concentrations of 1-OHP (0.15 ng/mL) than samples collected on other weekday mornings (0.30 ng/mL), indicating occupational exposure to PAHs. Controls and road pavers were non-smokers (Levin *et al.*, 1995).

Toraason *et al.* (2001) examined urinary concentrations of 1-OHP at the beginning and end of the same working week (4 days later) of 26 workers who applied hot bitumen products in the USA. Urinary concentrations of 1-OHP were significantly increased at the end of the working week. [The Working Group noted potential confounding by exposure to coal tar and smoking.]

Heikkilä *et al.* (2002) measured pre- and post-shift urinary concentrations of 1-OHP in 32 road pavers at 13 paving sites. The workers had been exposed to 11 different asphalt mixtures. The results showed that concentrations of 1-OHP were significantly higher among pavers than among controls, and twice as high among pavers who were smokers than in non-smokers.

Campo *et al.* (2006) monitored asphalt workers ($n = 100$) in Italy, exposed to bitumen fume and diesel exhausts, and road-construction workers ($n = 47$) exposed to diesel exhausts only. Concentrations of 1-OHP were determined in spot samples of urine collected after 2 days of vacation (baseline), before and at the end of the monitored work-shift, in the second part of the working week. Median airborne concentration of the sum of 15 PAHs (in both vapour and particulate phases) during the working shift was 607 ng/m^3, with values for individual PAHs

ranging from < 0.1 to 426 ng/m^3. Median excretion values of 1-OHP in baseline, before- and end-shift samples were 228, 402, and 690 ng/L for the asphalt workers and 260, 304 and 378 ng/L for the road-construction workers. Lower values were found in non-smokers than in smokers (e.g. for asphalt workers, 565 and 781 ng/L *versus* 252 and 506 ng/L in before-shift and end-shift samples, respectively). These results showed that asphalt workers experienced occupational exposure to airborne PAHs, resulting in a significant increase of urinary concentrations of 1-OHP during the working day and the working week. The contribution of working activities to internal dose was in the same order of magnitude as the contribution from cigarette smoking.

Several other studies performed in different countries – Turkey (Burgaz *et al.*, 1998), the United Kingdom (Hatjian *et al.*, 1995), Sweden (Järvholm *et al.*, 1999), and Germany (Marczynski *et al.*, 2006, 2007) – reported the same increase in urinary 1-OHP concentrations in workers exposed to bitumen.

Increases in urinary concentrations of 1-OHP were also reported for asphalt workers who wore gloves, safety shoes and disposable respirators in post-shift measurements (Karaman & Pirim, 2009).

McClean *et al.* (2004b) designed a study to evaluate the total effect of exposure by inhalation and dermal exposure to PAHs among road-paving workers. Urinary concentration of 1-OHP was used as a measure of total absorbed dose in a study population that included two groups of highway-construction workers: 20 paving workers who worked with hot-mix bitumen, and six milling workers who did not. During multiple consecutive working shifts, personal air and dermal samples were collected from each worker and analysed for pyrene. During the same working week, urine samples were collected pre-shift, post-shift and at bedtime each day and analysed for 1-OHP. The paving workers had inhalation (mean, 0.3 μg/m^3) and dermal (mean, 5.7 ng/cm^2) exposures to pyrene that were significantly higher than those of the milling workers. At pre-shift on Monday morning, after a weekend away from work, the pavers and millers had the same mean baseline urinary concentration of 1-OHP of 0.4 μg/g creatinine. The mean urinary 1-OHP concentrations among pavers increased significantly from pre-shift to post-shift during each working day, while it varied little among millers. Among pavers there was a clear increase in the pre-shift levels during the working week, such that the average pre-shift levels on day 4 were 3.5 times higher than those on day 1. The impact of dermal exposure was approximately eight times that of inhalation exposure. Furthermore, dermal exposure that occurred during the preceding 32 hours had a statistically significant effect on urinary 1-OHP, while the effect of inhalation exposure was not significant.

In a further study, McClean *et al.* (2007a) investigated dermal exposure to PAHs among bitumen-roofing workers and used urinary concentrations of 1-OHP as a measurement of dermal exposure for total absorbed dose. The study population included 26 roofing workers who performed three primary tasks: tearing off old roofs (tear-off), putting down new roofs (put-down), and operating the kettle at ground level (kettle). During multiple consecutive work shifts (90 working days), dermal patch samples were collected from the underside of each worker's wrists and were analysed for PAHs, pyrene and benzo[*a*]pyrene. During the same working week, urine samples were collected at pre-shift, post-shift, and bedtime each day and were analysed for 1-OHP (205 urine samples). Dermal exposures were found to vary significantly by roofing task (tear-off > put-down > kettle) and by the presence of an old coal-tar pitch roof (pitch > no pitch). For each of the three analytes, the adjusted mean dermal exposures associated with tear-off were approximately four times higher than exposures associated with operating the kettle. The pyrene measurements obtained during the working shift

were found to be strongly correlated with urinary 1-OHP measurements obtained at the end of that shift as well as at bedtime. The task-based differences that were observed while controlling for coal-tar pitch suggested that exposure to bitumen contributes to dermal exposures.

(b) Other metabolites

In asphalt-mastic workers, the increase in concentrations of 1-, 2+9-, 3-, 4-hydroxyphenanthrene (OHPhe) in post-shift urine samples was greater than those of 1-OHP, compared with pre-shift samples. It was noted that the presence of urinary 1-OHP and OHPhe reflects recent exposure (Marczynski *et al.*, 2006, 2007).

The recent study of Raulf-Heimsoth *et al.* (2011a) reported urinary PAHs metabolites of six bitumen workers handling mastic and mastic asphalt in two consecutive weeks at the same construction site in a tunnel. Median personal shift concentration of vapours and aerosols of bitumen was 1.8 mg/m^3 (range, 0.9–2.4 mg/m^3) during the application of rolled asphalt and 7.9 mg/m^3 (range, 4.9–11.9 mg/m^3) when mastic asphalt was applied. Area measurement of vapours and aerosols of bitumen revealed higher concentrations than the personal measurements of mastic asphalt (mastic asphalt, 34.9 mg/m^3; rolled asphalt, 1.8 mg/m^3). Processing mastic asphalt was also associated with higher concentrations of PAH. Urinary 1-hydroxypyrene and the sum of 1-, 2+9-, 3- and 4-hydroxyphenanthrene increased slightly during the shift, without clear differences between mastic and rolled-asphalt applications. However, the post-shift urinary concentrations of PAH metabolites did not reflect the different levels of PAH exposure during mastic and rolled-asphalt applications. Individual workers could be identified by their spirometry results, indicating that these data reflected long-term rather than acute effects.

In the study by Buratti *et al.* (2007), the urinary excretion of OH-PAHs was measured among asphalt workers (road pavers). Total PAHs and 15 individual PAHs in inhaled air were measured by personal sampling. In addition, the OH-PAHs 2-naphthol, 2-hydroxyfluorene, 3-hydroxyphenanthrene, and 1-OHP were quantified in urine samples collected at three different time-points during the week. Specifically, the median cencentrations of vapour-phase polycyclic aromatic compounds, PACs (5.5 µg/m^3), PAHs (≤ 50 ng/m^3) and OH-PAHs (0.08–1.11 µg/L) were significantly higher in asphalt workers than in controls, except in the case of naphthalene and 2-naphthol. The urinary concentrations of OH-PAHs increased with time: median concentrations for 2-hydroxyfluorene, 3-hydroxyphenanthrene and 1-OHP were 0.29, 0.08 and 0.18 µg/L at baseline; 0.50, 0.18 and 0.29 µg/L pre-shift; and 1.11, 0.44 and 0.44 µg/L post-shift, respectively. Each OH-PAH showed a characteristic profile of increase, reflecting differences in half-lives of individual constituents of bitumen emissions. In non-smoking subjects, positive correlations were found between vapour-phase PACs or PAHs and OH-PAHs, both in pre- and post-shift samples. Smokers had concentrations of OH-PAHs that were two to five times higher than those of non-smokers.

Väänänen *et al.* (2006) investigated the occupational exposure of road pavers to asphalt that contained waste plastic and tall oil pitch (WPT). Traffic controllers distant from the paving site served as controls. Exposure was monitored over one working day at four paving sites among 16 road pavers who used mixtures of conventional asphalt, stone-mastic asphalt (SMA) and asphalt concrete (AC), or mixtures containing waste material (SMA-WPT, AC-WPT). The concentrations of 11 aldehydes in air at the SMA-WPT and AC-WPT worksites were 3 and 13 times greater than at the corresponding worksites where conventional asphalt was used. Eight OH-PAH biomarkers were quantified in pre- and post-shift urine, as a measure of exposure to naphthalene, phenanthrene and pyrene. The post-shift concentrations (mean ± SD, µmol/mol

creatinine) of 1- and 2-naphthol, combined 1-,2-,3-,4-, 9-phenanthrol and 1-OHP were: 6.0 ± 2.3, 1.70 ± 0.72 and 0.27 ± 0.15 µmol/mol for conventional asphalt workers (non-smokers), respectively. For WPT-asphalt workers (non-smokers), the concentrations were 6.8 ± 2.6, 2.35 ± 0.69 and 0.46 ± 0.13 µmol/mol. As noted in other studies, concentrations of PAH metabolites were significantly higher in smokers than in non-smokers.

Pasquini *et al.* (1989) measured the urinary excretion of thioethers and D-glucaric acid in road workers exposed to bitumen emissions from a hot mixture of cracked rocks and petroleum bitumen. Urinary excretion of D-glucaric acid was also determined to investigate the potential of bitumens to induce enzymes. Thio-ethers were higher only in subjects exposed simultaneously to bitumens and cigarette smoke. Excretion of D-glucaric acid did not increase significantly.

4.2.4 Effect on pulmonary cytochrome P450 in rats

The effects of exposure to bitumen-fume condensate (BFC) on pulmonary cytochrome P450 (Cyp450) were studied by Ma *et al.* (2002). Male Sprague-Dawley rats were treated intratracheally with saline or with BFC at 0.45, 2.22 or 8.88 mg/kg for three consecutive days and euthanized the following day. Lung microsomes were isolated and microsomal protein levels, NADPH cytochrome-c reductase activity, and the activities and protein levels of the isozymes Cyp1a1 and Cyp2b1 were quantified.

Exposure of rats to BFC did not significantly affect total Cyp450 content or cytochrome-c reductase activity in the lung. The amount and activity of Cyp2b1 were not significantly affected by exposure to BFC. In contrast, Cyp1a1 levels and activity were significantly increased in microsomes isolated from BFC-exposed lungs. Exposure to bitumen emissions may alter the metabolism of PACs by the Cyp system in the rat lung, which may contribute to BFC-induced genotoxic effects (see Section 4.3).

4.3 Genetic and related effects

4.3.1 Studies in humans

Studies of genotoxicity in workers exposed to bitumen are presented in Table 4.2.

(a) Urinary mutagenicity

A comparison of urinary mutagenicity was carried out in 17 bitumen road pavers (exposed to emissions from a hot mixture of cracked rocks and petroleum bitumen at a concentration of 0.6–0.8 mg/m^3) and 27 control subjects (Pasquini *et al.*, 1989). Five workers were also exposed to diesel exhaust. All 15 smokers (six exposed to bitumens, nine non-exposed) had mutagenic urine. Among the non-smokers, 9 out of 11 exposed workers (82%) had mutagenic urine, compared with 5 out of 18 (28%) non-exposed controls. This difference was statistically significant ($P < 0.025$). Urinary mutagenicity was detectable only with *S. typhimurium* TA98 with S9 fraction, but not with TA100.

(b) DNA damage

DNA damage, as measured by alkaline elution, in peripheral mononuclear blood cells of bitumen-exposed workers (roofers exposed to class 2 bitumens; pavers exposed to class 1 bitumens; bitumen painters exposed to class 1 bitumens) was measured at the end of the working week and again at the beginning of the subsequent week (Fuchs *et al.*, 1996). For roofers ($n = 7$; all smokers), levels of alkaline DNA strand breaks were 43% higher than in the 34 controls ($P < 0.002$); for road pavers ($n = 18$; 12 smokers) and bitumen painters ($n = 9$; eight smokers), DNA damage was similar to that in controls. Nevertheless, for pavers there was more DNA damage observed in samples collected on Friday

Table 4.2 Studies of genotoxicity in workers exposed to bitumen

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Mutagens in urine						
Urinary mutagenicity *S. typhimurium*, TA98 + S9	Pavers (cracked rocks, petroleum bitumen)	17 (6)	27 (9)	+	Among non-smokers, 82% of exposed and 28% of non-exposed had mutagens in urine ($P < 0.025$). Five workers were also exposed to diesel exhaust.	Pasquini *et al.* (1989) Italy
DNA damage						
DNA damage, mononuclear blood cells (alkaline elution)	Pavers Roofers Bitumen painters	18 (12) 7 (7) 9 (8)	34 (> 50%)	– + –	Samples taken on Monday and Friday. Roofers had higher levels of DNA-damage on Friday ($P < 0.05$) and higher levels *vs* control smokers ($P < 0.002$)	Fuchs *et al.* (1996) Germany
DNA damage, leukocytes (comet assay)	Roofers (bitumen ± coal-tar pitch)	26 (16)	15 (3)	+	Significant comet results for seven roofers who had no coal-tar exposure.	Toraason *et al.* (2001) USA
DNA damage in buccal leukocytes (comet assay)	Pavers (asphalt concrete and stone-mastic asphalt with or without waste plastic and tall-oil pitch)	15 (10)	5 (0)	–	No statistically significant differences in pre-shift *vs* post-shift samples, or in exposed vs controls. Nonetheless, DNA damage and urinary metabolites of PAHs (naphtol and 1-OHP) were correlated	Lindberg *et al.* (2008) Finland
DNA damage, blood lymphocytes, and leukocytes in sputum (comet assay)	Pavers (mastic asphalt)	42 (27)	NA	–	Pre-shift samples served as controls. No change in sputum leukocytes; decrease in lymphocytes.	Marczynski *et al.* (2010) Germany
DNA damage, lymphocytes (comet assay)	Pavers (mastic asphalt)	320 (199)	118 (61)	–	DNA damage decreased during shift in exposed and controls. No effect of smoking.	Marczynski *et al.* (2011) Germany
DNA damage, whole blood (comet assay)	Pavers (mixing/ paving)	36 (20)	37 (19)	+	Significant increase in exposed *vs* controls, in smokers (exposed *vs* controls) and non-smokers (exposed *vs* controls).	Sellappa *et al.* (2011) India

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Oxidative DNA damage						
Oxidative DNA damage (HPLC-electrochemical)	Roofers (bitumen ± coal-tar pitch)	26 (16)	15 (3)	–	Increase in oxidative damage was not significant; no effect of smoking.	Toraason *et al.* (2001) USA
Oxidative DNA damage in lymphocytes (comet assay, Fpg-modified)	Pavers (concrete asphalt; 160 °C)	19 (9)	22 (11)	+	$P = 0.008$, exposed *vs* controls; 7/19 (37%) had oxidative DNA damage.	Cavallo *et al.* (2006) Italy
Oxidative DNA damage (HPLC-electrochemical)	Pavers (mastic-asphalt) (exposure to coal-tar pitch was excluded)	320 (199)	118 (61)	+	Oxidative DNA damage (8-OH-dG) higher in exposed *vs* controls before shift ($P = 0.0001$) and after shift ($P = 0.0001$). No effect of smoking.	Marczynski *et al.* (2011) Germany
DNA/protein adducts						
DNA adducts, leukocytes (USERIA)	Roofers	28 (NR)	9 (2)	+	Seven of the roofers (four smokers) and two controls (both smokers) showed measurable BPDE-DNA adducts. No exposure information given.	Shamsuddin *et al.* (1985) USA
DNA adducts, leukocyte (^{32}P-postlabelling)	Roofers, incl. removal of old roofs	12 (8)	12 (8)	+	Detectable adducts in 10 roofers (83%), in 2 controls (17%), in the 4 exposed non-smokers, and in none of the control non-smokers. Removal of old roofs may involve coal-tar exposure.	Herbert et al. (1990a, b) USA
DNA adducts, mononuclear blood cells (^{32}P-postlabelling)	Pavers Roofers Bitumen painters	18 (12) 7 (7) 9 (8)	34 (> 50%)	±	Samples taken from 12 pavers and 2 painters; adduct-related spots detectable in 4 samples (3 pavers, 1 painter). No data given for other exposed or controls.	Fuchs *et al.* (1996) Germany
DNA adducts, mononuclear blood cells (^{32}P-postlabelling)	Pavers (hot-mix asphalt)	49 (11)	36 (13)	±	Samples taken in four seasons. No difference in pavers *vs* non-pavers. Adduct levels in pavers increased by weekday during work-season, not in off-season. Such increase was not seen in non-pavers.	McClean *et al.* (2007b) USA

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
DNA adducts, leukocytes (HPLC-fluorescence)	Pavers (mastic asphalt) (exposure to coal-tar pitch was excluded).	202 (133)	55 (23)	–	Detectable adducts in only 42% (pre-shift) and 40% (post-shift) of samples of 154 workers. No difference. No data on controls.	Marczynski *et al.* (2007) Germany
DNA adducts, leukocytes (HPLC-fluorescence)	Pavers (mastic asphalt) (exposure to coal-tar pitch was excluded).	320 (199)	118 (61)	–	Adducts measured in 227 exposed and 66 controls, of whom 110 and 27, resp., had detectable BPDE-adducts. No difference in exposed *vs* controls, or pre-shift *vs* post-shift.	Marczynski *et al.* (2011) Germany
Protein (albumin) adducts, blood plasma (ELISA)	Roofers	12 (NR)	12 (NR)	±	Monoclonal antibody recognizes PAH/protein adducts of BPDE; crossreacts with chrysene adducts.	Lee *et al.* (1991) USA
Sister-chromatid exchange						
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers and roofers	14 (3)	8 (0)	±	Pavers/roofers *vs* controls, $P < 0.05$; sister-chromatid exchange frequencies in pavers/roofers and 13 manual workers were similar.	Hatjian *et al.* (1995) United Kingdom of Great Britain and Northern Ireland
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (raking, bitumen production)	28 (16)	19 (12)	+	Exposed *vs* controls, $P < 0.05$ For non-smokers: exposed *vs* control, $P < 0.001$	Burgaz *et al.* (1998) Turkey
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers ("ordinary" road paving)	28 (0)	30 (0)	–		Järvholm *et al.* (1999) Sweden
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (hand-pavers, finishers, road pavers)	46 (26)	87 (46)	+	Hand-pavers and finishers *vs* industrial controls: $P < 0.05$ in 1996; sister-chromatid exchange frequency decreased in 1997 to 1999	Major *et al.* (2001) Hungary
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (concrete asphalt; 160 °C)	19 (9)	22 (11)	–		Cavallo *et al.* (2005, 2006) Italy

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (concrete asphalt; 170 °C)	26 (1)	24 (1)	+	Samples taken just after 2-wk holiday and post-shift at 14 days. Exposed *vs* controls, $P < 0.001$. Positive correlation with duration of exposure. All workers wore gloves, safety shoes, respirators.	Karaman & Pirim (2009) Turkey
Micronucleus formation						
Micronucleus formation, blood lymphocytes	Pavers (raking, asphalt production)	28 (16)	28 (18)	+	Exposed *vs* controls, $P < 0.0001$ For non-smokers: exposed *vs* control, $P < 0.01$.	Burgaz *et al.* (1998) Turkey
Micronucleus formation, blood lymphocytes	Pavers (road paving)	28 (0)	30 (0)	–		Järvholm *et al.* (1999) Sweden
Micronucleus formation, exfoliated urothelial cells, blood lymphocytes	Pavers	12 (6)	18 (6)	+	For both cell types: MN/1 000 cells, $P < 0.01$. No effect of smoking.	Murray & Edwards (2005) Australia
Micronucleus formation, blood lymphocytes	Pavers (mastic asphalt) (exposure to coal-tar pitch was excluded)	202 (133)	55 (23)	–	MN data given for 34 exposed and 14 controls.	Marczynski *et al.* (2007) Germany
Micronucleus formation, blood lymphocytes	Pavers (concrete asphalt; 170 °C)	26 (1)	24 (1)	+	Samples taken just after 2-wk holiday and post-shift at 14 days. Exposed *vs* controls, $P < 0.001$. Positive correlation with duration of exposure. All workers wore gloves, safety shoes, respirators.	Karaman & Pirim (2009) Turkey
Micronucleus formation, blood leukocytes	Pavers (mixing/ paving)	36 (20)	37 (19)	+	Significant increase in exposed *vs* controls, in smokers and non-smokers; also exposed to coal tar.	Sellappa *et al.* (2011) India
Micronucleus formation, blood lymphocytes	Mastic-asphalt workers	225 (139)	69 (41)	–		Welge *et al.* (2011) Germany

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Chromosomal aberrations						
Chromosomal aberrations, blood lymphocytes	Pavers (hand-pavers, finishers, road pavers) *vs* industrial controls	46 (26)	87 (46)	+	$P < 0.05$ in 1996 and 1997. Frequency decreased in 1998–99; also exposed to diesel exhaust	Major *et al.* (2001) Hungary
Chromosomal aberrations, blood lymphocytes	Pavers (hand-pavers, finishers, mixers) *vs* industrial controls	66 (45)	56 (24)	+	$P < 0.05$; also exposed to diesel exhaust	Tompa *et al.* (2007) Hungary

1-OHP, 1-hydroxypyrene; BPDE, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BrdU, bromodeoxyuridine; 8-OH-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; d, day; ELISA, enzyme-linked immunosorbent assay; Fpg, formamido-pyrimidine-glycosylase; HPLC, high-pressure liquid chromatography; MN, micronuclei; NR, not reported; S9, 9000 × *g* rat liver supernatant; USERIA, ultrasensitive enzymatic radioimmunoassay; *vs*, *versus*; wk, week

than on Monday, while the contrary was evident for the bitumen painters.

DNA damage, measured as alkali-labile lesions with the comet assay, were determined in peripheral blood leukocytes of 26 roofers (16 smokers) exposed to roofing bitumen [class 2], and 15 construction workers (three smokers), not exposed to bitumen during the past 5 years. A subgroup of 19 roofers (12 smokers) was exposed to coal tar during removal of existing roofs. There was statistically significantly more DNA damage in end-of-week samples from workers exposed to bitumens only, compared with start-of-week samples. This group also had elevated 1-OHP levels in the urine (Toraason *et al.*, 2001).

Comet-assay analysis of pavers (n = 19; nine smokers) and controls (n = 22; 11 smokers) in Italy, showed higher levels of oxidative DNA damage in the pavers (Cavallo *et al.*, 2006). Additional use of formamido-pyrimidine-glycosylase (Fpg) in the comet assay indicated that oxidative damage to DNA contributed in about one third of the samples from the pavers (7 out of 19), but in none of the 22 controls.

Buccal leukocytes collected from 15 pavers (10 smokers) were collected pre- and post-shift, analysed by the comet assay, and compared with 5 controls (all non-smokers). The pavers were exposed to asphalt concrete (without or with WPT, produced at 145–165 °C; [class 1, class 5]), and to stone-mastic asphalt (without or with WPT, produced at 151–157 °C; [class 1, class 5]) (Lindberg *et al.*, 2008). No significant differences were found between pre- and post-shift samples, or between the workers and the unexposed controls.

An analysis of 202 mastic-asphalt [class 1] workers exposed during high-temperature application at 240–260 °C, mainly indoors and in basement garages (exposure to coal-tar pitch was excluded) and 55 non-exposed construction workers (controls) found that DNA-strand breaks post-shift (mid-week), relative to pre-shift, were statistically significantly decreased in these larger groups ($P < 0.05$) (Marczynski *et al.*, 2007). Levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OH-dG) were significantly higher post-shift in both the exposed workers and the non-exposed controls ($P < 0.0001$), but significantly higher levels of 8-OH-dG and alkali-labile DNA lesions were found in the exposed workers than in the controls both before and after the working shifts.

This study has been expanded further to include 320 bitumen [class 1]-exposed workers and 118 non-exposed construction workers (Marczynski *et al.*, 2011). Blood lymphocytes were tested in the alkaline comet assay. Midweek pre-shift levels of DNA damage were significantly higher than post-shift in both the exposed group and the control group ($P < 0.0001$ and $P = 0.012$, respectively); the levels of damage in the exposed workers were significantly higher than in the controls at both sampling times ($P < 0.0001$). Levels of 8-OH-dG in lymphocytes were significantly higher in the exposed group at both sampling times ($P < 0.0001$) and the levels increased from pre- to post-shift in both groups ($P < 0.0001$ for exposed workers; $P = 0.0002$ for controls).

In a pilot study, the same investigators compared leukocytes from induced sputum with peripheral blood lymphocytes from 42 bitumen-exposed workers before and after shift (Marczynski *et al.*, 2010). There was no correlation between DNA damage in the two cell types, as measured by the comet assay (Spearman rank correlation coefficient: $r_s = -0.04$, $P = 0.802$ before shift; and $r_s = 0.27$, $P = 0.088$ after shift).

In a study of 36 bitumen-exposed road pavers in India (no protective equipment worn other than safety shoes) and 37 controls, DNA damage in peripheral blood leukocytes, analysed by the comet assay, was significantly greater ($P < 0.05$) in the exposed workers than in the controls (Sellappa *et al.*, 2011). In both the exposed and the control group, smokers and alcohol-drinkers had higher levels of damage than did non-smokers

and non-drinkers. [The Working Group noted that these workers were also exposed to coal tar.]

(c) DNA adducts and protein adducts

Antibodies raised against DNA modified by 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) were used to develop an ultrasensitive enzymatic radioimmunoassay (USERIA) to analyse leukocyte DNA from 28 roofers (no other information on occupational exposures was given) and nine controls (Shamsuddin *et al.*, 1985). With a limit of detection of one adduct per 7.5×10^7 nucleotides, adducts were detected in seven of the roofers (three of these were smokers, three were non-smokers and one unspecified; no information was given on the smoking status of the other 21 roofers in whom adducts were not detected). Among the controls, two of nine samples were positive, both of them from smokers (the remaining seven individuals in the control group were non-smokers).

An analysis by ^{32}P-postlabelling of DNA isolated from leukocytes of roofers (n = 12; eight smokers) revealed detectable levels of bulky/aromatic adducts in ten roofers, compared with detectable levels in only two of twelve control subjects (eight smokers) (Herbert *et al.*, 1990b). The roofers were involved in the removal of old pitch roofs and replacement of each section with a new asphalt roof. Among the roofers, all four non-smokers had detectable levels of DNA adducts; among the controls, none of the non-smokers showed DNA adducts. Among the roofers, skin wipes taken post-shift contained concentrations of PAHs that correlated with the levels of DNA adducts.

In a subsequent study, serum samples from the same workers and controls were analysed for PAH–albumin adducts using an enzyme-linked immunosorbent assay (ELISA): the roofers had higher levels of adducts (5.19 fmol/μg versus 3.28 fmol/μg; marginally statistically significant, $0.1 > P > 0.05$) (Lee *et al.*, 1991). There was also a weak correlation between levels of PAH–albumin adducts and levels of DNA adducts measured in the earlier study ($0.1 > P > 0.05$).

Analysis of peripheral mononuclear cells from bitumen-exposed workers (twelve road-paving workers and two bitumen painters; nine smokers) revealed the presence of DNA adducts detected by ^{32}P-postlabelling analysis in ten workers (Fuchs *et al.*, 1996). [The Working Group noted that no results were reported on DNA adducts in control subjects.]

Forty-nine asphalt-paving workers [bitumen, class 1] were monitored for formation of DNA adducts over a 12-month period and compared with 36 non-paving construction workers, both during the working season and during the off-season (winter) (McClean *et al.*, 2007b). Although levels of DNA adducts – measured by ^{32}P-postlabelling – were increased in the lymphocytes of the exposed workers through the working week, they were not higher overall than in the non-exposed workers, and seasonal variations were such that levels were higher in the off-season.

A modified HPLC-FD method was used to determine adducts of benzo[*a*]pyrene diol-epoxide in leukocytes of 154 mastic-asphalt workers, and in road-construction workers not exposed to bitumens (Marczynski *et al.*, 2007). The method was based on determination of benzo[*a*]pyrene tetrol after acidic hydrolysis of DNA. A low level of adducts was reported in the workers, with no significant difference between pre- and post-shift samples. [The Working Group noted that no results were reported on DNA adducts in control subjects.] This study was expanded further to include 320 bitumen-exposed workers and 118 non-exposed construction workers (Marczynski *et al.*, 2011). Levels of DNA adducts were not significantly different between the two groups, both pre- and post-shift, with no change in levels during the shift.

(d) Sister-chromatid exchange

In a study from the United Kingdom, pavers and roofers (n = 14; three smokers) had significantly higher frequencies of sister-chromatid exchange in peripheral blood lymphocytes than did administrative staff (n = 8; all non-smokers) (P < 0.05), but so also did manual workers (n = 13; three smokers) with no known exposure to PAHs, and there was no significant difference between the pavers/roofers and the manual workers (Hatjian *et al.*, 1995).

In a Turkish study, sister-chromatid exchange was measured in lymphocytes from 28 bitumen-exposed workers (16 smokers), 21 of whom were employed as rakermen in road-paving operations, while 7 worked in an asphalt plant. The control group for sister-chromatid exchange analysis (n = 19; 12 smokers) was recruited from university and hospital staff (Burgaz *et al.*, 1998). The frequency of sister-chromatid exchange in the exposed group overall was statistically significantly higher than that in the control group (P < 0.05). The non-smokers in the exposed group had significantly higher sister-chromatid exchange levels than the non-smokers in the control group (P < 0.001).

Another study from Turkey included 26 asphalt workers and a matched control group of 24 administrative workers. The asphalt workers were involved in paving with concrete asphalt (170 °C), and they all wore personal protection devices. Blood samples were collected from the asphalt workers immediately after a 2-week holiday and before starting work, and after the Friday working shift 2 weeks later. The exposed workers had a significantly increased frequency of sister-chromatid exchange compared with controls (P < 0.001). The frequencies of sister-chromatid exchange in the two samples taken from the workers before and after the 14-day period of work were not statistically significantly different (Karaman & Pirim, 2009).

A study from Hungary in 1996–99 included eight road pavers (five smokers; working close to the fresh pavement as "hand-pavers"), 14 drivers of paving equipment (nine smokers; working in closed cabins as "finishers"), 24 other road pavers (12 smokers), eight workers (six smokers) in bitumen production, six non-exposed white-collar workers (four smokers), and a historical control group of 87 industrial workers (46 smokers). Frequencies of sister-chromatid exchange were initially (in 1996) higher (P < 0.05) in the exposed workers (hand-pavers, finishers) than in the controls, although subsequently they decreased to control levels (Major *et al.*, 2001), possibly as a result of changes in work practice and in the composition of asphalts.

In a Swedish study, no difference in the frequency of sister-chromatid exchange in peripheral blood lymphocytes was found between 28 non-smoking workers involved in "ordinary" road-paving operations, with exposure to PAHs at an average concentration of 2.3 μg/m^3 (range, 0.2–23.8 μg/m^3), and 30 non-smoking controls (Järvholm *et al.*, 1999). Likewise, no difference was observed in an Italian study between the frequency of sister-chromatid exchange in blood lymphocytes of 19 pavers (9 smokers) working with concrete asphalt (at 160 °C) and that in the 22 controls (11 smokers) (Cavallo *et al.*, 2005, 2006).

(e) Micronucleus formation

Studies on the frequency of micronucleus formation in bitumen and asphalt workers showed a mixture of positive and negative results.

In the comparison of 28 workers in Sweden workers [bitumen class 1] with 30 controls (see above), no difference in frequency of micronucleus formation in peripheral lymphocytes was observed (Järvholm *et al.*, 1999). Similarly there was no difference in micronucleus formation in 34 exposed German workers compared with 14 controls, except in "before-shift" comparisons (Marczynski *et al.*, 2007). Expansion of this study

group to include analysis of 225 exposed workers and 69 unexposed construction workers did not reveal any association between micronucleus frequency and exposure to bitumen emissions (Welge *et al.*, 2011). However, in the two Turkish studies mentioned above, frequencies of micronucleus formation were significantly higher in lymphocytes of the exposed workers compared with controls (Burgaz *et al.*, 1998; Karaman & Pirim, 2009). Also, in an Australian study that included 12 bitumen road-layers (6 smokers; [bitumen class 1]) and 18 non-exposed controls (6 smokers), the workers had statistically significantly higher ($P < 0.01$) frequencies of micronucleus formation in both peripheral lymphocytes and in exfoliated urothelial cells (Murray & Edwards, 2005). In an Indonesian study, micronucleus frequencies in the blood cells of bitumen-exposed road pavers ($n = 36$) were statistically significantly higher ($P < 0.05$) than in controls ($n = 37$) (Sellappa *et al.*, 2011). In both groups, higher frequencies were found in smokers and alcohol-drinkers than in non-smokers and non-drinkers (statistically significant in the exposed workers, $P < 0.05$).

(f) Chromosomal aberrations

In monitoring studies carried out in 1996–99 among road pavers and workers with related occupations in Hungary (see above), the frequencies of chromosomal aberrations were initially higher in the road pavers than in the controls, but the levels declined over time in the pavers until they were the same in both groups (Major *et al.*, 2001; Tompa *et al.*, 2007); this may have been the consequence of changes in work practices and composition of asphalts. [The Working Group agreed with the authors who declared that "increase in chromosomal aberrations yields can be attributed to the use of genotoxic agents other than 'asphalt fumes', mainly diesel exhausts, and crude oil (frequently used for cleaning the equipment in Hungary)."]

(g) HPRT mutations

The frequencies of *HPRT* variants in peripheral blood lymphocytes of "tar-free" asphalt road-pavers were not higher than in controls (Major *et al.*, 2001).

(h) Unscheduled DNA synthesis

Peripheral blood lymphocytes from "tar-free" asphalt road-pavers and other bitumen-exposed workers ($n = 111$) did not differ in their response to ultraviolet-induced unscheduled DNA synthesis compared with control workers ($n = 93$) (Major *et al.*, 2001).

4.3.2 Mutagenicity in bacteria

Studies of mutagenicity in *Salmonella typhimurium* exposed to bitumen and bitumen emissions are presented in Table 4.3.

In the Ames test, using *S. typhimurium* strains TA98 and TA100 in the presence or absence of metabolic activation from S9, petroleum bitumen paints were not mutagenic, while coal-tar paints gave positive results after metabolic activation with S9. Nonetheless, both types had tumour-initiating activity on mouse skin (coal tar being more active than bitumen) (Robinson *et al.*, 1984).

DMSO-extracted oils, including bitumen vacuum residue, were also found to be mutagenic in *S. typhimurium* TA98 (Booth *et al.*, 1998).

Bitumen-fume condensate [derived from bitumen class 1] was mutagenic in *S. typhimurium* TA98, TA100, YG1041 and YG1042 in the presence of metabolic activation from S9 from rat liver, but 15–600 times less active than coal-tar fume condensate (De Méo *et al.*, 1996).

Bitumen and asphalt particulates and fumes were tested in *S. typhimurium* TA98 and YG1024 (Heikkilä *et al.*, 2003). Bitumens containing coal-fly ash or waste plastics [bitumen class 5] were heated to paving temperatures in the laboratory. The vapour fractions were negative for mutagenicity, but the particulate fractions were mutagenic

Table 4.3 Studies of mutagenicity in *Salmonella typhimurium* treated with bitumen, bitumen fume or their condensates

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
S. typhimurium, TA98, TA100, TA1535, TA1537, TA1538	Petroleum-bitumen paints (n = 4): bitumen cut-backs (64% solid, diluted with 1–3% xylene); 0.005–10 µL/plate.	–	–	No toxicity. Some of these paints were mouse-skin carcinogens	Robinson *et al.* (1984)
	Coal-tar paints (n = 4): coal-tar pitch/xylene (67/33); coal-tar pitch/clay talc/solvent (47/16/37 or 37/42/21 or 39/30/30); 0.005–10 µL/plate.	–	+ positive in all strains, except in TA1535	Toxicity at high dose; all paints were mouse-skin carcinogens	
S. typhimurium, TA98 (pre-incubation assay)	Petroleum oils (n = 13), DMSO-extracted; 20% solution in EGDE	NT	+ (n = 6) – (n = 7)	Mutagenicity correlated with skin carcinogenicity	Blackburn *et al.* (1984)
S. typhimurium, TA98	Bitumen, vacuum residue; various petroleum-derived oils	NT	± bitumen	Mutagenicity index correlated with DNA adducts in mouse skin	Booth *et al.* (1998)
S. typhimurium, TA98, TA100, YG1041, YG1042 (plate-incorporation assay)	Condensates of bitumen fume generated at 160 °C or 200 °C	–	+ condensates positive in all strains	Mutagenicity 15–600 times lower than for coal-tar condensates (110 °C/160 °C).	De Méo *et al.* (1996)
S. typhimurium, TA98, YG1024	Bitumen B120; bitumen B80 with 66% coal fly ash; bitumen B120 with 10% waste plastics. Samples taken at 170–180°C; C_2Cl_4 extract of filters; 0.03–0.5 mg/plate tested.			Note: samples correspond to class 5 bitumen.	Heikkilä *et al.* (2003)
	Vapour fractions of the different samples	–	–		
	Particle fractions of the different samples	+	+		
	Stone-mastic asphalt fume collected during paving and remixing, +/– coal fly ash (10%) or lime (10%); C_2Cl_4 extract of Teflon filters; only particles tested.	+	+	Sampling temperature 160–210 °C (paved asphalt) and 150–350 °C (remixed asphalt)	

Table 4.3 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
S. typhimurium, TA98, YG1024	*Laboratory samples:* Fumes from stone-mastic asphalt ± waste plastics and tall oil pitch, produced in the laboratory at 150 °C. *Collected during paving:* Asphalt concrete ± waste plastics and tall oil pitch, produced at 145–165 °C; Stone-mastic asphalt ± waste plastics and tall oil pitch, produced at 151–157 °C (filters all extracted with C_2Cl_4/DMSO).	–	–	Samples also contain coal fly ash and lime. Note: samples corresponded to class 1 and 5 bitumens	Lindberg *et al.* (2008)
S. typhimurium, TA98 (pre-incubation assay)	Fumes from roofing (n = 4) and paving bitumens (n = 18) were generated at 232–316 °C and 163 °C, respectively. The oil phase of the fume condensates was extracted with DMSO.	NT	+	For some samples, the mutagenic response correlated with the 3- to 7-ring PAH content.	Machado *et al.* (1993)
S. typhimurium, TA98 (pre-incubation assay)	Condensate of bitumen fume, drawn from a storage tank at 147–157 °C	NT	–		Reinke *et al.* (2000)
	Condensate of bitumen fume from a laboratory generator at 149 °C and 316 °C.	NT	+	More 3–4-ring *S*-heterocyclic PAH in high-temperature samples	
S. typhimurium, TA98 (ASTM Standard Method E 1 687–95)	Copenhagen mastic-asphalt core samples 1952–91; DMSO extracts (n = 11)	NT	++ early samples (before 1970) + later samples	Higher mutagenicity in early samples was consistent with the presence of coal tar.	Kriech *et al.* (1999a)
S. typhimurium, TA98 (ASTM Standard Method E 1 687–95)	Laboratory-generated roofing bitumen-fume condensates, various fractions; DMSO extracts	NT	+	Mutagenic effects correlated with skin carcinogenicity in the mouse.	Kriech *et al.* (1999b)

C_2Cl_4, tetrachloroethylene; DMSO, dimethyl sulfoxide; EGDE, ethylene glycol dimethyl ether; NT, not tested

in the presence and absence of S9 in both strains. In addition, the particulate fractions of bitumen fumes collected in the field during paving with stone-mastic asphalts (with either lime or coal-fly ash as filler) and during remixing of stone-mastic asphalt or asphalt concrete, were tested. These were also mutagenic in the presence and absence of S9 in both strains. The field samples were more mutagenic than the laboratory-generated fumes without S9, and the remixing fumes were more potent than the normal paving fumes and the laboratory-generated fumes with S9.

Another study of field samples of fumes from asphalt concrete (without or with WPT, produced at 145–165 °C; [bitumen class 1, class 5]) and from stone-mastic asphalt (without or with WPT, produced at 151–157 °C; [bitumen class 1, class 5]) found the materials to be non-mutagenic in *S. typhimurium* strains TA98 and YG1024 with or without metabolic activation (Lindberg *et al.*, 2008).

Asphalt-fume condensate (derived from class 2 bitumen) was reported to be mutagenic in the modified Ames test with *S. typhimurium* TA98 in the presence of S9, but was about 100 times less mutagenic than coal-tar pitch fume condensate (Machado *et al.*, 1993). The weak-to-moderate potency observed in this study broadly correlated with PAH content, in particular for three- to seven-ring PAHs.

The mutagenic activity in the modified Ames assay of several fume condensates from bitumens K and E [class 1] was found to correlate with the total content of three- to six-ring PACs, as determined by extraction with DMSO followed by GC (Brandt, 1994). This was found to be a better measure of mutagenic potential than comparisons with either the concentrations of single compounds or with the sum of the concentrations of 14 compounds.

In another study, condensate of laboratory-generated fumes of 85/100 grade paving bitumen [class 1] was tested with the modified Ames test; the sample generated at 316 °C was more mutagenic than the material generated at 149 °C, reflecting higher three- and four-ring *S*-heterocyclic PAH content (Reinke *et al.*, 2000).

When roofing-bitumen [class 2] fume condensate ("NIOSH fumes") were fractionated by HPLC, the two subfractions that were carcinogenic on mouse skin were also the fractions that were mutagenic in the modified Ames assay. These subfractions showed relatively high fluorescence intensities at 415 mn, consistent with the presence of four- to six-ring PACs (Kriech *et al.*, 1999b). Furthermore, there was good correlation between the biological activity of the subfractions, or various combinations of subfractions, and their PAC content, as determined by fluorescence emissions.

In an Ames test that included S9 prepared from the lungs of rats exposed to high concentrations of bitumen fumes ([class 1]; 1150 ± 63 mg.h/m^3; generated at 170 °C), the mutagenic activity of 2-aminoanthracene, but not of benzo[*a*]pyrene, was statistically significantly enhanced compared with when S9 from control (non-exposed) rats was used (Zhao *et al.*, 2004).

4.3.3 Genotoxicity in mammalian systems

Genotoxicity data in mammalian systems *in vivo* and *in vitro* are presented in Table 4.4.

(a) DNA damage

Exposure of plasmid DNA to bitumen (100 µg/mL) plus ultraviolet A (UVA) did not reveal the formation of single- or double-strand breaks, but reactive oxygen formation was demonstrated by incubation with deoxyguanosine, resulting in the formation of 8-OH-dG (Hong & Lee, 1999). However, the combination of bitumen (10 µg/mL; obtained from distillation of crude oil) and UVA (1.5, 3.0 or 6.0 mJ/cm^2) on the human promyelocytic leukaemia cell-line HL 60 showed a significant increase in DNA–protein crosslinks over the modest increase produced by either exposure alone (Hong & Lee, 1999).

Table 4.4 Studies of genotoxicity in mammalian systems *in vitro* and *in vivo* treated with bitumen or bitumen fume

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
In vitro					
DNA strand-breaks, λ DNA	Bitumen (100 µg/mL) + UVA (24 mJ/cm^2)	–	NT		Hong & Lee (1999)
DNA adducts (^{32}P-postlabelling), calf thymus DNA	Condensates of bitumen fume generated at 160 °C or 200 °C	NT	+	DNA-adduct formation correlated with mutagenicity in *S. typhimurium*	De Méo *et al.* (1996)
DNA adducts (^{32}P-postlabelling, ^{32}P-HPLC), calf thymus DNA	Bitumen fume sampled at hot storage tanks	NT	+	Activation by rat or human liver microsomes	Akkineni *et al.* (2001)
Formation of oxidative DNA damage (8-OH-dG), λ DNA	Bitumen (100 µg/mL) + UVA (6 mJ/cm^2)	+	NT		Hong & Lee (1999)
	Bitumen (only) 10 µg/mL	±	NT		
Micronucleus formation, Chinese hamster lung V79 cells	Fume of type I and III bitumen, generated at 316 ± 10 °C. Fume condensates were tested at 0–250 µg/mL.	+	NT	Immunostaining for kinetochores suggests activity as aneuploidogen	Qian *et al.* (1996)
Micronucleus formation, Chinese hamster lung V79 cells	Fume of type III bitumen, generated at 316 ± 10 °C. HPLC of condensates: five fractions tested up to 250 µg/mL[a]	+	NT	The four most polar of the five fractions were positive	Qian *et al.* (1999)
Chromosomal aberrations, Chinese hamster ovary cells	Condensate of bitumen fume, drawn from a storage tank at 147–157 °C (5–120 µg/mL for up to 18 h).	–	–		Reinke *et al.* (2000)
	Condensate of bitumen fume from a laboratory generator at 149 °C and 316 °C (5–120 µg/mL for up to 18 h).	–	–		
DNA–protein cross-links, HL60 human promyelocytic leukaemia cell-line	Bitumen (10 µg/mL) combined with UVA (1.5 mJ/cm^2)	+	NT		Hong & Lee (1999)
	Bitumen (only) 10 µg/mL	±	NT		
DNA strand-breaks (comet assay), BEAS 2B human bronchial epithelial cells	Laboratory-generated SMA-WPT fume	+	–		Lindberg *et al.* (2008)

Table 4.4 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
DNA adducts (^{32}P-postlabelling), adult and fetal human skin samples	Bitumen paint, applied topically on the epidermis: 4% or 20% in THF (3 or 15 mg bitumen); 24-h treatment	+	NT	Similar adduct pattern found in skin of mice treated with bitumen, coal-tar or creosote	Schoket *et al.* (1988b)
Micronucleus formation, BEAS 2B human bronchial epithelial cells	*Laboratory samples:*			Sightly toxic at concentrations > 10 µg/ml	Lindberg *et al.* (2008)
	Fumes from SMA produced at 150 °C (40 µg/mL)	+	–		
	Fumes from SMA-WPT produced at 150 °C (10 µg/mL)	+	–		
	Field samples collected during paving:			Slightly toxic at 40 µg/ml	
	Asphalt concrete ± WPT, produced at 145–165 °C	–	–		
	SMA produced at 151–157 °C (20 µg/mL)	+	–		
	SMA-WPT produced at 151–157 °C (10 µg/mL)	+	–		
In vivo					
DNA strand-breaks (comet assay), rat (female Sprague-Dawley) alveolar macrophages	Bitumen fume (class 1) generated at 170 °C; inhalation exposure (353, 641 and 1150 mg.h/m^3)	+	NT	Dose-dependent increase	Zhao *et al.* (2004)
DNA adducts (^{32}P-postlabelling), male Parkes mouse skin and lung	Bitumen paint (57% bitumen) applied to mouse skin (15 mg). Killed after 24 h	+	NT	Adducts found in the treated epidermis and lungs	Schoket *et al.* (1988a)
DNA adducts (^{32}P-postlabelling), BD4 rat (age, 7–8 wk), skin, lung and lymphocytes	Bitumen-fume (160 °C, 200 °C) condensate (class 1) trapped on glass-fibre filter and XAD-2 resin, extracted with benzene and diethylether, respectively. Applied topically on skin, twice.	+	NT	Bitumen-specific DNA adduct found in skin, lung and lymphocytes	Genevois *et al.* (1996)

Table 4.4 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
DNA adducts (^{32}P-postlabelling), female CD1 mouse (age, 8–11 wk), skin	Bitumen, vacuum residue; dermal application	+	NT	Adduct levels correlated with mutagenicity in *S. typhimunium*	Booth *et al.* (1998)
DNA adduct (^{32}P-postlabelling), male CD rat lung cells and blood leukocytes	Fumes of type I and III bitumen, generated at 316 ± 10 °C. Fume condensates were intratracheally instilled, 3×/24 h, at 250–2000 mg/kg bw. Killed 6 h after last administration.	+	NT	Adducts found in lung cells but not in leukocytes	Qian *et al.* (1998)
DNA adducts (^{32}P-postlabelling), Sprague-Dawley BD6 rat (age, 8 wk), lung	Bitumen fume, generated at 200 °C; particles at 5 or 50 mg/m^3; nose-only inhalation, 6 h/d for 5 d	+	NT	Single DNA adduct detected in high-particle sample (50 mg/m^3).	Genevois-Charmeau *et al.* (2001)
DNA adducts, (^{32}P-postlabelling), Big Blue mouse, lung	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (particles, 100 mg/m^3); 6 h/d, 5 d; expression period, 30 d	–	NT		Micillino *et al.* (2002)
DNA adduct (^{32}P-postlabelling), B6C3F$_1$ mouse, lung	Bitumen-fume condensates generated at 180 °C; inhalation (whole-body exposure) 4 h/d for 10 days, 152–198 mg/m^3.	+	NT	BPDE-dG, -dA, and -dC adducts identified by nanoflow-LC/Q-TOF-MS.	Wang *et al.* (2003b)
DNA adducts, two-months old Big Blue® male rat	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (100 mg/m^3 particles); 6 h/d, 5 d.	±	NT	Bitumen-specific DNA adduct.	Bottin *et al.* (2006), Gate *et al.* (2007)
DNA adducts (^{32}P-postlabelling), (young adult SPF-Wistar) rat lung, nasal, and alveolar epithelium	Bitumen-fume condensate, 4, 20, 100 mg/m^3; 6 h/d, 5 d, 30 d, 12 mo inhalation.	+	NT	Adducts found in lung, nasal epithelium and alveoli. Highest adduct level in nasal epithelium.	Halter *et al.* (2007)
cII and *lacI* mutation in lung DNA, Big Blue mouse	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (100 mg/m^3 particles); 6 h/d, 5 d; expression period 30 d.	–	NT		Micillino *et al.* (2002)
cII mutation in lung DNA, Big Blue® male rats (age, 2 mo)	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (particles, 100 mg/m^3); 6 h/d, 5 d	–	NT	Mutation spectrum associated with increase of G:C → T:A and A:T to C:G	Bottin *et al.* (2006), Gate *et al.* (2007)

Table 4.4 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, male Sprague-Dawley rat bone-marrow polychromatic erythrocytes	Asphalt-fume condensate, collected at 160 °C; intratracheal instillation, 0.45–8.88 mg/kg bw, 3 d, killed after 24 h	+	NT	Significant increase in micronuclei at the highest dose	Ma *et al.* (2002)
Micronucleus formation, rat (female Sprague-Dawley) bone-marrow polychromatic erythrocytes	Bitumen fume (class 1) generated at 170 °C; inhalation, higher exposure, 1733 mg.h/m^3.	–	NT		Zhao *et al.* (2004)
Micronucleus formation, young adult SPF-Wistar rat, peripheral blood erythrocytes and bone-marrow polychromatic erythrocytes	Bitumen-fume condensate, 4, 20, 100 mg/m^3; 6 h/d, 5 d, 30 d, 12 mo inhalation.	–	NT		Halter *et al.* (2007)
Micronucleus formation, Wistar rat bone-marrow erythrocytes	Roofing bitumen-fume condensate (class 3); nose-only, inhalation, 30, 100 and 300 mg/m^3, 28 d	–	NT		Parker *et al.* (2011)

[a] There is a confusion throughout the article with the units of concentration; the most plausible unit (µg/mL) is given in the Table.

8-OH-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; bw, body weight; d, day; h, hour; LC/Q-TOF-MS, liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry; mo, month; NT, not tested; SMA, stone-mastic asphalt; THF, tetrahydrofuran; WPT, waste plastics and tall oil pitch
[The exact names of the three adducts are as follows: dA-BPDE, N^6-deoxyadenosine-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide; dC-BPDE, N^4-deoxycytidine-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide; dG-BPDE, N^2-deoxyguanosine-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide.]

Laboratory-generated stone-mastic asphalt fumes, with WPT, produced at 150 °C [class 5] induced DNA damage in the cultured human bronchial epithelial cells BEAS 2B without metabolic activation from rat liver S9, but not after incubation with S9 mix. The laboratory-generated stone-mastic asphalt fumes alone [bitumen class 1], however, gave negative results in the comet assay (alkali-labile damage) with and without metabolic activation. None of the asphalt fumes collected in the field at the paving sites induced DNA damage in BEAS 2B cells with or without metabolic activation (Lindberg *et al.*, 2008).

In an experiment *in vivo* in which rats were exposed by inhalation to bitumen fumes generated at 170 °C under simulated road-paving conditions for 1 hour or 6 hours, the comet assay revealed DNA damage in alveolar macrophages obtained from the rats by bronchoalveolar lavage (Zhao *et al.*, 2004).

(b) DNA adducts

The adduct-forming ability of condensates of bitumen fume generated at 160 °C or 200 °C, when incubated with calf-thymus DNA and rat liver S9, correlated with their bacterial mutagenicity (De Méo *et al.*, 1996). There was also a correlation between DNA adduct-forming ability in female CD1 mouse skin and mutagenicity in *S. typhimurium* for a series of petroleum products, including bitumens (Booth *et al.*, 1998).

Application of black bitumen paint (57% bitumen) to the skin of male Parkes mice resulted in the formation of DNA adducts (detected by ^{32}P-postlabelling analysis) in the treated epidermis and in the lungs of the animals (Schoket *et al.*, 1988b). The pattern of adducts indicated a complex mixture of different species and was similar to the profile produced by coal tar and creosote, although the levels of adduct formation were lower for bitumen. Similar patterns, and levels, of adducts were formed in samples of adult and fetal human skin maintained in short-term organ culture and treated topically with bitumen paint (Schoket *et al.*, 1988a).

Bitumen fumes (160 °C, 200 °C) were trapped on glass-fibre filters and XAD-2 resin, extracted with benzene and diethylether, and the condensates applied twice to the skin of BD4 rats aged 7–8 weeks. After this treatment, complex patterns of DNA adducts were detected in the skin, lung and lymphocytes of the rats. It was noted that the patterns of adducts obtained with bitumen fume were qualitatively different to those induced by coal-tar fume condensate (Genevois *et al.*, 1996).

In another study, microsomes from rat liver and human microsomes both activated components of bitumen to form DNA adducts *in vitro* (Akkineni *et al.*, 2001). Studies with liver microsomes from different strains of mice and with yeast microsomes expressing human CYPs were conducted to investigate mechanisms of metabolic activation of the active components of bitumen (45/60 PEN hardness, derived from heavy Venezuelan crude oil; bitumen fume produced at 160 °C and 200 °C) (Genevois *et al.*, 1998). The findings demonstrated that CYP1A isoforms were partially, but not wholly, responsible for activating the genotoxic components of bitumen; other enzymes under the control of AhR may also play a role.

Several studies have investigated the formation of DNA adducts in mouse or rat lung after exposure to bitumen and bitumen emissions by inhalation. One study did not detect gene mutations at the *cII* and *lacI* loci, or DNA adducts, in the lungs of Big Blue mice exposed to bitumen fume (100 mg/m^3) for 6 hours per day for 5 days, followed by a 30-day fixation period (Micillino *et al.*, 2002). In Big Blue rats treated under the same regimen, and from which there was some evidence of exposure-related mutations, there was also evidence for DNA-adduct formation that appeared to be bitumen-specific (Bottin *et al.*, 2006; Gate *et al.*, 2007). This bitumen-specific DNA adduct was similar to the adduct found in skin, lung and lymphocytes after bitumen

skin-painting of BD4 rats (Genevois *et al.*, 1996) and shown to be different from both major and minor benzo[*a*]pyrene adducts (Bottin *et al.*, 2006; Gate *et al.*, 2007).

DNA adducts were detected in the lungs and in nasal and alveolar epithelium of rats exposed to inhaled bitumen-fume condensate, which increased in a time- and dose-dependent manner (Halter *et al.*, 2007). Nose-only inhalation of bitumen fume by rats also resulted in DNA-adduct formation in the lung at a high level of exposure (50 mg/m^3, 6 hours per day, for 5 days) but not at a lower dose (5 mg/m^3) (Genevois-Charmeau *et al.*, 2001).

Similarly, inhalation by rats of bitumen-fume condensate resulted in DNA-adduct formation in the lung. The presence of adducts formed by benzo[*a*]pyrene was shown by MS (Wang *et al.*, 2003b).

When bitumen-fume condensate was given to rats by intratracheal instillation, DNA adducts were formed in lung tissue, but not in leukocytes (Qian *et al.*, 1998).

(c) Mutagenicity in vivo

In a study in which Big Blue mice were exposed to bitumen fume (100 mg/m^3; CAS No. 8052-42-4; generated at 170 °C; [class 1]) for 6 hours per day for 5 days, followed by a 30-day fixation period, no increase in either the frequency of *cII* mutation, or in the level of bulky DNA adducts detected by ^{32}P-postlabelling, was found in exposed mice compared with non-exposed mice (Micillino *et al.*, 2002). In an analogous experiment in Big Blue rats, the frequency of *cII* mutation was also similar in exposed and non-exposed lungs, although the exposed rats had a slight, non-statistically significant, increase in G:C to T:A and A:T to C:G transversions (Bottin *et al.*, 2006; Gate *et al.*, 2007).

(d) Micronucleus formation

Whole fume condensates of two types of roofing bitumen (type I and type III; fume generated at 316 °C) caused a significant (> five–six times) increase in the frequency of micronucleus formation in Chinese hamster lung fibroblasts (V79 cells). About 70% of the micronucleated cells induced by bitumen-fume condensate carried kinetochore-positive micronuclei, which indicated that the cytogenetic damage caused by the condensates was primarily at the level of the spindle apparatus of the exposed cultured cells (Qian *et al.*, 1996). In a subsequent study, one of the condensates (type III) was separated – on the basis of polarity – into five fractions, four of which showed activity (Qian *et al.*, 1999). The two most active (most polar) fractions contained alkylated benzo- and dibenzothiophenes, and alkylated benzonaphthothiophenes in one case, and alkylated phenylethanones and dihydrofuranones in the other.

In another study, field samples of bitumen fume collected from a paving site (asphalt concrete without or with WPT, produced at 145–165 °C; [class 1, class 5]; stone-mastic asphalt without or with WPT, produced at 151–157 °C; [class 1, class 5] were tested for induction of micronuclei in BEAS 2B human bronchial epithelial cells, with positive results for both types of bitumen, without metabolic activation from S9 (Lindberg *et al.*, 2008).

Male Sprague-Dawley rats were intratracheally instilled with bitumen-fume condensate at 0.45–8.88 mg/kg bw, collected at the top of a paving-bitumen storage tank, in sterile saline for three consecutive days and killed on the next day (Ma *et al.*, 2002). The frequency of micronucleated polychromatic erythrocytes in bone marrow was determined. The mean numbers of cells with micronuclei per 1000 polychromatic erythrocytes was statistically significantly increased at the highest dose (2.9 ± 0.6 *versus* 1.5 ± 0.4 for saline controls, $P < 0.05$).

In another study *in vivo*, exposure of rats to high total levels of bitumen fume by inhalation (1733 mg h/m^3) did not result in detectable micronucleus formation in bone-marrow polychromatic erythrocytes (Zhao *et al.*, 2004). No micronuclei were detected in erythrocytes or polychromatic erythrocytes of the bone marrow of rats exposed to bitumen fume at up to 100 mg/m^3 for 6 hours per day, for 5 days, 30 days, or 12 months (Halter *et al.*, 2007).

Micronucleus formation was not detected in bone-marrow polychromatic erythrocytes of Wistar rats given [class 3] roofing bitumen-fume condensates by inhalation (nose-only) with up to 300 mg/m^3 (the highest concentration tested) for 28 days (Parker *et al.*, 2011).

(e) Chromosomal aberrations

No chromosomal aberrations were induced in Chinese hamster ovary (CHO) cells exposed, with or without exogenous metabolic systems, to condensate of asphalt fumes collected from the head space of an operating hot-mix asphalt storage tank or to laboratory-generated bitumen-fume condensate (Reinke *et al.*, 2000).

4.4 Other effects relevant to carcinogenesis

4.4.1 Activation of AhR-associated pathways

Besides the direct genotoxic effects of the parent compound or of their metabolites, PAHs that are present in bitumen fume and condensate may have more pleiotropic effects that may lead to carcinogenesis by acting through activation of AhR (Schmidt & Bradfield, 1996; Yamaguchi *et al.*, 1997; Puga *et al.*, 2009). The basic information on AhR-mediated mechanisms in relation to the biochemical and toxicological effects of PAHs, some of which also are present in bitumen fume and condensate, is briefly summarized at the beginning of this chapter, and was previously developed in Volume 92 of the *IARC Monographs* (IARC, 2010).

PAHs are indeed among the best characterized high-affinity exogenous AhR ligands, which include a variety of toxic and hydrophobic chemicals (Stejskalova *et al.*, 2011).

AhR is a ligand-dependent transcription factor that regulates a wide range of biological and toxical effects in many species and tissues. In addition to the regulation of the CYP1 family of xenobiotic metabolizing enzymes by AhR via exogenous ligands, the recent recognition of endogenous AhR ligands has helped to understand that AhR also plays a role in many physiological functions, such as regulation of the cell cycle and proliferation, immune response, circadian rhythm, expression of enzymes involved in lipid metabolism, and tumour promotion (Puga *et al.*, 2009; Stevens *et al.*, 2009).

Activation of AhR by high-affinity PAH ligands results in a wide range of cell-cycle perturbations, including G0/G1 and G2/M arrest, diminished capacity for DNA replication, and inhibition of cell proliferation. At present, all available evidence indicates that AhR can trigger signal-transduction pathways involved in proliferation, differentiation and apoptosis by mechanisms dependent on xenobiotic ligands or on endogenous activities that may be ligand-mediated or completely ligand-independent. These functions of AhR coexist with its well characterized toxicological functions involving the induction of phase I and phase II genes for detoxification of foreign compounds (Puga *et al.*, 2009).

Transcriptional activation of targeted genes in response to AhR is not only species- and tissue-specific, but also ligand-specific. Interaction between a ligand and a receptor is characterized by several variables and the final cellular response is dependent on the combination of these variables. In other words, activation of the receptor by two different compounds will result in different quantitative and qualitative outcomes

in terms of the cellular response. It was shown that the majority of exogenous AhR ligands are partial agonists that never elicit maximal response, even if all receptors are occupied, and importantly some partial agonists can behave as functional antagonists, i.e. when combining full agonist with partial agonist, the effect of the full agonist is diminished by partial agonist, hence, displaying antagonistic behaviour (Stejskalova *et al.*, 2011).

The ligand-specificity of AhR responses is an important notion to consider for complex PAH mixtures such as bitumen fume and condensate. As inducers of CYP xenobiotic-metabolizing and conjugating enzymes, individual PAHs present in bitumen fume and condensate that are AhR partial agonists normally stimulate their own metabolism and that of other carcinogens (Stejskalova *et al.*, 2011). Nonetheless, several other carcinogenic PAHs (e.g. benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, dibenzo[*a,c*]anthracene) were also found to be potent inhibitors of *CYP1A2* and *CYP1B1* (Shimada & Guengerich, 2006).

CYP monooxygenases, which are expressed in basically all tissues, albeit at different levels, play an essential role in the metabolic activation of the many constituents of bitumen, including aliphatic, aromatic, and PACs. Their metabolites may also interact with cellular processes and interfere with cellular functions, resulting in cellular stress and/or dysregulation of biological processes (Shimada & Guengerich, 2006).

4.4.2 Changes in gene and protein expression

(a) Workers exposed to bitumen

Changes in protein expression were investigated in skin punch biopsies from 16 bitumen-exposed road pavers (non-smokers; [exposure to bitumen class 1]) and of 10 age-matched controls. Overexpression of BAX and underexpression of BCL2 were observed in skin that had been exposed to bitumen in the long term, suggesting that bitumen fume induces activation of apoptosis (Loreto *et al.*, 2007). Moreover, the overexpression of the proteins TRAIL, DR5 and CASP3, also involved in apoptosis, was also observed as detected by immunohistochemistry in the skin of the same 16 workers – who reported having worn protective gloves, shoes and clothing (Rapisarda *et al.*, 2009). Furthermore, the activation of programmed cell death was demonstrated in the skin by enhanced terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL) positivity.

Skin punch biopsies from 16 road pavers, daily exposed to asphalt and bitumen, were investigated by immunohistochemistry for levels of HSP27, a member of the heat-shock protein family of chaperone proteins, which are involved in cellular defence mechanisms (Fenga *et al.*, 2000). A total of 25 biopsies from the 16 workers were compared with 5 biopsies from unexposed controls. In the worker samples, immunostaining for HSP27 was homogeneously detected in the whole epidermis, including the basal cell layer, and more intense than in the control samples, indicating that HSP27 was upregulated in the workers' skin.

(b) Experimental animals

Microarray analysis of gene-expression changes was performed on tissue from the lungs of rats exposed by nose-only inhalation to bitumen fume generated at 170 °C for 5 days, 6 hours per day. The analysis identified increased expression of many genes involved in lung inflammatory and immune responses (see Section 4.4.3), as well as genes encoding enzymes involved in the metabolism of PAHs and other xenobiotics. The PAH-inducible genes *Cyp1a1* and *Cyp1b1* genes were the most overexpressed in exposed rat lungs. In contrast, another phase I metabolism enzyme, *Cyp2f2*, was downregulated in bitumen-exposed lungs. Other inducible genes with AhR binding site in their promoter, including the antioxidant genes *Nqo1*, *Aldh3a1* and *Gsta5*

were also significantly upregulated in exposed lungs. Moreover, various genes involved in the cellular response to oxidative stress, including superoxide dismutase 2 (*Sod2*) heat-shock 70 kDa protein 1A (*Hspa1a*), haemo oxygenase 1 (*Hmox1*), glutathione peroxidase 1 (*Gpx1*) and metallothionein 1a (*Mt1a*) have been found to be overexpressed in treated animals. Furthermore, genes associated with protease activity and inhibition were differentially modulated in bitumen-exposed and non-exposed rats. In total, 363 out of the 20 500 probes were differentially expressed (Gate *et al.*, 2006).

Analysis by RT-PCR of the expression of selected genes was carried out on lung tissue from rats exposed to bitumen fume by inhalation for up to 12 months. *Cyp1a1* and *Cyp2g1* were up- and downregulated, respectively. Also significantly modulated, although not in a dose-dependent manner, were genes encoding cathepsin K and D, cadherin 22 and the regulator of G-protein signalling. With the CYP monooxygenases *Cyp1a1* and *Cyp2g1*, a dose-dependent regulation in respiratory epithelium of the upper (nasal) and lower (lung) airways was observed. In addition, there was a dose-dependent (not statistically significant) regulation of genes associated with immune response, inflammation and extracellular matrix remodelling, albeit at different levels when lung and nasal epithelium were compared (Halter *et al.*, 2007).

Exposure to an extract of bitumen fume (generated at 150 °C; typical for paving asphalt) of JB6 P^+ cells (mouse epidermal cell line), and in cultured primary keratinocytes (from AP-1-luciferase reporter transgenic mice) resulted in statistically significant increases in the activity of AP-1, a transcription factor that regulates many genes including some involved in cell growth, proliferation and transformation (Ma *et al.*, 2003a). Downstream effects included activation of the PI3K/Akt pathway, which has been shown to play a critical role in tumour promotion. Furthermore, topical application of bitumen fume by painting the tail skin of mice increased AP-1 activity by 14 times (Ma *et al.*, 2003a).

4.4.3 Alteration of the immune system, inflammation, and risk of cancer

Chronic inflammation increases the risk for cancer, in part as a result of enhanced production of reactive oxygen species, inflammatory mediators, and proteolytic enzymes that can both damage DNA and lead to increases in reparative cell proliferation rates (Grivennikov *et al.*, 2010).

Exposure of the immune system to bitumen and bitumen emissions results in a complicated interplay between individual constituents that have the potential to bind to endogenous AhR of immune competent cells and to metabolize as part of the detoxification programme; this results in induction of oxidative stress and/or the removal of reactive metabolites via secondary metabolic processes. As with many tissues, the immunotoxicity of PAHs present in bitumen emissions is dependent on exposure levels to circulating parent compounds and metabolites, cell type-specific expression of AhR and the balance between bioactive *versus* detoxified metabolites. The dose and route of exposure to PAHs present in bitumen and bitumen emissions are important determinants of immunotoxicity in animals and possibly humans. In general, the total cumulative dose of exposure to PAHs correlates well with immunotoxicity in mice. For individual PAHs (e.g. benzo[*a*]pyrene), a biphasic dose–response curve has been reported, whereby low doses stimulated immune responses and high doses caused inhibition (Burchiel & Luster, 2001; Booker & White, 2005).

As summarized in Volume 92 of the *IARC Monographs* (IARC, 2010), the overall effects of PAHs on the immune and haematopoietic systems result from activation of both genotoxic and non-genotoxic (epigenetic) pathways. Because of the heterogeneity of lymphoid and myeloid cell populations and the complex interplay between

different types of cells and secreted products, the mechanisms of action of individual constituents of bitumen fume and condensate have not been assessed as yet. Nonetheless, many of the PAHs clearly exert effects on the developing as well as the mature immune system and some correlation exists between the carcinogenicity of PAHs and their ability to produce immunosuppression.

There is evidence that immunosuppressive PAHs, some of which are present in bitumen fume, function as AhR ligands and this receptor plays an important role in the development of the immune system in mice (Fernandez-Salguero *et al.*, 1995). The precise mechanism whereby the activation of AhR leads to immunotoxicity is not known. Furthermore, as many of the PAHs and their metabolites are moderate to strong (high-affinity) AhR ligands, it is difficult to distinguish between the action of a parent compound, such as benzo[*a*]pyrene, and that of metabolites that are formed in response to AhR binding and the induction of metabolic enzymes. Ligand-dependent AhR activation can lead to immune effects via interaction of AhR with regulatory sequences, i.e. xenobiotic response element/xenobiotic dioxide response element (XRE/XDRE), which are also found in genes coding for innate and adaptive immune response (Esser *et al.*, 2009). Thus, regulation via AhR activation of genes containing XRE/XDREs may well be correlated with immunotoxicity, as observed for several halogenated aromatic hydrocarbons and many PAHs (for review, see IARC, 2010).

Lung tissue from rats exposed to bitumen fume by inhalation had increased expression of many genes involved in the inflammatory and immune response, as shown using microarray technology (Gate *et al.*, 2006). The inflammatory cytokines (interleukins *IL6* and *IL8*) and chemokines (*CCL2*/*MCP1*, *CXCL1*/*CINC1* and *CXCL2*/*M1P2*) were among the most strongly upregulated genes in exposed lungs. In addition, among the 363 differentially regulated genes, about two dozen were associated with the inflammatory process. Furthermore, bronchiolar lavage cells from exposed rats showed increased expression of pulmonary inflammatory-response genes (*TNFα, IL2β, MIP2*) (Gate *et al.*, 2006). While short-term exposure of rats to bitumen fume by inhalation did not produce acute lung damage or inflammation (Ma *et al.*, 2003b), long-term exposure for 1 year produced inflammatory responses (Fuhst *et al.*, 2007).

As bitumen fume and condensate consist of mixtures of PAHs and other PACs, it is difficult to attribute the relative contributions of individual PAHs to the overall immunotoxic effects.

Data concerning the irritative effects of exposure to bitumen emissions on the airways in humans are limited. Raulf-Heimsoth *et al.* (2007) investigated the irritant effects of bitumen on the airways by monitoring inflammatory processes in the upper and lower airways of 74 mastic-asphalt workers exposed to bitumen emissions and of workers in a reference group. All workers were examined immediately before and after shift. At both time-points, nasal lavage fluid (NALF), induced sputum and spot urine were collected and analysed. Exposure to bitumen emissions was monitored by personal air sampling. Significantly higher concentrations of IL-8, IL-6, nitrogen oxide (NO) derivatives, and total protein were determined in sputum collected before and after shift in exposed workers, especially in those that were highly exposed. Thus, irritative effects in response to exposure to fumes and aerosols of bitumen on the upper and lower airways were demonstrated, especially in mastic-asphalt workers with exposure > 10 mg/m^3.

In a further study by the same investigators, the irritative effects caused by vapours and aerosols of bitumen were assessed in a cross-shift study comparing 320 bitumen-exposed mastic-asphalt workers, with 118 road-construction workers as the reference group (Raulf-Heimsoth *et al.*, 2011b). The induced sputum concentrations of IL-8, matrix metalloproteinase-9, and total protein were significantly higher in

bitumen-exposed workers than in the reference group, suggesting potentially (sub)chronic irritative inflammatory effects in the lower airways of bitumen-exposed workers. These investigators also reported an association between genotoxic and inflammatory effects in the lower airways, which they had compared simultaneously with DNA-strand breaks in induced sputum and blood of bitumen-exposed workers (Marczynski *et al.*, 2010).

In the study of Ellingsen *et al.* (2010), several biomarkers of systemic inflammation and endothelial activation were studied during a working season in 72 pavers, 32 asphalt-plant operators and 19 asphalt engineers. Among the bitumen-exposed workers, smokers had lower concentrations of Clara cell protein (CC-16) and surfactant protein A, but higher concentrations of surfactant protein D, IL-6, C-reactive protein, fibrinogen and intercellular adhesion molecule (ICAM)-1 than non-smokers. [The Working Group noted that in this study no evidence for increased systemic inflammation and endothelial activation in bitumen-exposed workers throughout the season could be determined and that several identified confounders such as smoking habits, body mass index and the level of physical activity needed to be considered.]

In lung and nasal respiratory epithelium of rats exposed to bitumen fume, the expression of chitinase – a candidate gene associated with asthma – as well as other genes coding for immune response and of chronic obstructive pulmonary disease was significantly altered (Halter *et al.*, 2007).

4.4.4 Inhibition of gap-junction intercellular communication

In the study of Sivak *et al.* (1997), different laboratory-generated bitumen-roofing fume fractions [bitumen class 2] were produced and tested for inhibition of gap-junction intercellular communication (Toraason *et al.*, 1991; Wey *et al.*, 1992). All fractions inhibited intercellular communication in Chinese hamster lung fibroblasts (V79 cells) as defined by inhibition of the transfer of toxic phosphorylated metabolites of 6-thioguanine (6TG) from wildtype 6TG cells to 6TG-resistant cells via gap junctions. Induction of 6TG-resistant colonies was considered to be inhibitory of intracellular communication (Toraason *et al.*, 1991). Similarly, Wey *et al.* (1992) examined the effect of these fractions on gap-junction intercellular communication in human epidermal keratinocytes. All fractions inhibited gap-junction intercellular communication in a concentration-dependent fashion. Modulation of gap junctions in functional intercellular communication has been implicated in the promotion of tumour growth. The inhibition of gap-junction intercellular communication may disconnect preneoplastic cells from the regulatory signals of surrounding cells, leading to the development of neoplasms.

4.5 Mechanistic considerations

Bitumens, like many petroleum-based products, are complex mixtures that contain a variety of different chemical compounds that can contain carbon, hydrogen, sulfur, nitrogen and oxygen (IARC, 1985). PAHs containing two to seven aromatic, fused-ring systems have been detected in bitumen-fume samples. Several of these PAHs and related agents are known to be genotoxic and/or carcinogenic in experimental systems. These include benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,i*]pyrene, indeno[1,2,3-*cd*]pyrene and naphthalene. Benzo[*a*]pyrene, an IARC Group 1 carcinogen, is also found in some bitumen-fume samples (IARC, 2010). Non-substituted and substituted thiaarenes containing two to four rings, including dibenzothiophene, benzo[*b*]naphtho[2,1-*d*]thiophene, benzo[*b*]naphtho[1,2-*d*]thiophene and benzo[*b*]naphtho[2,3-*d*]thiophene were also detected in

bitumen fumes (Brandt *et al.*, 2000; Reinke *et al.*, 2000; Binet *et al.*, 2002). The azaarene quinoline, and the oxyarene dibenzofuran, were also detected (Brandt *et al.*, 2000).

Studies in experimental systems on the genotoxic activities of bitumen-fume samples have given mixed results that have been ascribed to the differing compositions of the bitumen samples used, the temperature at which fumes are generated, and experimental conditions used to collect the fume condensates. These genotoxic activities included DNA-adduct formation, bacterial mutagenicity, DNA damage and chromosomal effects. Many of these studies used laboratory-generated bitumen-fume samples and a group of these studies showed positive results in tests for genotoxicity. Field-simulated samples were generally negative for genotoxicity. One study used field-collected samples and gave positive results for bacterial mutagenicity (Heikkilä *et al.*, 2003). Bitumen-fume condensate was mutagenic in bacterial assays in the presence of metabolic activation (Machado *et al.*, 1993; De Méo *et al.*, 1996; Kriech *et al.*, 1999a; Reinke *et al.*, 2000; Heikkilä *et al.*, 2003). Bitumen-fume condensate and several of its fractions induced micronucleus formation in mammalian cells (Qian *et al.*, 1996, 1999). Bitumen fume induced DNA damage (comet assay) in rat lung and pulmonary alveolar macrophages after inhalation exposure (Zhao *et al.*, 2004) and induced micronucleus formation in polychromatic erythrocytes from rats treated by intratracheal instillation with bitumen-fume condensate (Ma *et al.*, 2002).

Lung tissue from rats exposed to bitumen fume and condensates by inhalation had increased expression of *Cyp1a1, Cyp1b1, Cyp2g1* genes and other genes involved in PAH metabolism (Gate *et al.*, 2006; Halter *et al.*, 2007). Nasal tissues showed increased expression of *Cyp1a1* (Halter *et al.*, 2007). These results suggested that bitumen-fume exposure activated AhR, a ligand-dependent transcription factor that regulates a wide range of biological and toxical effects. Increases in rat microsomal lung Cyp1a1 protein levels and enzymatic activity were also observed (Ma *et al.*, 2002).

Many studies using ^{32}P-postlabelling techniques have shown the presence of bulky aromatic DNA adducts in mammalian cells, isolated tissues or tissue samples from experimental animals treated dermally with bitumen-fume condensate or by inhalation of bitumen fume (Table 4.4). In a study of inhalation of bitumen fume in mice, MS of DNA isolated from lung tissue identified three specific benzo[*a*]pyrene–DNA adducts that were derived from *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide: *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-deoxyguanosine; *anti*-benzo[*a*]-pyrene-7,8-dihydrodiol-9,10-epoxide-deoxyadenosine and *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-deoxycytosine. Rats exposed under similar experimental conditions produced detectable urinary levels of benzo[*a*]pyrene-7,8-dihydrodiol, benzo[a]pyrene-7,8,9,10-tetrol and 3-hydroxybenzo[a]pyrene (Wang *et al.*, 2003a, b).

Lung tissue from rats exposed to bitumen fume by inhalation showed increased expression of many genes involved in the inflammatory and immune responses, as shown using microarray technology (Gate *et al.*, 2006). Bronchiolar lavage cells from exposed rats showed increased expression of genes involved in the pulmonary inflammatory response (*Tnfα, Il2β, Mip2*) (Gate *et al.*, 2006). While short-term exposure of rats to bitumen fume by inhalation did not produce acute lung damage or inflammation (Ma *et al.*, 2003b), long-term exposure for 1 year produced inflammatory responses (Fuhst *et al.*, 2007).

Humans are exposed to bitumen emissions dermally and by inhalation. Over the years, conflicting results have been reported in studies on biomarkers of exposure and effects using populations of bitumen-exposed workers. Confounding variables such as differences in population characteristics (e.g. roofers *versus*

pavers), population size, changes in exposure levels due to improved safety practices and potential confounding factors such as age, smoking, nationality, time of measurement, diet, and exposures from other sources may have contributed to the disparate results reported. Overall, there was evidence from studies of occupationally exposed populations that workers exposed to bitumen fume have been exposed to a group of PACs, some of which are genotoxic and carcinogenic. This conclusion was based on studies that measured the mutagenicity of urine samples in bacteria (Pasquini *et al.*, 1989), urinary 1-OHP and other OH-PAHs (Burgaz *et al.*, 1992; Toraason *et al.*, 2001; Campo *et al.*, 2006; Buratti *et al.*, 2007; Raulf-Heimsoth *et al.*, 2007; Pesch *et al.*, 2011), and bulky aromatic DNA adducts and PAH–albumin adducts in peripheral blood (Herbert *et al.*, 1990a, b; Lee *et al.*, 1991). Workers exposed to bitumen had higher levels of oxidative DNA damage measured as 8-OH-dG adducts in peripheral blood lymphocytes and increased levels of DNA damage, sister-chromatid exchange, micronucleus formation and chromosomal aberrations in leukocytes (see Table 4.2). The qualitative and quantitative analyses of bulky aromatic DNA adducts in leukocytes of bitumen-exposed populations established that these populations had been exposed to PACs.

Constituents of bitumen fume also interfere with intercellular gap-junctional communication. Inhibition of intercellular communication may disconnect a pre-neoplastic cell from the regulatory signals of surrounding cells to possibly foster tumour development (see Section 4.4.4).

In summary, bitumen fume contains PAHs and heterocyclic PACs. Many of the PAHs are mutagenic and carcinogenic and have produced many of the same genotoxic activities as those reported for bitumen-fume condensates (IARC, 2010). One of these PAHs, benzo[*a*]pyrene, has been detected in bitumen-fume condensates and in the lungs (as benzo[*a*]pyrene-diol-epoxide–DNA adducts) of mice and in the urine (as benzo[*a*]pyrene metabolites) of rats exposed to bitumen fume. It is noted that while bitumen-fume condensates induced skin tumours in mice after dermal application (Sivak *et al.*, 1997; Clark *et al.*, 2011; Freeman *et al.*, 2011), there are no adequate studies of bitumen fume and cancer in mice exposed by inhalation, and a study of bitumen fume and cancer in rats exposed by inhalation was considered negative, even though a nasal tumour, defined as an adenocarcinoma, was reported (Fuhst *et al.*, 2007). While there was evidence for the role of benzo[*a*]pyrene in the genotoxicity of some bitumen fumes in experimental systems, the lack of definitive studies linking the genotoxic effects induced by exposure to bitumen fume to other specific PAHs or heterocyclic PACs prevents the identification of a clear role for these agents in the mechanism of genotoxic or carcinogenic action of bitumen fume.

There is conclusive evidence that bitumen fume and condensate cause cellular stress and disrupt cellular defence programmes. This leads to overproduction of reactive oxygen species, which perpetuates inflammatory signalling as a result of AhR-mediated or AhR-independent signalling. An imbalance in the detoxification of reactive oxygen species stimulates the immune response. Inflammation affects immune surveillance and immune cells infiltrate tumours to engage in an extensive and dynamic crosstalk with cancer cells. Bitumen fume and condensate induce inflammatory signalling, but may also function as immunosuppressants, possibly via AhR-mediated immunotoxicity (Burchiel & Luster, 2001; Gate *et al.*, 2006; Puga *et al.*, 2009; Stevens *et al.*, 2009).

On the basis of the weight of evidence from studies in experimental systems, it is highly probable that a mechanism involving genotoxicity is responsible for the tumorigenic effects of exposure to bitumen in mouse skin.

In studies in humans, exposure to bitumen fume resulted in more mutagenic urine, 8-OH-dG in DNA – a measure of reactive oxygen species – DNA damage, unidentified bulky aromatic DNA adducts, PAH–albumin adducts, sister-chromatid exchange, micronucleus formation, and chromosomal aberrations. Associations have been reported between genotoxicity and inflammatory effects in the lower airways of humans.

These positive findings in humans are consistent with a mechanism involving genotoxicity that is responsible for the tumorigenic effects of exposure to bitumen.

5. Summary of Data Reported

5.1 Exposure data

Bitumens are a complex mixture of organic compounds of high relative molecular mass that are manufactured in large quantities as residuum of crude oil in petroleum refineries, and that also occur naturally in petroleum-rich regions of the world. The major classes of bitumen are straight-run bitumens (class 1), oxidized bitumens (class 2) cutback bitumens (class 3), bitumen emulsions (class 4), modified bitumens (class 5) and thermally cracked bitumens (class 6). Oxidized bitumens are further divided into semi-blown (or air-rectified) bitumens, with applications similar to class 1 bitumens, and fully oxidized bitumens. Global annual consumption of bitumens is estimated to be more than 100 million tonnes, the vast majority of which is used for road paving (85%) and roofing (10%). Straight-run bitumens mixed with mineral aggregates are used in road paving, generally at 110–160 °C. Oxidized bitumens are used in hot-roofing applications (180–230 °C). Mastic asphalt, a subclass of class 1 bitumens, is used in specialized applications at higher temperatures (200–250 °C). These applications result in emissions of fume (the aerosolized fraction of total emissions, i.e. solid particulate matter, condensed vapour, and liquid petroleum droplets), vapours and gases. Although most polycyclic aromatic compounds are removed during the manufacturing process, these fumes and vapours contain a mixture of two- to seven-ring polycyclic aromatic compounds varying in composition and concentration by application temperature. There is strong evidence that higher application temperatures are associated with higher exposures.

More than one million workers in road paving, roofing, manufacture of bitumen and bitumen products and other specialized applications may be exposed to bitumen by dermal contact and to bitumen emissions by inhalation. The levels of exposure to bitumen and bitumen emissions vary by type of application, job, type of bitumen used and over time. The highest concentrations of bitumen fumes and vapours have been measured during mastic-asphalt application and for roofers applying hot bitumen, while asphalt-mixing plant workers and pavers are exposed to lower concentrations.

Both pavers and roofers may be coexposed to coal tar. Exposure to coal tar among roofers was associated with a 35-times increase in dermal exposure to benzo[*a*]pyrene and a 6-times increase in dermal exposure to polycyclic aromatic compounds. Similarly, exposure by inhalation to benzo[*a*]pyrene among road pavers was estimated to be a factor of 5 higher when coal tar was present. Both roofers and road-paving workers may also have coexposure to engine exhausts, mineral dusts, diluents of bitumens, and thermal degradation products of modified bitumens.

Time-trend data are primarily available for road paving in Europe, where exposures to bitumen fume, bitumen vapour and benzo[*a*]pyrene have decreased by a factor of 2–3 each decade since 1970. In the USA and Europe, the recent introduction of warm-mix asphalts with a lower application temperature (100–140 °C) will further lower exposures to bitumen fumes and

vapours for road pavers. Similarly, cold and soft applications have been developed to lower exposure to bitumen emissions in roofers.

5.2 Human carcinogenicity data

The Working Group reviewed all the available studies to evaluate the risks of cancer in workers occupationally exposed to bitumens. The Working Group summary of the findings emphasized a meta-analysis published in 1994 that included eight case–control and eleven cohort studies. Of those studies not included in the meta-analysis, the IARC multicentre cohort study was considered to be the most informative because of its large size and detailed evaluations of exposure to bitumen and potentially confounding exposures. The risk ratios from the 1994 meta-analysis provided the starting point for the evaluation of exposure to bitumens and risk of cancer. Next, results from studies not included in the meta-analysis were considered. Finally, findings from the IARC multicentre study were considered. The meta-risk ratio from the more recent meta-analysis of the literature was not used for the evaluation because it included the IARC multicentre study. When making its assessments, the Working Group took into consideration that there was some overlap in the populations included in some of the individual studies, the meta-analysis and the IARC multicentre cohort study. Four major occupational categories exposed to bitumens were identified, namely, road paving, roofing, mastic-asphalt applications and diverse occupations involving exposure to bitumens, including asphalt products manufacturing, mixing plants , and unspecified occupations.

5.2.1 Road-paving workers

(a) Cancer of the lung

Several studies have assessed the risk of cancer of the lung among road-paving workers. A meta-analysis published in 1994 calculated a meta-risk ratio of 0.87 (95% CI, 0.76–1.08) for lung cancer based on four studies (three case–control and one cohort) of pavers and road-maintenance workers.

Two independent case–control studies were not included in the meta-analysis. A study from Northern Germany reported an unstable odds ratio of 3.7 (95% CI, 1.06–13.20), while the other, which pooled two other German case–control studies, found only a small excess (odds ratio, 1.20; 95% CI, 1.0–1.5). The occupational group examined in the German studies combined road pavers with excavating workers and pipe-layers and therefore probably involved exposure to asbestos and silica dust in addition to bitumen, and therefore the effect of bitumens may have been diluted. Risk ratios among road pavers varied between 0.8 and 1.6 in the cohort studies published after 1994.

The IARC multicentre cohort study, by far the largest study reporting data for road-paving workers, observed increased mortality from cancer of the lung among road-paving workers in comparison to the general population (SMR, 1.17; 95% CI, 1.01–1.35), which was attenuated when an internal group of ground and building construction workers was used as the referent (RR, 1.08; 95% CI, 0.89–1.34). While internal referents may give risk estimates that are less likely to be confounded by tobacco smoking, they may be exposed to other occupational carcinogens (e.g. quartz, asbestos) that may negatively bias the risk estimates for bitumen and thus underestimate an association. A large variation in the risk of cancer of the lung was present for the internal controls between countries, which calls for additional caution when comparing risks obtained with the external *versus* internal referents.

Exposure response was investigated within the group of road-paving workers in the IARC multicentre cohort study, using quantitative exposure estimates (cumulative exposure, average exposure, and exposure duration) for bitumen fume, organic vapours and benzo[*a*] pyrene. In a comparison of different quantitative measures of bitumen exposure in road-paving workers, average exposure to bitumen fume improved the model fit compared with cumulative exposure and duration in both lagged and unlagged analyses and was significantly associated with mortality from cancer of the lung. In the case–control study nested within a part of the IARC cohort that excluded earlier workers exposed to coal tar but exposed to higher levels of bitumens, there were no indications of a positive trend in risk of cancer of the lung with average or cumulative exposure to bitumen.

The Working Group evaluated a group of studies to focus on exposure to bitumens and bitumen emissions in road pavers. These studies partly overlapped with those reviewed for the evaluation of road paving with coal-tar pitch, which has previously been classified by IARC as a Group 1 carcinogen. Overall, the evidence for an increased risk of cancer of the lung among road pavers and road-maintenance workers exposed to bitumens was inconsistent and observed relative risks were – if anything – only marginally elevated.

(b) Cancer of the urinary bladder

The 1994 meta-analysis reported a meta-risk ratio of 1.20 (95% CI, 0.74–1.83). Among the four additional studies not included in this meta-analysis, two reported slight deficits for bladder cancer, and one a slight excess, while a twofold relative risk was reported in the extended German part of the IARC multicentre cohort study. The IARC multicentre cohort study reported an increased risk of bladder cancer among pavers associated with exposure to benzo[*a*]pyrene, but not with bitumens specifically. Although there was a suggestion of an association between bladder cancer and bitumen exposure among road pavers in some studies, the data were inconclusive.

(c) Cancer of the upper airway and upper digestive tract

A study of proportionate mortality in the USA found a deficit for cancers of the buccal cavity and pharynx, and of the oesophagus among road pavers. Mortality for cancer of the larynx was about as expected. In the IARC multicentre cohort study, the standardized mortality ratio for cancer of the head and neck was 1.30 (95% CI, 0.99–1.68), and a risk ratio of 1.24 (95% CI, 0.91–1.68) was found when using an internal control group. Overall, the data regarding these cancers and bitumen exposure among pavers were inconclusive.

(d) Other cancer sites

An association between cancer at other sites (e.g. stomach, kidney, leukaemia and skin) and exposure to bitumens as a road-paving worker was investigated in several other studies. The data were inconclusive.

5.2.2 Roofing workers exposed to bitumens

When assessing risk of cancer associated with roofing, it should be noted that the proportion of roofers actually applying or removing bitumen roofs or involved in waterproofing with bitumens varies between studies and between countries. The occupational category "roofer" may be a poor proxy for exposure to bitumen in population-based studies conducted in countries where most roofs are covered with shingles or clay-based roof tiles. Industry-based studies have a better ability to restrict cohorts to the exposure of more specific interest. Roofers are more likely to be exposed to coal tar than are road pavers, since a common task for roofers on a gentle slope or flat roofs is to remove old roofs that may contain coal tar – a procedure that

involves high exposure to coal-tar dusts. Roofers may be exposed to other agents such as asbestos, e.g. while removing corrugated asbestos cement plates. Roofers are typically exposed to higher emissions of bitumen than are road pavers due to the higher application temperatures in use.

(a) Cancer of the lung

Twelve publications (eight cohort studies and four case–control studies) provided information on risks of cancer of the lung among roofers. A meta-analysis published in 1994 that included four cohort studies and three case–control studies showed a meta-risk ratio of 1.78 (95% CI, 1.50–2.10), with virtually no difference between cohort and case–control studies, although the latter studies generally adjusted for smoking.

Four additional publications of cohort studies and one case–control study from Italy, all published after 1994, provided additional information on risk of cancer of the lung among roofers. Statistically significantly increased risks of cancer of the lung were observed among roofers in two studies of proportionate mortality from the USA, one based on the population in Washington State and the other on unionized roofers and waterproofers. In the IARC multicentre cohort study, a standardized mortality ratio of 1.33 (95% CI, 0.73–2.23) was observed among roofing and waterproofing workers.

The Working Group evaluated a group of studies to focus on exposure to bitumens and bitumen emissions among roofers. These studies overlapped with those reviewed for the evaluation of roofing with coal-tar pitch, which has previously been classified by IARC as a Group 1 carcinogen. There was a clear association between roofing and lung cancer in the studies reviewed here that was not likely to be a result of chance. Risk estimates were as high in the case–control studies that were adjusted for smoking as in the cohort studies, which suggested that the observed excess was not likely to be explained by uncontrolled confounding from smoking. Roofers have been exposed to other human lung carcinogens such as coal tar, and few studies assessed the magnitude of this potential confounding. Although it was unlikely that the excess risks are entirely explained by coal-tar exposure, confounding could not be ruled out with reasonable certainty.

(b) Cancer of the urinary bladder

Two independent cohort studies of roofers found excesses for bladder cancer, while a study of proportionate mortality reported deficits. The association of bladder cancer with employment as a roofer was assessed in a large multicentre case–control study in Europe, showing an odds ratio of 0.72 (95% confidence interval, 0.36–1.43) for roofers, after adjustment for smoking. A subsequent follow-up of the Scandinavian part of the IARC multicentre cohort showed no overall excess incidence of cancer of the bladder among roofers, but indicated a non-statistically significant association with time since follow-up. Overall, cancer of the bladder did not appear to be associated with exposure to bitumens in these studies of roofers.

(c) Cancer of the upper airway and upper digestive tract

Four cohort studies that assessed the risk of cancers of the upper aerodigestive tract among roofers all showed elevated risks. These studies showed relative risks ranging from 1.3 to 3.3 for cancers of the buccal cavity, pharynx, larynx and oesophagus, although possible confounding by tobacco, alcohol or other occupational exposures could not be ruled out.

(d) Other cancer sites

The Working Group considered several other cancer sites (e.g. stomach, kidney, leukaemia and skin) associated with occupation as a roofer, with some studies showing excesses, but in general the results were inconsistent.

5.2.3 Mastic-asphalt workers

The highest reported exposures to bitumen emissions occured among mastic-asphalt workers, and it is noteworthy that the temperature at which this material is applied is generally 200–250 °C. Informative data about the association of cancer with mastic-asphalt work were provided by a cohort study of mastic-asphalt workers in Denmark who were followed for cancer incidence and mortality, showing a standardized incidence ratio of 3.44 (95% CI, 2.27–5.01) for cancer of the lung. The risk for cancer of the lung was higher (SIR, 8.57; 95% CI, 1.77–25.05), although imprecise when the cohort was restricted to time periods during which no coal tar was used. Results similar to the incidence study were found in a subsequent mortality study of the same cohort, and remained substantially elevated after group-level adjustment for smoking. This study was noteworthy because of the distinctive exposures among mastic-asphalt workers and the efforts to control confounding by coal tar and tobacco smoking. Excess risks were noted for several other cancers, notably cancers of the upper aerodigestive tract (mouth, larynx, oesophagus).

The IARC multicentre cohort study also showed increased risks of cancer of the lung among workers identified as mastic-asphalt workers (SMR, 2.39; 95% CI, 0.78–5.57).

5.2.4 Other occupational groups

Exposure to bitumens has been studied in several occupations other than roofing, paving and mastic-asphalt work, namely during asphalt-product manufacturing and fibreglass manufacturing. In addition, case–control studies often reported information on self-reported or assigned exposures integrated across a wide range of occupations that were not specifically reported (potentially including exposures encountered through employment in paving, roofing and mastic-asphalt work). Few studies have reported on individual cancer sites and the findings were inconsistent. The Working Group considered studies of these diverse occupational groups to be uninformative regarding the carcinogenicity of exposure to bitumens.

5.3 Animal carcinogenicity data

Straight-run bitumens (class 1), oxidized bitumens (class 2), pooled mixtures of class 1 and class 2 bitumens, fumes generated from class 1, class 2 or pooled mixtures of class 1 and class 2 bitumens, cutback bitumens (class 3), and thermally cracked bitumens (class 6) have been tested for carcinogenicity in experimental animals.

Straight-run bitumens (class 1) have been studied as the neat material in one skin-painting study in mice, one skin-painting study in rabbits and one study of subcutaneous injection in mice. All three of these studies were considered to be inadequate for the evaluation of carcinogenicity. Straight-run bitumen (class 1) has been studied by application in a vehicle solvent in four skin-painting studies in mice, one study of intramuscular injection in mice and one study of intramuscular injection in rats. Two of the studies of skin-painting in mice were considered to be inadequate for the evaluation of carcinogenicity. Eight different "road-grade asphalts" (bitumens class 1) applied in benzene in one of the skin-painting studies in mice did not produce an increase in the incidence of skin tumours. Another of the skin-painting studies of two different class 1 bitumens applied in mineral oil to mice did not produce skin tumours. The study of intramuscular injection in mice of four different "road petroleum asphalts" (bitumen class 1) diluted in tricaprylin was considered to be inadequate for the evaluation of carcinogenicity. Straight-run bitumen (class 1) fume condensates have been studied in two skin-painting studies and one study of subcutaneous injection in mice. One of the skin painting studies and the study

of subcutaneous injection were considered to be inadequate for the evaluation of carcinogenicity. A "field matched" paving bitumen (class 1) fume condensate collected at 148 °C applied in mineral oil in one skin-application study in mice did not produce an increase in the incidence of skin tumours.

Oxidized bitumen (class 2) has been studied as the neat material in three skin-painting studies in mice, one skin-painting study in rabbits, and one study of subcutaneous injection in mice. Two of the skin-painting studies in mice, the skin-painting study in rabbits and the subcutaneous-injection study in mice were all considered to be inadequate for the evaluation of carcinogenicity. Application of a neat "built-up type III roofing 'steep' asphalt" (bitumen class 2) did not produce an increase in the incidence of skin tumours in mice. Oxidized bitumen (class 2) has been studied by application in a vehicle solvent in two skin-painting studies in mice. An "air-refined petroleum asphalt" (bitumen class 2) applied in toluene in one of the skin-application studies in mice produced a significant increase in the incidence of malignant skin tumours. In the other skin-painting study in mice, a "standard roofing-petroleum asphalt" (bitumen class 2) applied in toluene did not produce skin tumours. Oxidized bitumen (class 2) fume or fume condensates have been studied in seven skin-painting studies in mice, and three inhalation studies in rats and guinea-pigs. One of the skin-painting studies in mice and the studies of inhalation in rats and guinea-pigs were considered to be inadequate for the evaluation of carcinogenicity. Six of the skin-painting studies in mice were conducted with oxidized bitumen (class 2) fume condensates generated by heating bitumen at temperatures ranging from 199 °C to 316 °C, collecting the resultant fume condensate and applying it either neat or in a vehicle (mineral oil). Significant increases in the incidence of skin tumours in treated animals were observed in these six studies. For example, type I and type III "built-up roofing 'steep' asphalts" (bitumen class 2) were heated to 232 °C or 316 °C, and the laboratory-generated fume condensate collected, applied in cyclohexane/acetone (1:1) to two strains of mice and caused a significant increase in the incidence of malignant and benign skin tumours in both strains. A "field-matched" type III "built-up roofing asphalt" (bitumen class 2) fume condensate collected at 199 °C and applied in mineral oil in a skin-application study in mice produced an increase in the incidence of malignant skin tumours. A laboratory-generated type III "built-up roofing asphalt" (bitumen class 2) fume condensate collected at 232 °C also applied in mineral oil in a skin-application study in mice, produced a significant increase in the incidence of malignant skin tumours. A "field-matched" type II built-up roofing asphalt (bitumen class 2) fume condensate collected at 199 °C and applied in mineral oil gave positive results as an initiator in a skin-painting initiation–promotion study in mice.

Pooled samples of straight-run bitumens (class 1) and oxidized bitumens (class 2) applied in solvents have been studied in one skin-painting study and one subcutaneous-injection study in mice. Pooled samples of bitumens of class 1 and class 2 have also been used to generate fumes for two studies of inhalation in mice and one study of inhalation in rats. The skin-painting study in mice was considered to be inadequate for the evaluation of carcinogenicity. Subcutaneous injection of a pooled sample of six class 1 and class 2 bitumens suspended in olive oil caused a significant increase in the incidence of injection-site sarcoma. The studies of inhalation of pooled samples of straight-run bitumens (class 1) and oxidized bitumens (class 2) in mice were considered to be inadequate for the evaluation of carcinogenicity. A study of inhalation in rats of a bitumen-fume condensate generated at 175 °C and comprised of a majority (> 70% mass) of air-rectified bitumen (bitumen class 2) with the

remainder being straight-run vacuum residue (bitumen class 1) gave negative results.

Cutback bitumens (class 3) have been studied in two skin-painting studies in mice. An "asphalt cutback" (a solid petroleum-bitumen material cut back to 64% solid with mineral spirits; bitumen class 3) applied in mineral spirits in one skin-application study in mice did not produce an increase in skin tumours. In a skin-painting initiation–promotion study in mice that was conducted on four different cutback materials (bitumen class 3), two of the samples promoted the formation of skin tumours.

Thermally cracked bitumens (class 6) have been studied by applying them in a vehicle solvent in a skin-painting study in mice that was considered to be inadequate for the evaluation of carcinogenicity.

5.4 Mechanistic and other relevant data

Bitumen fume contains PAHs and heterocyclic polycyclic aromatic compounds. Many of the PAHs are mutagenic and carcinogenic and have produced many of the same genotoxic activities as those reported using bitumen-fume condensates. One of these PAHs, benzo[*a*]pyrene, has been detected in bitumen-fume condensates and in the lungs (as benzo[*a*]pyrene-diol-epoxide–DNA adducts) of mice and in the urine (as benzo[*a*] pyrene metabolites) of rats exposed to bitumen fume. It is noted that while bitumen fume induced skin tumours in mice treated dermally, there were no adequate studies of cancer in mice exposed to bitumen fume by inhalation. A study of carcinogenicity in rats exposed to bitumen fume by inhalation was considered negative; even though a nasal tumour, defined as an adenocarcinoma, was reported. While there was evidence for the role of benzo[a]pyrene in the genotoxicity of some bitumen fumes in experimental systems, the lack of definitive studies linking the genotoxic effects induced by bitumen-fume exposures to other specific PAHs or heterocyclic polycyclic aromatic compounds prevented the identification of a clear role for those agents in the mechanism of genotoxic or carcinogenic action of bitumen fume.

In experimental studies, exposure to bitumen fume produced bulky aromatic DNA adducts and specific benzo[*a*]pyrene–DNA adducts related to *anti*-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide. Bitumen fume was mutagenic in bacteria, induced DNA damage and induced cytogenic alterations (micronucleus formation and sister-chromatid exchange).

There was conclusive evidence that bitumen fume and condensate cause cellular stress and disrupt cellular defence programmes. This leads to overproduction of reactive oxygen species, which perpetuates inflammatory signalling as a result of AhR-mediated or AhR-independent signalling. An imbalance in the detoxification of reactive oxygen species stimulates the immune response. Inflammation affects immune surveillance and immune cells that infiltrate tumours to engage in an extensive and dynamic cross-talk with cancer cells. Bitumen fume and condensate induce inflammatory signalling, but may also function as immunosuppressant, possibly via AhR-mediated immunotoxicity.

On the basis of the weight of evidence from studies in experimental systems, it is highly probable that a mechanism involving genotoxicity is responsible for the tumorigenic effects of exposure to bitumen in mouse skin.

In studies in humans, higher levels of mutagenic urine, 8-oxo-deoxyguanosine in DNA (a measure of reactive oxygen species), DNA damage, unidentified bulky aromatic DNA adducts, PAH–albumin adducts, sister-chromatid exchange, micronucleus formation and chromosomal aberrations were observed in workers exposed to bitumen emmissions compared with unexposed workers. Associations have been reported

between genotoxicity and inflammatory effects in the lower airways of humans.

These positive findings in humans are consistent with a genotoxic mechanism for the tumorigenic effects of exposure to bitumens.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of occupational exposures to bitumens and bitumen emissions during roofing and mastic-asphalt work. A positive association has been observed between occupational exposures to bitumens and bitumen emissions during roofing and mastic-asphalt work and cancers of the lung and the upper aerodigestive tract (buccal cavity, pharynx, oesophagus and larynx).

There is *inadequate evidence* in humans for the carcinogenicity of occupational exposures to bitumens and bitumen emissions during road paving.

6.2 Cancer in experimental animals

There is *inadequate evidence* in experimental animals for the carcinogenicity of straight-run bitumens class 1 and fume condensates generated from straight-run bitumens class 1.

There is *limited evidence* in experimental animals for the carcinogenicity of oxidized bitumens class 2.

There is *sufficient evidence* in experimental animals for carcinogenicity of fume condensates generated from oxidized bitumens class 2.

There is *limited evidence* in experimental animals for the carcinogenicity of pooled samples of straight-run bitumens class 1 and oxidized bitumens class 2.

There is *inadequate evidence* in experimental animals for the carcinogenicity of fume condensates generated from pooled samples of straight-run bitumens class 1 and air-rectified bitumens class 2.

There is *limited evidence* in experimental animals for the carcinogenicity of cutback bitumens class 3.

There is *inadequate evidence* in experimental animals for the carcinogenicity of thermally cracked bitumens class 6.

6.3 Mechanistic and other relevant data

6.3.1 Pavers

In studies of pavers, bitumen emissions produced higher levels of mutagenic urine, increased DNA damage, and increased levels of sister-chromatid exchange, micronucleus formation and chromosomal aberrations in human lymphocytes compared with control populations. These positive genotoxic findings in pavers provided strong evidence for a genotoxic mechanism for a tumorigenic effect of exposures to bitumens and bitumen emissions in pavers.

6.3.2 Roofers and mastic-asphalt workers

In studies of roofers exposed to bitumens and bitumen emissions, there was increased DNA damage compared with control populations. In mastic-asphalt workers, there was increased DNA damage and higher levels of 8-OH-dG in lymphocyte DNA – a measure of reactive oxygen species. These positive genotoxic findings in roofers and in mastic-asphalt workers provide weak evidence for a genotoxic mechanism for the tumorigenic effects of exposures to bitumens and bitumen emissions. There was also an association between genotoxic and inflammatory effects in the lower airways in mastic-asphalt workers.

6.4 Overall evaluation

Occupational exposures to oxidized bitumens and their emissions during roofing are *probably carcinogenic to humans (Group 2A)*.

Occupational exposures to hard bitumens and their emissions during mastic-asphalt work are *possibly carcinogenic to humans (Group 2B)*.

Occupational exposures to straight-run bitumens and their emissions during road paving are *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

In making the overall evaluation for occupational exposures to straight-run bitumens and their emissions during road paving, the Working Group considered the following mechanistic results and other relevant data from independent studies in exposed workers:

- Increased levels of DNA damage
- Increased levels of sister-chromatid exchange
- Increased levels of micronucleus formation
- Increased levels of chromosomal aberration.

Many of these events are known to be associated with human neoplasia. In addition, data in experimental systems *in vitro* and *in vivo* support these findings.

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