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Bitumen (vapours and aerosols from high-temperature processing)

MAK Value Documentation, supplement – Translation of the German version from 2019

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the classification of bitumen [8052-42-4; 64741-56-6; 64742-93-4] in Carcinogen Category 2 considering all toxicological end points. New data allowed a separate assessment of the harmful properties of vapours and aerosols from high-temperature processing of 2 bitumen groups.

Straight-Run and Air-Rectified Bitumen

For the derivation of a maximum concentration at the workplace (MAK value), a 2-year inhalation study in rats exposed to vapours and aerosols from a mixture of straight-run and air-rectified bitumen is used. Due to an increased incidence of bronchioalveolar hyperplasia in the lungs and of inflammatory cells in the nasal epithelium, the study resulted in a NOAEC of 6 mg/m^3. Taking into account the extrapolation from an animal study and the increased respiratory volume at the workplace compared with the exposure of the animals at rest, a MAK value of 1.5 mg/m^3 (sum of vapour and inhalable fraction) is derived for straight-run and air-rectified bitumen based on bitumen condensate standard. The MAK value is one-third of the mean concentration at which 3 out of 12 inflammation markers are elevated in the sputum of exposed workers. However, the clinical relevance of this finding is unclear and the margin to the MAK value is considered sufficient. The effect on the lung is the most sensitive endpoint, so vapours and aerosols of bitumen are assigned to Peak Limitation Category II with an excursion factor of 2. All in all, the investigated straight-run and air-rectified bitumens did not exhibit distinct genotoxic or carcinogenic properties. However, due to the wide range

in the chemical composition, harmful emissions of carcinogenic and mutagenic substances during high-temperature processing cannot be completely excluded. Therefore, vapours and aerosols of straight-run and air-rectified bitumens, as commonly used in road paving, are regarded as suspicious carcinogens and classified in Carcinogen Category 3 B. As no data are available for developmental toxicity they are assigned to Pregnancy Risk Group D. Skin contact may contribute significantly to systemic toxicity and the "H" notation is confirmed. Sensitization is not expected from the available data.

Oxidized Bitumen
There are no inhalation studies available for evaluation of the carcinogenicity of oxidized bitumen ("Roofing Bitumen"). The new animal studies published since 2001 and most of the previous studies show the carcinogenicity of condensates of vapours and aerosols of oxidized bitumens in skin painting studies in mice, so that a significant number of tested oxidized bitumens must be considered carcinogenic. The positive animal experiments on the carcinogenicity are consistent with the results of a genotoxicity study in roofing workers with increased DNA strand break rates. Vapours and aerosols of oxidized bitumen are classified in Carcinogen Category 2. A MAK value could not be derived. Mutations in bacteria and higher levels of benzo[*a*]pyrene in oxidized bitumen condensates compared to those of straight-run and air-rectified bitumen and the systemic availability of inhaled vapours and aerosols of oxidized bitumen support suspicion of germ cell mutagenicity. Therefore, vapours and aerosols of oxidized bitumen are classified in Category 3 B for germ cell mutagens. Skin contact may contribute significantly to systemic toxicity and the "H" notation is confirmed. Sensitization is not expected from the available data.

Straight-run Bitumen/Air-rectified Bitumen

MAK value (2018)	**1.5 mg/m³ [a)]**
Peak limitation (2018)	**Category II, excursion factor 2**
Absorption through the skin (2001)	**H**
Sensitization	**–**
Carcinogenicity (2018)	**Category 3 B**
Prenatal toxicity (2018)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BAT value	**–**

Oxidized Bitumen

MAK value	**–**
Peak limitation	**–**
Absorption through the skin (2001)	**H**
Sensitization	**–**
Carcinogenicity (2018)	**Category 2**
Prenatal toxicity	**–**
Germ cell mutagenicity (2018)	**Category 3 B**
BAR/BLW/EKA	**–**

[a)] sum of vapour and the inhalable fraction based on the bitumen condensate standard

Note: The substance can occur simultaneously as vapour and aerosol.

Since the documentation was last revised in 2001 (Greim 2002), studies have been published which have made a re-evaluation necessary. On the basis of these new data, a more differentiated assessment can be made of the harmful properties of vapours and aerosols formed during the high-temperature processing of bitumen or of products containing bitumen. A separate evaluation of the emissions of 2 groups of bitumen has thus been carried out.

This documentation examines the following 2 groups of bitumen:

Straight-run bitumens, commonly also referred to as "vacuum residue", and **air-rectified bitumens**, or "semi-blown bitumens", and mixtures of these, which are used mainly in road paving applications.

Oxidized bitumens, or "blown/fully-blown bitumens", are used primarily for roofing applications. The areas of application differ from one type of bitumen to another according to their technical properties, in particular with respect to hardness, sensitivity to temperature, ductility or viscosity.

However, technical developments are increasingly broadening the areas of application for straight-run and air-rectified bitumens to include those for which once only oxidized bitumens were used.

In the absence of adequate occupational health and toxicological data, an evaluation of other kinds of bitumen and products containing bitumen cannot be made.

Many types of bitumen are listed with individual CAS numbers and the designations assigned to them in North America, and, if applicable, are registered under REACH with an EINECS number. Examples are the CAS numbers 8052-42-4 (asphalt; EINECS number 232-490-9), 64741-56-6 (residues (petroleum), vacuum; EINECS number 265-057-8) and 64742-93-4 (asphalt, oxidized; EINECS number 265-196-4). It should be noted that although both air-rectified bitumens and oxidized bitumens are registered under the CAS number 64742-93-4, this documentation considers the 2 types separately for the toxicological assessment. According to Regulation (EC) No. 1272/2008 (CLP-Regulation), bitumens themselves are not regarded as hazardous substances or mixtures and do not contain hazardous constituents in concentrations relevant for classification that would make them subject to mandatory labelling requirements. Thus, suppliers are not required to provide safety data sheets for bitumen products or include a list of the relevant CAS numbers in a voluntarily supplied safety data sheet.

A number of different analytical methods are used to determine the vapours and aerosols of bitumen (see Section "Analytical methods for determining the vapours and aerosols of bitumen"). The MAK value of 1.5 mg/m^3 for straight-run bitumens and air-rectified bitumens from high-temperature processing was derived according to the German IFA Method 6305 and using "bitumen condensate" as the standard for calibration (bitumen condensate standard, method 6305/2). Values determined using the reference standard "mineral oil for spectroscopy" for calibration (mineral oil standard, method 6305/1), which has been obsolete since 01 January 2007, can be converted by multiplying the respective value by a factor of 1.5.

Introduction

Bitumens are produced on an industrial scale as a residue of the distillation of crude oils suitable for this purpose. The bitumen fraction of crude oil can range from 0 to 60% (for example: Boscan Venezuela: 58%, Nigerian Light: 1%). Only a quarter of the approximately 1500 known types of crude oil can be used for the production of bitumen (Sörensen and Wichert 2009).

The properties of the final products can be modified to meet the specific requirements of an application by the crude oil chosen, the use of certain chemical reactions, additional refining and extraction steps, the blending of different types of bitumen or through the addition of other agents ("blends"). These factors influence also the chemical composition of the different types of bitumen. At standard ambient temperature, bitumens are solid to brittle and non-volatile. When heated, they become soft to fluid. Bitumens are primarily used for products for the building industry (roofing sheets and damp-proof sheeting, insulating paint, building protection agents, screed, joint sealing compounds, adhesives) and as binding agents for aggregates in road paving. In Europe, bitumen mixed with fine or coarse aggregates

is called asphalt, while in North America, the terms “asphalt” or “asphalt binder” are generally used to refer only to the bitumen itself. “Asphalt concrete” is used to describe the surface layer applied to roads.

The exposure concentration at the workplace is dependent not only on the processing temperature, but also on the ventilation conditions.

Parameters for describing technical requirements and for distinguishing between air-rectified bitumens and oxidized bitumens

The technical characterization of bitumens is not based on their chemical composition or the type of crude oil used, but on physical properties such as hardness and their performance at the temperatures required for the intended purpose. These properties can be modified by the crude oil chosen, the use of special refining processes, by blending different bitumen products or by adding aggregates and additives. In some cases, European application standards have been developed that specify the technical requirements.

A range of tests are available to determine the technical properties of different types of bitumen, such as the depth of needle penetration and the ring-and-ball softening point. Needle penetration is determined by measuring the depth to which a standard needle under a load of 100 g penetrates into a bitumen sample in 5 seconds. For testing, the sample is generally kept at a temperature of 25 °C (in the case of very soft bitumens: 15 °C). The results are given as the penetration grade (PEN) in 1/10 millimetres. The specifications for a bitumen product usually state a tolerance range (penetration range) for the depth of penetration. As an example, bitumen 160/220 has a penetration depth of between 16 and 22 mm. The ring-and-ball softening point refers to the temperature at which a ball weighing 3.5 g deflects a bitumen layer to a distance of 25.4 mm. The bitumen layer is poured into a metal ring. The bitumen is heated at a controlled rate and deforms under the weight of the ball. The 2 values are used in combination to designate types of oxidized bitumen: bitumen 115/15 is a grade of bitumen that has a ring-and-ball softening point of 115 °C and reaches a penetration depth of 1.5 mm.

The penetration index is calculated from the values obtained for needle penetration and the ring-and-ball softening point using a formula provided in European standard EN 12591, Appendix A. The penetration index expresses the thermal sensitivity of bitumen and is used in Europe to distinguish between air-rectified bitumens, which have a penetration index ≤ 2.0, and oxidized bitumens, which have a penetration index > 2.0.

“Superpave” is another specification system for bitumen that is commonly used in the United States. “Performance grades” (PG) specify performance requirements at high and low temperatures. Performance grades for road paving applications are determined based on the climate zone and specific traffic load (for example PG 64-22: expected average 7-day maximum road surface temperature: 64 °C, expected average 7-day minimum road surface temperature: –22 °C).

Types of bitumen according to manufacturing process

Straight-run bitumen and high vacuum bitumen

Straight-run bitumen is produced from the residues from the atmospheric distillation of petroleum crude oil by further distillation under vacuum (temperatures of 350 °C to 380 °C), solvent precipitation or a combination of these processes. Straight-run bitumen is the undistillable fraction that remains. It occurs as a dark, thermoviscous fluid. This type of bitumen is soft to medium-hard; hard grade bitumen is produced by high-vacuum distillation.

Air-rectified bitumen and oxidized bitumen

Depending on the reaction conditions, either air-rectified bitumen (semi-blown bitumen) or oxidized bitumen (blown bitumen) are formed by blowing air into straight-run bitumen under controlled conditions and in special reactors at temperatures between 232 °C and 277 °C. This process modifies the material’s resistance to cold, heat and ageing and

its hardness and rigidity, thereby extending the application areas of straight-run bitumen in the hard range. At the same time, this increases the softening point. On a molecular level, these effects can be explained by condensation reactions of macromolecules thereby increasing molar mass and polarity. Additionally, lighter hydrocarbons and carbon dioxide are formed during this process. Air-rectified bitumen is produced by briefly introducing air into hot, soft straight-run bitumen under controlled, moderate conditions. By contrast, oxidized bitumen is formed only after an intense oxidation process that involves passing 85–140 m^3/min of air through the feedstock (Asphalt Institute and Eurobitume 2015); this results in a product that has a higher softening point and is harder than air-rectified bitumen.

Precipitated bitumen

Low-boiling hydrocarbons can be extracted from heavy crude oils by adding liquefied gases such as propane or butane. The precipitated bitumen that forms as a residue of this process is a relatively hard form of bitumen.

Thermally cracked bitumen

The visbreaking (viscosity breaking: reduction of viscosity) of straight-run bitumen involves the formation of highly volatile hydrocarbons from saturated long-chain hydrocarbons by short-term cracking under pressure at 440 to 500 °C and subsequent removal by distillation. The residue of this process is thermally cracked bitumen (visbreaker vacuum residue, CAS number 92062-05-0), a hard, high-molecular material used as a blending component. This type of bitumen is used to modify other products to meet specific technical requirements.

Modified bitumen

Modified bitumen generally contains 3% to 15% by weight of special additives such as polymers or rubber granules that modify the properties of the bitumen. According to REACH-terminology, modified bitumens are defined as mixtures and have not been assigned individual CAS numbers. Instead, they are listed as the individual substances. The type most commonly used in Europe for surfaces with a particularly high traffic load in road and airport construction and for high-quality roofing sheets and damp-proof sheeting are polymer modified bitumens as defined by the European standard EN 14023. Polymer modified bitumen is made by mixing straight-run bitumen with additives such as polypropylene or styrene–butadiene–styrene rubber.

Rubber modifications are rarely used in Germany. They should not be heated to temperatures above 190 °C. No European application standards have been developed for rubber modified bitumens.

Cutback and fluxed bitumen

As specified by European standard EN 15322, cutback or fluxed bitumen is produced by adding agents to straight-run bitumen or oxidized bitumen to reduce their viscosity. The products can thus be used at lower processing temperatures. Fluxed bitumen is prepared by adding flux oils to bitumen to reduce its viscosity (EN 12597: bitumen and bituminous binders – terminology, 2013; section 2.9). Flux agents or flux oils are liquids with a relatively low volatility (oils; EN 12597; section 2.7.1) that are either mineral oil or plant-based. However, the designation fluxed bitumen is used only for bitumens that have been prepared with mineral oil-based flux agents, which are typically oils with varying distillation ranges (EN 12597; section 2.9.3). The viscosity of cutback bitumen is reduced by adding a diluent (EN 12597; section 2.8); one such example is repair asphalt. These diluents are fractions of crude oil distillation with a fairly high volatility such as naphtha or kerosine (EN 12597; section 2.7.2). CAS numbers have not been assigned to cutback and fluxed bitumens because they are different blends of various products.

Cationic bitumen emulsions

Cationic bitumen emulsions (EN 13808) are fine bitumen droplets dispersed in water. They are prepared from straight-run bitumen, cutback bitumen and modified bitumen and emulsifiers such as the hydrochlorides of organic amines. Bitumen emulsions are not used at temperatures above 100 °C and almost no heat-related vapours and aerosols are

produced during their application. In addition to cationic bitumens, there are also anionic and non-ionic bitumen emulsions, which are not commonly used for road paving applications in Europe and are not regulated by a European standard.

Viscosity modified bitumen

The processing temperature can be decreased by adding additives such as zeolites, Fischer-Tropsch paraffins or amide wax to straight-run bitumen. This produces "low temperature" bitumens. Rolled asphalt can be applied at temperatures of 100 °C to 140 °C instead of 140 °C to 160 °C and mastic asphalt at 230 °C instead of 250 °C.

Natural asphalts

Natural asphalts are not distillation products of crude oil and therefore not strictly bitumens. They are extracted from natural deposits and often occur mixed with mineral constituents. They have similar properties to those of bitumen and can therefore be used for the same applications. They are relatively hard, practically solid at 25 °C, but viscous at 175 °C. Natural asphalts can, for example, be found in asphalt lakes in Trinidad (La Brea Pitch Lake) and Venezuela (Lago Bermúdez or Lago Guanoco) or in the oil sands of Canada. There are also deposits in countries such as Cuba, the United States, Argentina, Syria and Egypt.

Applications of different types of bitumen

Special manufacturing processes and further refining steps are used to modify the properties of bitumen to meet the technical specifications required for specific applications. The most important applications are described below together with the types of bitumen that are primarily used for them.

Standardized applications

In some cases, standards have been developed that specify the technical properties that bitumens are required to have when used for the applications described below. The specifications are defined using parameters such as the ring-and-ball softening point, the grade of penetration or penetration index (or ranges for these values). The standards do not stipulate the type of bitumen or manufacturing process to be used. Not every type of bitumen can be extracted from every crude oil source by means of a specific manufacturing process. Likewise, bitumen 50/70 (with a penetration grade of 50 to 70 (× 0.1 mm); earlier designation B 65) may occur as a pure straight-run bitumen or as an air-rectified bitumen. In addition, certain specifications can be met by blending different types of bitumen. Examples of bitumen specifications are shown in Table 1. Not all of the types of bitumen listed are used in Germany; depending upon the application, there are many other types that meet a range of different specifications.

Tab. 1 Examples of different types of bitumen

	Type	Penetration at 25 °C [in 0.1 mm]	Ring-and-ball softening point [in °C]
paving grade bitumen[a), b)] [EN 12591]			
	160/220	160–220	35–43
	70/100	70–100	43–51
	50/70	50–70	46–54
	40/60	40–60	48–56
	35/50	35–50	50–58
	30/45	30–45	52–60
	20/30	20–30	55–63

Tab. 1 *(continued)*

	Type	Penetration at 25 °C [in 0.1 mm]	Ring-and-ball softening point [in °C]
multigrade paving grade bitumen [EN 13924-2]			
	MG 50/70–64/74	50–70	64–74
	MG 35/50–59/69	35–50	59–69
	MG 20/30–54/64	20–30	54–64
hard paving grade bitumen[a), b)] [EN 13924-1]			
	15/25	15–25	55–71
	10/20	10–20	58–78
	5/15	5–15	60–76
polymer modified bitumen [EN 14023]			
	120/200–40	120–200	≥ 40
	45/80–50	45–80	≥ 50
	40/100–65	40–100	≥ 65
	25/55–55	25–55	≥ 55
	10/40–65	10–40	≥ 65
oxidized bitumen [EN 13304]			
	100/40	35–45	95–105
	95/35	30–40	90–100
	110/30	25–35	105–115
	85/25	20–30	80–90
	115/15	10–20	110–120
hard industrial bitumen [EN 13305]			
	H 80/90	< 10	80–90
	H 85/95	< 10	85–95
	H 90/100	< 10	90–100
	H 100/110	< 10	100–110
	H 155/165	< 10	155–165
high vacuum bitumen			
	HVB 85/95	4–11	85–95
	HVB 90/100	2–7	90–100
	HVB 130/140	1–7	130–140

[a)] also used in the production of roofing sheets and damp-proof sheeting
[b)] also used in the production of insulating and damping materials for applications in particular in the automotive or household appliance industry

Paving applications

Road paving is the primary area of application for straight-run bitumens and air-rectified bitumens used as binders for aggregates. In this area, processing temperatures can reach 190 °C. Softer types are prepared with only straight-run bitumen, harder types may additionally or exclusively be made of air-rectified bitumen. Polymer modified bitumen is being used for a growing number of applications, such as the maintenance and repair of airfields.

Mastic asphalt (as specified by EN 13108-6) is used to pave surfaces with high traffic load, bridges, underground car parks, pavements and cycle paths. This type of asphalt is prepared with hard straight-run or air-rectified bitumens. Other additives are polymers, waxes or pigments and, in rare cases, also natural asphalt. In 2008, a maximum temperature of 230 °C was set for mastic asphalt processing in Germany. Mastic asphalts are pourable, can readily be spread

at high temperatures and form a lasting surface layer that is impermeable to water, cavity-free and does not require further compaction. In comparison with mastic asphalt screed, the mastic asphalt used for road paving applications contains coarser aggregates or fillers.

In a number of European countries and regions such as France, Switzerland or the Benelux countries, but not in Germany, multigrade bitumens that meet the specifications of multigrade paving grade bitumens as delineated in EN 13924-2 are used for special paving applications (for example for the prevention of rutting and fatigue). They are less sensitive to temperature than paving grade bitumens and have a positive penetration index. As an example, multigrade bitumens are produced by air rectification, either with or without the addition of catalysts or additives. Other manufacturing processes are possible. Hard paving grade bitumens (EN 13924-1) have a high rigidity, but, like multigrade bitumens, are not used in Germany.

Oxidized bitumens are generally not used for road paving applications.

Applications in the building industry

The most important application area for oxidized bitumen is the production and installation of roofing sheets and damp-proof sheeting (roofing); polymer bitumen is likewise used in this area. In addition, oxidized bitumen is used as an adhesive and joint sealing compound, for corrosion resistance and the protection of outdoor pipes.

Mastic asphalts used for screed and sealing applications (surface layers, binder courses, protective and intermediate layers of bridges, tunnels and gutters) in building and industrial construction may contain different hard bitumens such as thermally cracked bitumen (visbreaker residue), precipitated bitumen, hard straight-run bitumen (high vacuum bitumen") and air-rectified bitumen. In comparison with the mastic asphalts used in road paving applications, they are prepared with finer aggregates and other fillers, but otherwise have similar properties and may also be processed up to a maximum temperature of 230 °C. Possible additives are polymers in bridge construction, waxes or pigments. Asphalt mastic is used for these purposes and contains finer aggregates than the bitumen used for paving applications (in Germany < 2 mm) (EN 12970).

Bitumen products used for roofing applications can also be applied "cold" (installation of shingles containing bitumen). Furthermore, a distinction is made between soft applications (welding of bitumen sheeting with heated air or a gas torch) and hot applications (pouring hot bitumen on a low incline roof) (IARC 2013).

Chemical composition

Elemental analysis

The chemical composition of bitumen varies depending on the crude oil used and the manufacturing or refining processes. Bitumens contain primarily hydrocarbons (carbon 79%–88%, hydrogen 7%–13%), sulfur 7%–13%, oxygen 2%–8% and up to 3% nitrogen. In addition, bitumens contain metals such as nickel, vanadium, iron, manganese, calcium, magnesium and sodium (lower mg/kg to lower g/kg range) (IARC 2013).

Separation into solubility fractions

Bitumens are made up of organic compounds that are a complex mixture of saturated straight-chain and branched hydrocarbons (alkanes), monocyclic and polycyclic alkanes with alkyl side chains (naphthenes) and monocyclic and polycyclic aromatic compounds, which also occur linked with alkyl side chains or naphthenes. Alkanes, naphthenes and aromatic compounds may contain heteroatoms. This mixture can be separated into its constituents by SARA fractioning (SARA: Saturates, Aromatics, Resins, Asphaltenes): the asphaltene fraction (usually 5% to 20% in bitumen) consists of the residue that is insoluble in *n*-heptane. Asphaltenes have an average molar mass ranging from 800 to 3500 g/mol and are aggregates of several molecules. They consist of aromatic ring systems with alkyl side chains with heteroatoms, and metal atoms bound to porphyrin rings. The maltenes are the fraction that is soluble in *n*-heptane.

After additional extraction, the maltenes can be separated into fractions containing primarily saturated hydrocarbons (5% to 15%), aromatic hydrocarbons (30% to 45%) and resins (30% to 45%). The average molar mass of the fraction of (primarily) saturated hydrocarbons containing low levels of heteroatoms ranges from 470 to 880 g/mol. Resins are similar in structure to asphaltenes, but have a lower average molar mass (780 to 1400 g/mol). The average molar mass of the fraction of aromatic hydrocarbons with low proportions of aliphatic structures is between 570 and 980 g/mol (Lesueur 2009).

Polycyclic aromatic hydrocarbons (PAHs)

PAH levels in bitumen

PAH levels in bitumen vary depending on the type of bitumen. The types of bitumen commonly used in Germany contain the 16 EPA-PAHs, the polycyclic aromatic hydrocarbons used as indicator substances (see Table 5), in concentrations ranging from 25 to slightly under 100 mg/kg, with benzo[*a*]pyrene concentrations between 1 and 3 mg/kg. Analyses of emissions from 100 g of each of these bitumen types at a temperature of 180 °C have demonstrated that PAH levels vary considerably in the respective vapours and aerosols (see Table 2) (Gesprächskreis Bitumen 2009; Knecht et al. 1999).

Tab. 2 PAH content in bitumen and PAH emissions from bitumen (Gesprächskreis Bitumen 2009; Knecht et al. 1999)

Bitumen type	Content [mg/kg]		Emissions (at 180 °C)		
(new designations in parentheses)	EPA-PAH	benzo[*a*]pyrene	total [mg/h]	EPA-PAH [µg/h]	benzo[*a*]pyrene [µg/h]
HB 90/100 (hard bitumen 90/100)	30.0	1.2	6.6	26.3	0.1
B 45 (B 30/45)	29.8	2.1	13.0	22.7	0.1
B 65 (B 50/70)	26.7	1.7	2.2	3.7	n. d.
B 80 (B 70/100)	25.6	1.4	3.5	6.6	n. d.
B 200 (B 160/220)	32.1	1.8	7.0	4.1	n. d.
B 85/25 (Ox Bit 85/25)	52.2	1.7	25.1	52.9	0.2
B 95/35 (Ox Bit 95/35)	93.5	2.7	35.2	79.0	0.3
Trinidad Epuré, purified natural asphalt	33.8	2.0	42.6	10.3	0.1

n. d.: not detected

The effects of oxidation on PAH levels in bitumen were investigated. The study analysed 12 different bitumens from European refineries and bitumens (air-rectified bitumen and oxidized bitumen) from the same bitumen feedstock modified by the injection of air at temperatures between 204 °C and 277 °C. Six of the 12 types of bitumen contained flux oils. The authors pointed out the difficulties in analysing PAH levels in bitumen because of a lack of uniform standards and generally recognized test methods. To ensure that the results were derived from a broader evidence base, the study combined findings from 3 different laboratories that used different procedures to prepare the samples (see Tables 3 and 4). The addition of flux oils (see Section "Types of bitumen according to manufacturing process") increased PAH levels in both the feedstock and the bitumen modified by the injection of air. On the basis of their findings, the authors concluded that the blowing operation leads to a 10% to 30% reduction in PAH levels by "stripping" from the liquid phase of bitumen. The possible formation of reaction products was not investigated (Bolliet et al. 2013). According to the data, the highest reduction in PAH levels was found in the fraction of 2-ring PAHs and, in the case of bitumens containing flux oil, also the fraction of 3-ring PAHs. Conversely, 4-ring PAH and benzo[*a*]pyrene levels were increased in bitumens modified by the injection of air that did not contain flux oils. However, the reliability of the analysed values is limited by high standard deviations.

Tab. 3 PAH content in straight-run bitumens and oxidized bitumens without additives according to Bolliet et al. (2013)

	Feedstock without flux oil[a), b)]			Bitumen after oxidation (n = 5 oxidized bitumens; n = 1 air-rectified bitumen) without flux oil[a), b)]		
	% BDL	mg/kg	SD mg/kg	% BDL	mg/kg	SD mg/kg
naphthalene	39	6.6	12.5	57	0.9	2.7
acenaphthalene	83	0.0	0.0	88	0.0	0.0
acenaphthene	56	0.4	0.6	67	0.0	0.0
fluorene	28	0.7	0.6	43	0.7	0.5
sum of 2-ring PAHs		**7.7**	**12.5**		**1.7**	**2.7**
phenanthrene	22	2.1	1.9	24	1.9	1.6
anthracene	61	0.2	0.4	67	0.1	0.2
fluoranthene	44	0.6	0.8	38	0.9	0.9
sum of 3-ring PAHs		**2.9**	**2.1**		**2.9**	**1.9**
pyrene	50	0.6	0.7	40	1.0	0.9
benzo[*a*]anthracene	64	0.2	0.3	45	1.0	1.4
chrysene	88	1.1	0.1	57	3.0	2.6
benzo[*k*]fluoranthene	100	–	0.0	86	0.6	0.2
benzo[*k*+*b*+j]fluoranthene	42	0.3	0.3	36	1.3	1.3
sum of 4-ring PAHs		**2.2**	**0.8**		**6.2**	**3.4**
benzo[*a*]pyrene	61	0.3	0.4	52	0.7	0.8
dibenzo[*ac*+*ah*]anthracene	67	0.4	0.5	67	0.5	0.3
indeno[1,2,3-*cd*]pyrene	67	0.3	0.5	69	0.2	0.2
benzo[*g*,*h*,*i*]perylene	39	2.9	4.3	60	1.3	0.8
sum of 5- to 6-ring PAHs		**3.8**	**4.3**		**2.7**	**1.2**
sum of 16 PAHs	**54**	**16.6**	**13.3**	**58**	**13.6**	**5.5**

[a)] number of analysed samples: 6
[b)] number of determinations: 24 to 42
BDL: below the limit of detection; SD: standard deviation

Tab. 4 PAH content of straight-run bitumens and oxidized bitumens with additives according to Bolliet et al. (2013)

	Feedstock with flux oil[a), b)]			Bitumen after oxidation (n = 5 oxidized bitumens; n = 1 air-rectified bitumens) with flux oil[a), b)]		
	% BDL	mg/kg	SD mg/kg	% BDL	mg/kg	SD mg/kg
naphthalene	39	6.6	15.3	61	2.8	9.4
acenaphthalene	81	0.7	0.7	89	0.0	0.0
acenaphthene	67	0.1	0.1	61	0.2	0.5
fluorene	28	1.1	1.3	17	1.1	0.7
sum of 2-ring PAHs		**8.7**	**15.4**		**4.1**	**9.4**
phenanthrene	6	7.3	9.7	6	4.0	4.0
anthanthrene	50	0.4	0.5	56	0.3	0.2
fluoranthene	31	1.5	2.3	19	1.2	1.4
sum of 3-ring PAHs		**9.2**	**10.0**		**5.5**	**4.3**

Tab. 4 *(continued)*

	Feedstock with flux oil[a), b)]			Bitumen after oxidation (n = 5 oxidized bitumens; n = 1 air-rectified bitumens) with flux oil[a), b)]		
	% BDL	mg/kg	SD mg/kg	% BDL	mg/kg	SD mg/kg
pyrene	17	3.2	4.6	17	2.2	2.6
benzo[*a*]anthracene	11	2.0	2.3	11	1.9	1.5
chrysene	17	6.3	6.2	17	6.7	7.6
benzo[*k*]fluoranthene	42	1.3	0.8	50	1.1	0.6
benzo[*k*+*b*+j]fluoranthene	0	3.1	2.2	8	2.7	1.8
sum of 4-ring PAHs		**14.7**	**8.3**		**13.4**	**8.4**
benzo[*a*]pyrene	22	2.7	2.3	17	2.2	2.3
dibenzo[*ac*+*ah*]anthracene	50	1.7	1.4	61	1.6	0.9
indeno[1,2,3-*cd*]pyrene	39	1.6	1.7	67	0.6	0.3
benzo[*g*,*h*,i]perylene	33	4.7	3.1	44	3.7	2.1
sum of 5- and 6-ring PAHs		**10.6**	**4.4**		**8.1**	**3.3**
sum of 16 PAHs	**33**	**43.1**	**30.7**	**37**	**31.1**	**20.0**

[a)] number of analysed samples: 6
[b)] number of determinations: 24 to 36;
BDL: below the limit of detection; SD: standard deviation

Tab. 5 PAH content in bitumen in mg/kg, cited from earlier publications according to Bolliet et al. (2013)

	Data available in the literature						Study results							
	paving grade		roofing grade		coal tar pitch		feedstock without flux oil		oxidized bitumen without flux oil		feedstock with flux oil		oxidized bitumen with flux oil	
number of samples	**31**		**12**		**3**		**6**		**6**		**6**		**6**	
	min	**max**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**	**min**	**max**
naphthalene	0.1	0.8	0.4	3.7	70 000	70 000	0.1	39.7	0.1	12.6	0.2	44.9	0.1	33.6
acenaphthalene	0.0	0.7	0.0	0.4	n. r.	n. r.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	1.6	< 0.1	< 0.1
acenaphthene	0.0	0.4	0.0	0.7	12 000	12 000	< 0.1	1.9	< 0.1	0.1	0.1	0.4	< 0.1	1.8
fluorene	0.0	2.1	0.8	3.4	15 000	15 000	< 0.1	1.8	0.2	2.3	0.1	4.6	0.3	2.5
phenanthrene	0.2	7.3	0.3	22.8	19 800	50 000	0.1	6.2	0.4	5.4	0.4	37	0.4	16
anthanthrene	0.0	0.4	0.0	6.0	18 000	76 000	< 0.1	1.4	< 0.1	0.5	0.1	1.7	< 0.1	0.7
fluoranthene	0.0	1.0	0.2	8.6	22 000	36 000	< 0.1	2.2	0.1	2.7	0.1	7.9	0.2	5.2
pyrene	0.1	2.6	0.2	11.0	5000	27 200	0.1	2	0.1	3.8	0.2	17	0.2	11.4
benzo[*a*]anthracene	0.1	10.1	0.2	8.6	20 400	24 500	< 0.1	1.0	0.1	4	0.1	10	0.2	5.9
chrysene	0.2	8.6	0.5	10.8	11 200	22 700	< 0.1	1.2	1	10	< 0.1	19.6	< 0.1	27
benzo[*b*]fluoranthene	0.4	7.5	1.8	11.7	5250	60 010	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
benzo[*k*]fluoranthene	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	< 0.1	< 0.1	< 0.1	0.8	n. r.	2.9	n. r.	2.5
benzo[*a*]pyrene	0.2	3.4	0.4	4.2	11 400	15 200	< 0.1	1.3	0.1	3.8	0.3	8.3	0.2	9.1
indeno[1,2,3-*cd*]pyrene	0.1	2.0	0.3	2.4	n. r.	n. r.	< 0.1	0.6	0.2	1	0.3	4.4	0.3	3
dibenzo[*ah*]anthracene	0.3	3.6	1.1	3.3	3430	3530	< 0.1	0.6	0.1	0.5	0.2	5.1	0.1	1
benzo[*g*,*h*,*i*]perylene	1.1	9.8	1.2	9.4	n. r.	n. r.	0.2	21	0.7	3.4	1	12.3	1	9
sum of 16 EPA-PAHs	**4.4**	**37.9**	**3.7**	**69.7**	**185 780**	**290 840**	**0**	**46**	**0**	**28.1**	**2**	**111.6**	**0.8**	**82.7**

n. r.: not reported

The reduction of PAH levels by oxidation was demonstrated by analysing dimethyl sulfoxide (DMSO) extracts from bitumen samples at different levels of oxidation by fluorescence spectroscopy and gas chromatography/time-of-flight mass spectrometry (GC/TOFMS) (Trumbore et al. 2011). The chemical composition of the samples was analysed and the mutation indices then determined (see Section 5.6.1.1). This involved the gradual conversion of bitumen samples of different origins to products with an increasing level of oxidation (softening points 54 °C, 71 °C, 88 °C and 104 °C) and analysis of the samples.

PAH exposure

As part of the German Human Bitumen Study, exposure levels of EPA-PAHs generated by the high-temperature processing of mastic asphalt were determined by stationary air sampling. The concentrations of benzo[*a*]pyrene in air were in the range from 6 to 460 ng/m^3, the median was 45 ng/m^3. The median of the sum of EPA-PAHs determined was about 2.5 µg/m^3 (Breuer et al. 2011). The sum of all EPA-PAHs determined during the processing of mastic asphalt on a tunnel construction site was 5.2 µg/m^3 and thus about twice as high as the sum determined during the processing of rolled asphalt (Raulf-Heimsoth et al. 2011 a).

An exposure matrix for workers in the asphalt industry was developed for an international epidemiological cohort study that took into account the data from several European countries. Overall, the data cover the time period from prior to 1960 up to 1996. The data for pavers for the time period between 1990 and 1996 concur well with the data for persons exposed to mastic asphalt from the German Human Bitumen Study. The maximum concentration of benzo[*a*]-pyrene found in the study was 403 ng/m^3. The arithmetic mean was 24 ng/m^3 (Burstyn et al. 2003).

Dermal and inhalation exposure of 20 paving workers, 12 millers and 6 roadside construction workers was investigated in Boston between the years 1999 and 2000. The latter group was not exposed directly during the high-temperature processing of bitumen. During work operations, also recycled bitumen was handled at higher processing temperatures. The analysis was optimized for PAHs with 4 or more rings. The geometric mean for inhalation exposure to PAHs was 4.1 µg/m^3 for paving workers and 1.4 µg/m^3 for millers (maximum values 40 µg/m^3 and 6.7 µg/m^3, respectively). The maximum concentration for benzo[*a*]pyrene was 30 ng/m^3 (McClean et al. 2004 a). The paving workers were exposed to an arithmetic mean concentration of pyrene in the air of 0.3 µg/m^3 (median 0.2 µg/m^3) and a maximum concentration of 1.7 µg/m^3 (McClean et al. 2004 b).

The determination of PAH exposure levels during the performance of various tasks involved in the high-temperature processing of paving bitumen in the United States demonstrated that lowering the processing temperature from 149 °C to 127 °C and substituting petroleum-based diesel with biodiesel as a cleaning or separating agent markedly reduced exposure levels (Cavallari et al. 2012).

PAHs are ubiquitous in the environment. As specified in Section 20 of the 39th Ordinance for the Implementation of the German Federal Immission Control Act, the concentration levels of benzo[*a*]pyrene and other relevant PAHs in air are monitored at sampling points of the German Environment Agency. In 2014, 9156 readings were taken for benzo[*a*]pyrene (determined in PM_{10}) at 113 test points located across the federal states of Germany. The annual mean values at the individual test points (rural to industrial areas) were in the range from 0.02 to 1.49 ng/m^3; the highest value determined was 29.5 ng/m^3.

Evaluation of PAH exposure: Comparative analyses

The vapours and aerosols generated during the high-temperature processing of bitumen contain PAHs. In the German Human Bitumen Study (Raulf-Heimsoth et al. 2011 c), stationary determinations of benzo[*a*]pyrene levels were taken at 15 different building sites. A rough estimate of exposure levels was calculated from these data (Breuer et al. 2011). The median concentration of benzo[*a*]pyrene in air was 45 ng/m^3. In order to draw conclusions about potential adverse health effects resulting from exposure, this value was compared with the exposure–risk relationship derived by the German Committee on Hazardous Substances (*Ausschuss für Gefahrstoffe*) for the development of lung cancer caused by exposure to benzo[*a*]pyrene (AGS 2011).

On the basis of this exposure–risk relationship, an excess risk for lung cancer of 26 : 100 000 (statistical probability: 26 additional cases of cancer per 100 000 workers after 40 years of daily exposure during the working week) was calculated by linear extrapolation for exposure to benzo[*a*]pyrene at a concentration of 45 ng/m^3 over a working lifetime of 40 years. The median is a valid reference value because long-term cumulative exposure is a decisive factor for the development of lung cancer.

The Human Bitumen Study also determined the sum of the vapours and aerosols of bitumen. The median of the values determined by personal air monitoring was 5.08 mg/m^3 using bitumen condensate as the reference standard. Assuming a linear relationship between the level of exposure to benzo[*a*]pyrene and the vapours and aerosols of bitumen, the benzo[*a*]pyrene concentration would correspond to an excess risk of about 8 : 100 000 for exposure to vapours and aerosols at the level of the MAK value of 1.5 mg/m^3 (related to the bitumen condensate standard).

To estimate the cancer risk for roofing workers, Rhomberg et al. (2015) carried out a number of comparative analyses that took into account not only the toxicity data for benzo[*a*]pyrene, for example, but also the animal studies discussed below (Clark et al. 2011; Fuhst et al. 2007). The authors concluded that the cancer risk from dermal and inhalation exposure to the vapours and aerosols from built-up roofing asphalt (BURA) is within the range deemed acceptable by regulatory frameworks (10^{-4} to 10^{-6}) (Rhomberg et al. 2015).

Volatile organic compounds (VOCs)

In addition to PAHs, bitumens contain large quantities of different VOCs. The VOC emissions profile of 4 different bitumens (designated as vacuum residue, 160/220, 50/70 and 35/50) was investigated by means of the dynamic headspace GC/MS method. The samples were heated to 180 °C, the vapours and aerosols were fed through a sorbent trap with a carrier gas (helium) at 30 °C and then desorbed (260 °C–270 °C). The levels of most of the analysed VOCs in the emissions were found to increase with the degree of oxidization, from the lowest, "vacuum residue", to the highest, 35/50 bitumens. Some of the substances, such as benzene and acetone, were detected only in oxidized bitumens. Other compounds that were present in significant quantities were, for example, octane, alkylated benzenes, toluene, acetic acid or various aldehydes such as 2-hexenal, butanal or methylbenzaldehyde (Boczkaj et al. 2014).

Metals

There are no data available for the concentrations of metal elements in the vapours and aerosols generated during the high-temperature processing of bitumen. The levels of the relevant metals vanadium, nickel and iron were determined in bitumen materials; the maximum concentration of vanadium was 1600 mg/kg, that of nickel 140 mg/kg and that of iron 150 mg/kg (Goodrich et al. 1986). The metals may occur as colloids, salts or in more complex forms (for example as porphyrin complexes).

Assuming that emissions contain the same mass fraction of vanadium and nickel as the bitumen material, a conservative estimate would be 0.21 µg nickel/m^3 and 2.4 µg vanadium/m^3 at a concentration in air of 1.5 mg/m^3 (bitumen condensate standard). According to Technical Rule TRGS 910 (AGS 2017), the "acceptable concentration" for nickel compounds is 6 µg/m^3. Therefore, the theoretical concentration calculated for nickel levels in emissions of about 0.21 µg/m^3 lies below the acceptable concentration. The corresponding concentration calculated for vanadium levels in emissions of 2.4 µg/m^3 is markedly below the occupational exposure limits (OELs) for vanadium compounds of 30 µg/m^3 (inhalable dust fraction) and 5 µg/m^3 (respirable dust fraction) set in the TRGS 900 (AGS 2015).

For iron, the corresponding value is calculated to be 0.23 µg/m^3, which lies below the current lowest international limit value for iron salts of 1 mg/m^3 (IFA 2018). No limit values have been set in Germany for iron salts (MAK values, OELs, or risk-based tolerable or acceptable concentrations).

Analytical methods for determining the vapours and aerosols of bitumen

Volatile compounds are emitted in large quantities during the high-temperature processing of bitumen (see above). They are released in vapour form and in some cases condense in the air to form aerosols (Breuer et al. 2011). The vapours and aerosols contain all of the emissions generated by heating bitumen and are determined as a composite parameter of exposure. A number of analytical methods have been developed; the methods described below are those used by the studies discussed in this documentation.

IFA method

IFA Method 6305/2 (Breuer 2008 b) analyses the vapours and aerosols of bitumen on the basis of data collected both by stationary determinations and by personal air monitoring using a German GGP sampling head. In this method, a pump draws a defined volume of air through the sampling system at a constant volumetric flow rate of 3.5 l/min. The system is equipped with a 37 mm glass fibre filter to collect the aerosols. Vapours are trapped downstream by an XAD-2 adsorbent. Following extraction of the filter and the adsorbent with tetrachloroethylene, a separate, quantitative infrared spectroscopic analysis is carried out in the wave number range from 2800 to 3000 cm^{-1} using calibration curves. The levels of all organic compounds that contain aliphatic C-H groups are determined. The compounds cannot be differentiated by classes of substances. Up until the end of 2006, the levels were determined using "mineral oil for spectroscopy" as the calibration standard (Breuer 2008 a; IFA Method 6305/1). As of 01 January 2007, IFA analyses began to use "bitumen condensate" as the standard for calibration. In order to be able to compare the values determined by the 2 methods, the results obtained with the mineral oil standard have to be multiplied by a factor of 1.4689 (rounded to 1.5).

NIOSH method

NIOSH Method 5042 is used to determine aerosol and dust concentrations. Vapours are not included in the analysis. A pump draws a defined volume of air through a filter cassette at a volumetric flow rate of 2 l/min; the dusts and aerosols are deposited on a 37 mm polytetrafluoroethylene filter (PTFE). The total particulate matter (TPM) is determined by gravimetric analysis of the filter. The filter is then extracted with benzene. The fraction that is soluble in benzene is gravimetrically determined after evaporation of the solvent and designated as the benzene-soluble fraction (BSF).

Heritage method

The Heritage method extends the NIOSH method by integrating an XAD-2 adsorbent into the system downstream of the polytetrafluoroethylene filter to determine not only the benzene-soluble fraction, but also the levels of volatile compounds in bitumen emissions. The adsorbent is extracted with dichloromethane. After the extract is combined with the benzene-soluble fraction, the total organic matter (TOM) is quantitatively determined using a flame ionization detector and the kerosine standard for calibration.

Relationship between the findings determined by the IFA, NIOSH and Heritage methods

Field studies were conducted at different workplaces at which bitumen was processed. The studies used side-by-side sampling to compare the 3 methods for determining vapour and aerosol concentrations (Kriech et al. 2010). The authors then derived conversion factors from the field data to convert the exposure data obtained using the different methods. However, these conversion factors were intended for use only with large sets of data and are not suitable for the conversion of single values.

Paving:

$$[BSF_{(NIOSH)}] = 0.60 \times [aerosol_{(IFA)}] \qquad R^2 = 0.88$$

$$[TPM_{(NIOSH)}] = 1.01 \times [aerosol_{(IFA)}] \qquad R^2 = 0.83$$

$$[TOM_{(Heritage)}] = 0.44 \times [aerosol_{(IFA)} + vapour_{(IFA)}] \qquad R^2 = 0.72$$

Roofing:

$$[TOM_{(Heritage)}] = 0.74 \times [aerosol_{(IFA)} + vapour_{(IFA)}] \qquad R^2 = 0.91$$

Production volume

The types of bitumen most commonly used in Europe are straight-run, polymer modified and oxidized bitumen. In 2016, about 3.94 million tonnes of bitumen were produced in Germany, including about 287 000 tonnes of reclaimed bitumen. Of the around 2.1 million tonnes of bitumen sold in Germany in 2016 (about 1.8 million tonnes were exported, BAFA 2018), about 1.6 million tonnes were used for paving applications (about 75%), about 348 000 tonnes for the bitumen roofing and sealing membranes sector (16%) and just under 198 000 tonnes for other applications (9%). More than 29% of the bitumen used for paving applications in Germany was polymer modified bitumen (Eurobitume 2018).

IARC classification

The International Agency for Research on Cancer (IARC) has divided the different bitumens into classes for the toxicological evaluation of the vapours and aerosols of bitumen (IARC 2013). Publications often refer to these classes (see Table 6). This documentation does not make use of this classification system because it is irrelevant for the differentiated evaluation of straight-run/air-rectified bitumens on the one hand and oxidized bitumens on the other hand.

Tab. 6 Classification of bitumens according to IARC (2013)

IARC classes	Description
1	straight-run bitumen
2	oxidized bitumen
3	cutback or fluxed bitumen products
4	bitumen emulsions
5	modified bitumen
6	thermally cracked bitumen

1 Toxic Effects and Mode of Action

Bitumens are products made from crude oil and contain numerous individual compounds. The biological effects induced by the vapours and aerosols formed during the high-temperature processing of bitumen largely depend on its production, its exact chemical composition and thus on the feedstock (crude oil) used, the specific production method (for example, with respect to the degree of oxidation) required for the intended area of application (for example, paving, sealing, etc.) and the conditions of application (for example, processing temperature), possible additives and the route of absorption.

In general, the vapours and aerosols formed during the high-temperature processing of bitumen induce irritation and inflammatory effects in the lungs after inhalation exposure; the severity of the effects may differ depending on the workplace and activity carried out. Inflammatory effects in the upper and lower respiratory tract of rats and in the lungs of humans were observed after inhalation exposure to vapours and aerosols. In animals, the application of bitumen vapour condensate to the epidermis led to keratosis and hyperplasia. A relationship between these effects and specific hazardous substances or groups of hazardous substances contained in bitumen cannot be established as a result of the complexity of the composition of the various types of bitumen. There are insufficient detailed and differentiated studies for most of the harmful constituents of the various types of bitumen or for possible combination effects. The polycyclic aromatic hydrocarbons are the only exception.

Recent epidemiological studies did not find consistent evidence of a general increase in the incidence of mortality from cancer. Slight, but inconsistent increases in the lung cancer risk could not be attributed to a specific type of bitumen

or activity. However, animal and in vitro studies have demonstrated that carcinogenic and genotoxic effects are induced by oxidized bitumen. Condensates of the vapours and aerosols of oxidized bitumen were carcinogenic in skin painting studies; this was not found for the condensates of straight-run/air-rectified bitumens. In addition, inhalation of the vapours and aerosols generated from the condensates of a mixture of straight-run bitumen containing a 70% fraction of air-rectified bitumen was found not to be carcinogenic in animal studies. In human studies, an increase in DNA strand breaks was observed in workers involved in roof damp-proofing, but not or to a markedly lesser degree in paving workers. The existing studies indicate that the PAHs present in vapours and aerosols, but also other substances that are formed during the high-temperature processing of oxidized bitumen, may give rise to carcinogenic effects. No studies of developmental toxicity have been published. Studies of sensitization in animals did not yield positive results. No human data are available for this end point.

2 Mechanism of Action

The relevant effects induced by bitumen are inflammatory effects in the respiratory tract of rats and humans, genotoxicity and a carcinogenic potential in animals after dermal exposure to oxidized bitumen, but not to straight-run and air-rectified bitumen.

The exact chemical composition of bitumen varies depending on the crude source and on the individual production steps. Chemical analytical studies of potentially harmful substances contained in various types of bitumen focused on PAHs and metals. In comparison with the levels found in coal tar, low levels of PAHs occur in bitumen materials and in emissions produced during high-temperature processing.

In animal studies that investigated dermal exposure using skin painting tests, the condensates of emissions produced by heating oxidized bitumen contained more PAHs than those produced by heating straight-run bitumen. However, first of all, these bitumens were produced using different manufacturing processes and, secondly, the condensates had been generated at different temperatures. Consequently, other unidentified substances present in the condensates in varying concentrations may have contributed to the harmful effects. Therefore, it has yet to be resolved whether the marked differences in the dermal carcinogenicity of the condensates of straight-run and oxidized bitumen are caused by the varying levels of PAHs or other constituents. The same is true for the data available for irritation in the lungs; again, it remains unclear which substances contribute to the induced effects.

Direct genotoxic effects may be involved in the carcinogenicity of oxidized bitumens because of the PAHs these contain. Inflammatory effects in the respiratory tract may also be caused by oxidative stress, which may lead to indirect genotoxic effects.

In summary, the vapours and aerosols of straight-run and air-rectified bitumen primarily induce inflammatory effects in the respiratory tract while those of oxidized bitumen cause carcinogenic effects. However, neither the overall mechanism of action, nor the constituents responsible for these effects have been explained.

3 Toxicokinetics and Metabolism

Since the 2001 documentation was published (Greim 2002), additional studies have been carried out that specifically examined the absorption, metabolism and elimination of PAHs following exposure to the vapours and aerosols of bitumen. Studies to investigate other hazardous substances or classes of hazardous substances (epoxides, thioesters, etc.) occurring in complex bitumen mixtures have not been performed. As is known from other workplaces at which PAHs occur, PAHs, which are present in the different types of bitumen in varying amounts, are absorbed both via the lungs and through the skin (Serdar et al. 2012) and are excreted with the urine in the form of hydroxylated metabolites.

Ten male non-smokers equipped with a respirator mask were exposed at rest to the vapours and aerosols of bitumen for 8 hours at a concentration of 20 mg/m^3 (bitumen B65, 88% vapour fraction, bitumen condensate standard). The mask was used to ensure that only the absorption of bitumen through the skin was determined. Two of the subjects

were exposed also without a respirator mask. The urine was analysed for the metabolites of chrysene, phenanthrene and pyrene before, during and up to 24 hours after exposure. The fractions absorbed through the skin and by inhalation were thus determined based on the urinary excretion of the metabolites. The fraction of chrysene, phenanthrene and pyrene absorbed through the skin was about 50% to 60% of the total amount absorbed after exposure via the air (Walter and Knecht 2007).

4 Effects in Humans

4.1 Single exposures

Ten male non-smokers equipped with a respirator mask were exposed to the vapours and aerosols of bitumen for 8 hours at a concentration of 20 mg/m^3 (bitumen B65, 88% vapour fraction, bitumen condensate standard) and at temperatures up to 50 °C. They wore only shorts and shoes. Two of the volunteers were exposed also without a respirator mask. Irritation of the skin and upper respiratory tract was not observed. Other examinations were not carried out as the aim of this study was to determine the level of absorption through the skin and by inhalation (Walter and Knecht 2007).

4.2 Repeated exposure

A survey was carried out among 859 mastic asphalt workers exposed to the vapours and aerosols of bitumen, many of whom worked also with other types of asphalt. The control group was made up of 517 civil engineering workers without such exposure. The survey was carried out during a routine occupational medical examination in the form of a questionnaire-based interview. The questions addressed the subjective complaints of the workers (running nose, breathing difficulties, sneezing, irritation of the skin and eyes) and an overall assessment was made by the examining physician. At 40.6 years (SD 11 years), the average age of the mastic asphalt workers was similar to that of the control group (42.6 years, SD 10.9 years). There was no statistically significant difference in the number of smokers in the 2 groups. While 60% of the exposed group were smokers and 40% non-smokers, ex-smokers or cigar smokers, 57% of the control group were smokers and 43% non-smokers, ex-smokers or cigar smokers. The data recorded for 142 of the exposed persons were only of limited relevance because of a lack of exposure data or the physician's assessment (Rumler et al. 2007).

Workers in the exposed group complained significantly more frequently (11.2%) of breathing difficulties than persons not exposed (1.7%). Likewise, the other specific complaints (running nose, sneezing, skin irritation, eye irritation) occurred significantly more frequently among the exposed workers. The number of workers reporting complaints rose with the increasing duration of exposure. While mastic asphalt workers without complaints were exposed to the vapours and aerosols of mastic asphalt for about 64% of their working day, the workers with complaints were exposed for 72.2% of their work time. Specific respiratory tract complaints were reported by workers who were exposed to the vapours and aerosols of mastic asphalt for 65.4% of their work. Mastic asphalt workers who were exposed to the vapours and aerosols of mastic asphalt for 36.5% of their working day did not report complaints of the airways. Exposed smokers reported significantly fewer complaints than exposed non-smokers (21.2% compared with 29.7%, $p = 0.007$). Among the workers without exposure, smokers had fewer complaints than non-smokers (3.1% compared with 11.2%, $p = 0.001$). With respect to effects in the respiratory tract, exposed smokers had fewer complaints than exposed non-smokers (9.4% compared with 14.2%, $p = 0.035$). This effect was observed also in the control group (0.89% compared with 3.6%, $p = 0.034$). For 14.3% of the mastic asphalt workers, the physician expressed reservations or applied restrictions to the continuation of work. Among the controls, 8.5% were affected (Rumler et al. 2007).

The German Human Bitumen Study (Raulf-Heimsoth et al. 2011 c) used non-invasive methods to study irritation of the upper and lower respiratory tract in workers exposed to the vapours and aerosols of bitumen during the high-temperature processing of mastic asphalt.

Acute effects were reported by a subset of 123 workers, 74 of whom were exposed to the vapours and aerosols of bitumen at a median concentration of 6.4 mg/m^3 (mineral oil standard, median: 0.1–41.7 mg/m^3; bitumen condensate standard: 9.6 mg/m^3, median: 0.15–62.55 mg/m^3). The control group was made up of 49 roadside construction workers who had not been exposed to the aerosols or vapours of bitumen during the preceding 5 years. Exposure levels were determined by personal air monitoring and stationary determinations. The workers were exposed for 6.5 to 8 hours during the shift. The 28 workers of the high exposure group (> 10 mg/m^3 mineral oil standard, > 15 mg/m^3 bitumen condensate standard) had worked at the company for a median period of employment of 9 years, the 46 workers of the low exposure group (< 10 mg/m^3 mineral oil standard, < 15 mg/m^3 bitumen condensate standard) for a median of 5.5 years and the workers of the control group for a median of 6.5 years. The examinations were carried out from Tuesday to Thursday. A questionnaire was answered to assess potential confounders, chronic and acute health complaints and the occupational history. The workers were asked about acute symptoms of the lower respiratory tract, the nose and the eyes before, during and after the shift. Spirometry was performed and nasal lavage, induced sputum and urine samples (hydroxylated metabolites of phenanthrene and pyrene) were investigated before and after the shift. Of the workers of the high exposure group, 70% complained of coughing during the shift in comparison with 15% of the workers of the low exposure group and 8% of the control persons. The incidence of irritation and a burning sensation in the eyes was significantly higher in the workers of the high and low exposure groups (33% and 13%, respectively) compared with the levels in control persons (0%). The incidence of nasal irritation was not significantly increased. A significant increase in chronic symptoms such as wheezing and shortness of breath was not reported either before or after the shift (Raulf-Heimsoth et al. 2007). A complete analysis of the lung function parameters and of the nasal lavage data is to be found in the Human Bitumen Study (Raulf-Heimsoth et al. 2011 b).

The extensive Human Bitumen Study included not only the 320 exposed persons, but also a reference group of 118 road construction workers of a similar age who had not been exposed to the vapours and aerosols of bitumen during the preceding 5 years. Exposure levels were determined by personal air monitoring. Workers of the exposure group were exposed to the vapours and aerosols of bitumen at a median concentration of 5.08 mg/m^3 (related to the bitumen condensate standard, interquartile range 2.64–8.67 mg/m^3); the median concentrations for workers without exposure was 0.29 mg/m^3 (0.10–0.44 mg/m^3). The maximum exposure concentration was 61.22 mg/m^3 (related to the bitumen condensate standard) (Breuer et al. 2011). Lung function was assessed both before and after the shift and nasal lavage fluid (NALF) and induced sputum were collected. Biomarkers for inflammatory reactions were quantified in the NALF and induced sputum samples. The lung function parameters forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) were within the normal range in both the control group and the exposure groups before and after the shift. A slight decline in the FEV1 and FVC was observed during the shift. The FEV1 was not decreased in the non-smokers of the reference group. An analysis of the nasal lavage fluid for cellular composition and soluble inflammatory mediators did not reveal significant inflammatory changes in the upper airways in the workers exposed to bitumen compared with the findings in the reference group. No significant shift effect was observed. By contrast, markers of inflammatory effects such as interleukin-8 (IL-8), total protein and matrix metalloproteinase 9 (MMP9) were significantly higher in the induced sputum samples of workers exposed to bitumen than those of workers without exposure, both before and after the shift. IL-8 and total protein were less than doubled, MMP9 levels were about 4 times higher in exposed non-smokers than in non-smokers of the control group. Smoking caused a threefold increase in IL-8 levels; its effect was markedly stronger than that of exposure to bitumen. In a comparison of the group of non-smokers exposed to bitumen with the smokers of the control group, the total protein levels were equally high in both groups and the MMP9 concentrations were almost doubled in the exposed group. Among the non-smokers, a positive correlation was found between the total number of cells, the number of neutrophils and TNF-α post-shift and exposure to bitumen. As the irritant and inflammatory changes determined with induced sputum bore no relationship to the prevailing work shift concentration, they were regarded as evidence of (sub)chronic effects in the lower respiratory tract. No significant inflammatory changes were found in the upper respiratory tract. No relationship was found between the level of exposure to bitumen on the day of examination and the concentration of inflammatory markers. The use of a cross-shift design did not reveal a possible relationship between repeated or cumulative exposure and inflammatory marker concentrations (Raulf-Heimsoth et al. 2011 b).

A study investigated respiratory tract effects in 72 asphalt pavers, 32 operators in asphalt mixing plants (lower exposure to PAHs than pavers) and asphalt engineers (lowest exposure to PAHs of the 3 groups) shortly before the beginning and at the end of the paving season (Ellingsen et al. 2010). Biomarkers for changes in the pulmonary epithelium, pro-inflammatory cytokines, acute-phase proteins and proteins for endothelial activation and adhesion in the blood were compared with data for lung function (spirometry), self-reported symptoms of the respiratory tract, smoking habits and adiposity. Smoking and self-reported symptoms of the respiratory tract (wheezing) had an effect on the investigated biomarkers. A significant increase in club cell protein 16 (CC-16) levels was observed in all groups during the season; this increase was highest in the asphalt pavers. Increased CC-16 concentrations in the blood were attributed to a disruption of the alveolar-endothelial barrier as a form of damage to the lung epithelium. Lower CC-16 concentrations were determined in smokers who reported wheezing than in smokers who did not report wheezing; this may be the result of increased damage to the club cells caused by smoking. Reduced cholesterol, P-selectin and ICAM-1 (intercellular adhesion molecule) levels were attributed to increased physical activity during the season. Overall, the authors concluded that there was no evidence of increased systemic inflammation or endothelial activation during the season.

Spirometry tests were performed also in a Norwegian cross-sectional study (Randem et al. 2004 b). A group of 64 asphalt workers were compared with a control group of 195 outdoor construction workers. The study did not report when the determinations were made (pre-shift or post-shift). Inflammatory markers were not analysed. The volunteers were asked to complete a questionnaire about lower respiratory tract symptoms and allergies, a medically confirmed asthma diagnosis and smoking habits. The average ages of the asphalt workers and the workers in the control group differed only slightly (37 years compared with 40 years). However, the asphalt workers had worked at their current jobs for a considerably shorter period of time on average than the workers in the control group (11 years compared with 16 years). In both groups, 53% of the workers were smokers. The asphalt workers complained significantly more frequently of respiratory symptoms such as a medically confirmed asthma diagnosis, chronic obstructive lung diseases and eye irritation than the workers in the control group. According to the authors, the ratio between the FEV and the expiratory vital capacity was significantly lower in the asphalt workers than in the workers of the control group (78.1 (SD 7.2) compared with 80.0 (SD 7.0), $p = 0.01$).

In a cross-sectional study, data pertaining to respiratory complaints were collected by questionnaire among 74 Iranian asphalt workers and 110 control persons working in government agencies who were not exposed to the vapours and aerosols of bitumen. The lung function parameters vital capacity (VC), FVC, FEV1 and peak expiratory flow (PEF) were determined in exposed persons pre-shift after an exposure-free period of 72 hours and post-shift by spirometry. Lung function was determined only once in the reference group. Total particulate matter (TPM) and the benzene-soluble fraction (BSF) of bitumen emissions were determined once using NIOSH method 5042. The mean exposure levels were low at total particulate matter concentrations of $0.9 \pm 0.2\,mg/m^3$ and a benzene-soluble fraction of $0.3 \pm 0.1\,mg/m^3$. The incidence of respiratory complaints such as coughing, phlegm, wheezing, shortness of breath and chest tightness was significantly higher among exposed workers than among the workers in the control group. The FEV1/FVC ratio (Tiffeneau index) and the FEV1/VC ratio were significantly lower in the exposure group than in the control group. In the exposed persons, the VC, FVC, FEV1 and FEV1/FVC were significantly lower after the shift than before the shift. Exposure was found by multiple linear regression analysis to have an effect on the above-mentioned lung function parameters; however, this was significant only for FEV1/FVC and FEV1/VC. The reduced FEV1/FVC ratio indicates an obstruction of the airways (Neghab et al. 2015). A direct comparison of the level of exposure (TPM and BSF) with threshold limit values for vapours and aerosols, determined by means of the IFA method, was not possible because the NIOSH method cannot be used to determine vapour levels. Another point that needs to be considered is that the exposure levels in this study were determined in the winter and the asphalt workers are probably exposed to markedly higher levels in the summer. Cumulative exposure may therefore be a relevant factor, at least in a comparison of the findings with those from the control group.

In a longitudinal study, the lung function of 75 male asphalt pavers and 71 road maintenance workers who were not exposed to the vapours and aerosols of bitumen was determined by spirometry from 2005 to 2010. The baseline FVC and FEV1 levels were determined in 2005; further determinations were carried out at different time points and at varying intervals. Using mixed linear modelling and after adjusting for age, body mass index and the number of

cigarettes smoked (given in pack-years), it was found that the decline in the FVC and FEV1 levels related to the square of mean body height (FVC/h^2 and $FEV1/h^2$) was greater in asphalt workers than in control persons. The FEV1/FVC ratio increased more markedly in asphalt workers than in control persons. In addition, the subgroup of screedmen, a group that is assumed to have the highest level of exposure because of the nature of their work, was compared with the remaining asphalt workers and the control persons. The decline in FVC/h^2 and $FEV1/h^2$ levels and the increase in FEV1/FVC were more marked in screedmen than in the other asphalt workers, and in these, more marked than in control persons. The exposure parameters of total dust, oil mist, oil vapour, PAHs and NO_2 were determined by personal sampling. In addition, the levels of ultrafine particles were analysed by stationary measurements. The level of exposure to the vapours and aerosols of bitumen was not determined. The results of this study differ from those of other studies because when effects on lung function were observed in other studies, these were regarded as evidence of a decrease in the FEV1/FVC ratio (for example Randem et al. 2004 b) caused by an obstructive ventilation disorder. By contrast, this study reported an increase in the FEV1/FVC ratio that was probably related to a restrictive ventilation disorder. This finding cannot be explained in terms of pathophysiology. At the end of the observation period, the exposed workers could choose to be examined by high-resolution computed tomography (HRCT). A reticular pattern with subpleural distribution was found in 3 of the 75 persons examined; this was regarded as evidence of interstitial fibrosis. These persons had concurrent exposure to quartz. Other HRCT findings were not thought to be associated with exposure to asphalt (Ulvestad et al. 2017).

A study investigated whether toxic effects in the liver, kidneys or blood could be induced by exposure to the vapours and aerosols of bitumen in an exposed group of 80 persons and a reference group of 130 persons. External exposure levels were determined on the basis of 30 personal air samples by means of NIOSH method 5042. On the effect side, clinico-chemical parameters were determined in the blood and urine. The mean concentration of bitumen emissions was low with total particulate matter concentrations of 0.9 ± 0.2 mg/m^3 and a benzene-soluble fraction of 0.32 ± 0.09 mg/m^3. In the analysis of the parameters relevant for liver function, the authors determined that the mean values for serum albumin, total protein, direct and total bilirubin and alanine aminotransferase and aspartate aminotransferase activities were significantly higher and alkaline phosphatase activity was significantly lower in the group of exposed persons in comparison with the values determined in the group of reference persons. However, all values were within the normal range. With respect to the kidneys, the mean value for blood urea nitrogen (BUN) was significantly higher in the exposed persons than the mean value determined in the reference persons. The values were again within the normal range (Neghab et al. 2017). The significance of the changes observed is difficult to evaluate as no data were provided for the influencing variables that are relevant for the development of liver and kidney damage and the findings were not supported by the results from other studies.

4.3 Local effects on skin and mucous membranes

Studies of exposure to bitumen dust reported findings of hyperkeratosis, xeroderma and contact dermatitis, which was not described in more detail, and irritation of the skin and mucous membranes (see Greim 2002). In a few isolated cases, effects that were probably phototoxic were observed. However, the studies did not provide a conclusive explanation for the causality of the effects.

Three workers carried out renovation work involving the removal of soundproofing materials made of cork and bitumen by sandblasting. A large amount of dust was generated during this activity and the workers were dressed in a full overall and neoprene gloves. As the weather was hot and sunny, the workers spent several breaks outdoors. A day later, all 3 workers had developed erythematous skin changes that in some cases progressed to scaly skin changes localized on areas of the skin that had not been covered by the clothing worn under the overall (hands, forearms, neck). Only 1 of the 3 workers was later examined more closely when the changes to the skin could no longer be observed. No evidence was found that would indicate earlier increased sensitivity to light or that phototoxic medicines were being taken (Lindberg et al. 2015).

In 28 dock workers who were exposed to the dust produced by sandblasting paint containing bitumen during cleaning activities, after 3 to 8 hours erythematous, and in some cases oedematous, changes were found on the conjunctiva

and in other areas of skin exposed to light; these effects healed after 7 to 10 days (Davies 1996; see also Greim 2002). In addition, dermatitis resulting from photosensitization was found in several persons from a group of 50 Israeli workers who had been continuously exposed to "bitumen-asphalt vapour" for 8 hours a day for at least 6 months during production work or during road construction or roofing work. However, the authors did not provide any additional information about the possible causes of the exposure to the vapours and aerosols of bitumen (Schaffer et al. 1985; see also Greim 2002).

4.4 Allergenic effects

There are no data available.

4.5 Reproductive and developmental toxicity

There are no data available.

4.6 Genotoxicity

The findings pertaining to genotoxicity in workers after exposure to the vapours and aerosols of bitumen are shown in Table 7.

4.6.1 DNA adducts

A number of different studies used chemical analytical methods or ^{32}P-postlabelling to investigate anti-benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts (anti-BPDE–DNA adducts) in the blood of workers who had been exposed to the vapours and aerosols of bitumen. In comparison with ^{32}P-postlabelling, chemical analytical methods are of high specificity, but lower sensitivity.

4.6.1.1 Studies of paving workers and mastic asphalt workers

Over the course of a year, if possible, 4 blood samples were taken from 49 paving workers who handled hot mix asphalt, and from 36 construction workers (millers and roadside workers) without exposure to bitumen. The samples were examined for DNA adducts. Of the 4 samples, 3 (spring, summer, autumn) were collected during the season when the paving workers handled bitumen, and 1 (winter) during the off-season when exposure did not occur. The DNA adduct levels were determined in the mononuclear cells of the peripheral blood by ^{32}P-postlabelling. Adduct levels above the limit of detection of 1 adduct per 10^{10} nucleotides were determined in 60% of the 169 samples collected in total from the paving workers. The median was 11 adducts per 10^{10} nucleotides and did not differ from that of the reference group, in which the median from 103 samples in total (61% of these above the limit of detection) was 12 adducts per 10^{10} nucleotides. The mean levels differed more markedly (23 adducts per 10^{10} nucleotides in paving workers compared with 29 adducts per 10^{10} nucleotides in the reference group) because of a number of extremely high values in the reference group. The statistical analysis was based on linear regression models that included variables such as the day of the week and the activity. Smoking habits were taken into account either as a continuous variable as the number of cigarettes smoked or as a category variable (former, never or current smoker, current non-smoker). Another variable was the body mass index. A positive association was observed between the number of cigarettes smoked and DNA adduct levels; however, this was not statistically significant. When smoking habits were evaluated in 2 categories (current smoker, current non-smoker), the effects of smoking on adduct levels were statistically significant. During the work season, the adjusted mean values for paving workers increased continuously from Monday (mean value: 3 adducts per 10^{10} nucleotides) to Friday (46 adducts per 10^{10} nucleotides). This trend was not observed in the paving workers during the off-season (winter samples) or in the reference group. During the work season, the adjusted mean adduct levels were lowest for roller operators (7 adducts per 10^{10} nucleotides) and highest for screedmen (23 adducts per 10^{10} nucleotides). In the winter, no differences in DNA adduct levels were found in paving workers who carried out different work

tasks. The authors noted that these findings are consistent with the internal exposure levels determined in a subset of this group on the basis of urinary 1-hydroxypyrene levels; the results were discussed in an earlier publication (McClean et al. 2004 b). A regression model demonstrated that the season had a significant impact on DNA adduct levels with the highest levels determined in the winter (45 adducts per 10^{10} nucleotides) and lowest levels in the summer (13 adducts per 10^{10} nucleotides) (McClean et al. 2007 b).

In summary, the authors found that adduct levels increased continuously in exposed persons during the working week by a factor of 14; this was not observed in the off-season (winter) and in the control group. The adduct levels varied in the workers according to the different work operations; these differences were not significant. Overall, however, the adduct levels in the group of control persons fluctuated from 1 work day to the next by up to a factor of 11. The median adduct levels were of the same order of magnitude in exposed persons and in control persons; the mean level was lower in exposed persons than in control persons. For this reason, the conclusion cannot be drawn that exposure to bitumen can lead to an increase in adduct levels.

In the Human Bitumen Study (Raulf-Heimsoth et al. 2011 c), anti-BPDE–DNA adducts were investigated by high pressure liquid chromatography coupled with fluorescence detection (HPLC-FLD) in 320 workers exposed to the vapours and aerosols generated during the high-temperature processing of mastic asphalt and in a reference group of 118 paving workers without exposure. The group of bitumen workers was exposed to the vapours and aerosols at a concentration of 5.08 mg/m^3 (median, related to the bitumen condensate standard, interquartile range 2.64–8.67 mg/m^3). Overall, the BPDE-DNA adduct levels in exposed persons were low (median < limit of detection of 0.5 adducts per 10^8 nucleotides) and no differences were found in the adduct levels before and after the shift and between groups (Marczynski et al. 2011).

In 6 workers on a tunnel construction site who had handled rolled asphalt and mastic asphalt during consecutive weeks, BPDE–DNA-DNA adducts were determined in the white blood cells both pre-shift and post-shift by HPLC-FLD. A clear trend could not be found for either type of asphalt for the formation of adducts during the shift and there were no clear differences between the values determined for the 2 types of asphalt. A median of 2.0 adducts per 10^8 nucleotides was determined during the processing of rolled asphalt and of 3.6 adducts per 10^8 nucleotides during the processing of mastic asphalt (Raulf-Heimsoth et al. 2011 a). After handling the 2 types of asphalt, the median BPDE–DNA adduct levels determined in the workers were higher than those found in the study above, in which the median was under the limit of detection of 0.5 adducts per 10^8 nucleotides. The study is not relevant to the evaluation because of the small number of cases (n = 6); however, it provides evidence for possible maximum detectable BPDE–DNA-DNA adduct levels and thus a possible range for adduct formation.

4.6.1.2 Studies of roofers

In studies carried out in 1990 (see Greim 2002) on the basis of an identical data set (Herbert et al. 1990 a, b), PAH–DNA adducts were detected by ^{32}P-postlabelling in 10 of 12 exposed roofers, but only in 2 of 17 control persons. In the roofers, a positive association was found between PAH exposure in the air and DNA adduct levels. PAH–albumin adducts were also immunologically quantified in the same persons (Lee et al. 1991). However, there was co-exposure to coal tar, and the DNA and protein adducts cannot be attributed unequivocally to exposure to the vapours and aerosols of oxidized bitumen.

In an earlier, methodologically-oriented study, benzo[*a*]pyrene–DNA adducts were determined in roofers, foundry workers and reference persons using immunological methods (ELISA and USERIA). The adducts were detected in 7 of 28 samples from roofers. As the primary objective of this study was to investigate the formation of the adducts of benzo[*a*]pyrene in humans, the report did not provide any other details about the exposure levels of the roofers. The smoking habits of the exposed group was not discussed, but it was pointed out that adducts had been detected also in smokers from the reference group (Shamsuddin et al. 1985). As a result of the absence of data, the study cannot be used to evaluate genotoxic effects in roofers.

4.6.2 Oxidative stress

In addition to the development of BPDE–DNA adducts, another relevant mechanism that could lead to the development of cancer from exposure to bitumen or the potentially carcinogenic compounds it contains (including PAHs) is the induction of oxidative stress and the formation of unspecific 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dGuo) that this is associated with. For this reason, a number of studies have investigated 8-oxo-dGuo levels in exposed persons. However, when evaluating these data, it is to be taken into consideration that oxidative stress and the oxidative base modification 8-oxo-dGuo this is associated with are influenced both by exogenous sources and endogenous factors. Reactive oxygen species are formed endogenously not only as a natural by-product of breathing, but develop also during inflammatory processes. For this reason, they are not to be interpreted only in terms of the genotoxic or mutagenic effects of a hazardous substance.

4.6.2.1 Studies of paving workers and mastic asphalt workers

In the German Human Bitumen Study (Raulf-Heimsoth et al. 2011 c), higher levels of 8-oxo-dGuo adducts were detected pre-shift and post-shift in 320 workers exposed to the vapours and aerosols of mastic asphalt (exposure: median 5.08 mg/m^3, related to the bitumen condensate standard, interquartile range 2.64–8.67 mg/m^3) than in the reference group (118 paving workers). The levels of 8-oxo-dGuo per 10^6 dGuo increased significantly during the shift ($p < 0.001$) both in the exposed persons (median 3.72 pre-shift compared with 4.13 post-shift) and in the reference group (median 2.93 pre-shift compared with 3.28 post-shift). No association was found between the level of exposure to the vapours and aerosols of bitumen on the day of examination or the concentrations of PAH metabolites in the urine (1-hydroxypyrene, total hydroxyphenanthrenes, total hydroxynaphthalenes) and the level of 8-oxo-dGuo adducts. The authors concluded that the differences observed between the exposed persons and the reference persons at the group level should be regarded as evidence of increased oxidative damage in the exposed group; this can be interpreted both as a genotoxic effect and an inflammatory effect. The effects cannot be clearly attributed to exposure to the vapours and aerosols of bitumen because of a lack of a concentration–effect relationship (Marczynski et al. 2011).

The findings from a subset of this study (66 exposed persons, 49 reference persons) were published in 2006 (see Table 7) (Marczynski et al. 2006). The findings largely coincide with those of the overall study, which were published later (Marczynski et al. 2011).

A sub-project of the German Human Bitumen Study investigated a small group of 6 workers on a tunnel construction site who had first handled rolled asphalt and then mastic asphalt during 2 consecutive weeks. Examinations were carried out pre-shift and post-shift over the course of the 2 weeks. Exposure to the vapours and aerosols of bitumen was lower during the application of rolled asphalt (median 2.64 mg/m^3, related to the bitumen condensate standard, personal sampling) than during the application of mastic asphalt (median 11.6 mg/m^3). This was true also for total EPA-PAHs and most of the individual PAH compounds determined (exception: phenanthrene); however, the data were collected by stationary determinations. After the application of both types of asphalt, 8-oxo-dGuo adduct levels in the workers increased from comparable median baseline levels to again comparable post-shift levels. However, the individual values were increased in 5 of 6 workers during the laying of rolled asphalt and in only 2 of 6 workers during the laying of mastic asphalt (Raulf-Heimsoth et al. 2011 a). No generalizable conclusions can be drawn from a comparison of the exposure to mastic asphalt and to rolled asphalt during laying because of the small number of cases and the only slight differences in findings. In general, no conclusions can be drawn from this study about genotoxic or inflammatory changes induced by exposure during the laying of asphalt, also because of the number of cases and because of the lack of a specific control group for comparison.

A study of 19 paving workers and 22 reference persons in Italy analysed concentrations of 1-hydroxypyrene in the urine as a marker of internal exposure and investigated oxidative damage to DNA in lymphocytes. The paving workers handled concrete asphalt at a temperature of 160 °C. The frequency of strand breaks was determined by formamidopyrimidine–DNA–glycosylase (FPG)-modified comet assay (single-cell gel electrophoresis); therefore, the DNA strand breaks were probably a response to oxidative stress. In addition, the PAH levels in air samples collected in the breathing zone of paving workers on 3 consecutive days over the course of the entire work shift were analysed. Urine samples were

taken from the paving workers on Mondays before the shift and at the end of the shift on the first 3 days of work. Only 1 urine sample from the reference persons was analysed; this was collected pre-shift on Mondays. The blood samples from the paving workers were collected on the third day of the working week. The 1-hydroxypyrene concentrations in the urine were higher at the end of the workdays than on Monday before the shift and the mean concentration was significantly higher in the paving workers than in the reference persons. External exposure was found to influence the internal exposure data even after the results were statistically adjusted for smoking. In the comet assay, FPG-treated cells from paving workers had significantly higher tail moment values than those from reference persons; the differences between the groups were slight and insignificant in the cells not treated with FPG. The number of cells with a visible tail was significantly higher in the group of paving workers than in the group of reference persons, both for cells treated with FPG and for those not treated with FPG. As the criterion for oxidative damage, the authors determined a value of 2.0 for the ratio of the tail moment in cells treated with FPG to the tail moment in untreated cells. On the basis of this criterion, damage was found in 37% of the persons in the group of asphalt workers, but none in the group of reference persons (Cavallo et al. 2006). Overall, this finding is more likely evidence of an increase in the frequency of DNA strand breaks induced by indirect oxidative stress than evidence of direct damaging effects on the DNA.

In a study of 34 asphalt workers and 35 reference persons, all of whom were non-smokers, disulfide/thiol homoeostasis in serum was examined at the end of the working week as a marker for oxidative stress (Yilmaz et al. 2016). Factors that could potentially influence oxidative status (lifestyle factors, nutrition) and exposure (personal protective equipment, activities on the day of examination) were determined by questionnaire. The level of exposure was determined by means of the 1-hydroxypyrene concentration in the urine. Both the disulfide/thiol ratio and the 1-hydroxypyrene concentration were significantly higher in the group of exposed persons than in the group of reference persons. A positive correlation was found between the disulfide/thiol ratio and the 1-hydroxypyrene concentration in the urine. Other than the urinary concentration of the pyrene metabolite, further data for exposure, the duration of exposure, work activities and the temperature at which the asphalt was laid were not given. In addition, not enough information was provided about the reference group to determine whether the group was comparable to the exposure group with respect to other influencing factors such as physical activity.

4.6.2.2 Studies of roofers

In a study of 26 roofers, different parameters were determined to investigate oxidative damage (8-oxo-dGuo both in leukocytes and the urine by means of HPLC/ECD, 8-epi-prostaglandin2$_\alpha$ in the urine) and internal exposure (1-hydroxypyrene in the urine). The samples were taken on Mondays before the shift and on Wednesdays or Thursdays after the shift. Nineteen of the roofers were co-exposed to coal tar pitch dust during the removal of old roofing materials that contained tar. The reference group was made up of 15 construction workers who had not been exposed during the preceding 5 years. Air samples were collected in the breathing zone of 23 of the roofers, which were analysed for total particulate matter and the benzene-soluble fraction of the total particulate matter according to NIOSH method 5042. The PAH levels in the air were determined by NIOSH method 5800. After exposure for several days, the highest number of adducts was found in the leukocytes of the reference group and the lowest in the group of roofers who were exposed also to coal tar pitch dust. The values of the group of roofers who were exposed only to the vapours and aerosols of bitumen were between those of these 2 groups. Over the course of the working week, the adduct levels increased slightly in the roofers exposed only to the vapours and aerosols of bitumen, remained about constant in the reference group and decreased in the subset of roofers exposed also to coal tar pitch dust. No significant differences were found between smokers and non-smokers. Overall, with respect to urinary 8-oxo-dGuo levels, no significant differences were found between the roofers without additional exposure to coal tar pitch dust, those with exposure to coal tar pitch dust and the reference group. With respect to the urinary 8-epi-prostaglandin2$_\alpha$ concentrations of the 3 groups, significant differences were not found between the levels at the beginning of the working week and those at the end of the working week, or between the groups (Toraason et al. 2001). Therefore, the findings cannot be used as evidence of an association between oxidative stress and exposure to bitumen or PAHs.

In a cross-sectional pilot study with 19 roofers from Florida, the metabolites of different PAHs, including 1-hydroxypyrene and 9-hydroxyphenanthrene, were determined in the urine by HPLC-MS before and after a 6-hour work shift.

As a marker of oxidative DNA damage, 8-oxo-dGuo levels in the urine were analysed before and after the shift by ELISA. The external exposure in the air was not determined. A questionnaire was used to collect information about personal or lifestyle factors that could influence either 8-oxo-dGuo levels in the urine or internal PAH exposure. Which activities were performed, the use of personal protective equipment and the extent of skin contact with bitumen were ascertained on the basis of the work-related information that was collected. The exposure led to an increase in the concentration of PAH metabolites in the urine during the shift. The 8-oxo-dGuo levels were higher in smokers than in non-smokers and increased over the shift both in these 2 subgroups and in the total collective. The data were not related to creatinine, although the creatinine levels increased over the shift. The most important predictors of post-shift 8-oxo-dGuo levels were the post-shift levels of hydroxypyrene in the urine and the use of protective gloves. These variables explained 86.8% of the variation (Serdar et al. 2012). The study findings demonstrated the relevance of dermal exposure and the necessity of taking suitable preventative measures. All but 1 of the test persons were also involved in the removal of old roof coverings containing coal tar. Therefore, the increase in the excretion of 8-oxo-dGuo could not be clearly attributed to exposure to the vapours and aerosols of oxidized bitumen.

4.6.3 DNA strand breaks

4.6.3.1 Studies of paving workers and mastic asphalt workers

A study of 36 paving workers ("mixing and paving", no other details) and 37 control persons in India not only investigated the frequency of micronuclei (see below), but also DNA damage in the peripheral lymphocytes by single-cell gel electrophoresis (comet assay, without FPG). The mean tail length was chosen as the study parameter. Exposure was characterized by determining the levels of 1-hydroxypyrene in the urine. The mean tail lengths were significantly higher in the group of exposed persons. Stratification of the group according to smoking habits and alcohol consumption showed that the mean tail lengths of exposed smokers (> 5 cigarettes/day) and persons who consumed alcohol (> 120 g/day) were higher than those of non-smokers or persons with lower alcohol consumption. However, although smoking and alcohol consumption were shown to have an impact on the frequency of strand breaks, after all subgroups were stratified for smoking and alcohol consumption, significant increases in the mean strand break levels were still consistently found in the group of exposed persons in comparison with the levels determined in the control group (Sellappa et al. 2011). A regression analysis was not performed, probably because of the small size of the exposure groups.

In the Human Bitumen Study (Raulf-Heimsoth et al. 2011 c), DNA strand breaks in the peripheral lymphocytes were determined by single-cell gel electrophoresis (comet assay, without FPG) before and after a single workshift in 320 workers exposed to the vapours and aerosols generated during the high-temperature processing of mastic asphalt and in the 118 paving workers of the control group who were not exposed to bitumen (Marczynski et al. 2011). The group of exposed persons was exposed to the vapours and aerosols of bitumen at a median level of 5.08 mg/m^3, related to the bitumen condensate standard (interquartile range of 2.64–8.67 mg/m^3). As a group, exposed persons were found to have higher levels of DNA strand breaks, measured as the olive tail moment, in comparison with the levels determined in the control group, both before the shift and after the shift. No association was found between the levels of strand breaks and external exposure (vapours and aerosols of bitumen) or internal exposure to specific PAHs (1-hydroxypyrene, sum of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene, 1- and 2-hydroxynaphthalene in the urine). A statistically significant association was not found with the other genotoxicity biomarkers (8-oxo-dGuo and anti-BPDE) investigated in the study. The increase in the frequency of DNA strand breaks determined at the group level in workers exposed to bitumen was within the range found in other studies in healthy workers not specifically exposed to genotoxic substances. The authors did not attribute the observed effects to exposure to the vapours and aerosols of bitumen or the PAHs these contain because of the absence of an association between oxidative DNA damage and the level of exposure.

The data for a subset of the study described above were published in 2006 (Marczynski et al. 2006). The findings essentially coincide with the results determined after analysing the data for the total collective in 2011. However, in the subset, associations were made by simple correlation analysis of the levels of DNA strand breaks, determined as the olive tail moment in the comet assay, and the post-shift levels of 1-hydroxypyrene ($r_s = 0.32$, $p = 0.001$) and total

hydroxyphenanthrenes ($r_s = 0.27$; $p = 0.004$) in the urine; these associations were not confirmed by the findings in the collective as a whole.

In 6 workers on a tunnel construction site who had handled rolled asphalt during the first week of work and then mastic asphalt the following week, very similar frequencies of DNA strand breaks in the peripheral lymphocytes were determined post-shift in the comet assay for both types of asphalt; these were somewhat lower than those found in the Human Bitumen Study. A high variability in the frequency of DNA strand breaks was found in pre-shift samples (Raulf-Heimsoth et al. 2011 a). Conclusions cannot be drawn from a comparison of rolled asphalt and mastic asphalt because of the small number of test persons participating in this study.

In another study, lymphocytes from the oral mucosa of 15 paving workers who were exposed to the vapours and aerosols of different asphalt mixes that had been modified with, for example, waste plastic and tall oil pitch, were investigated for DNA strand breaks by comet assay. The findings were compared with the levels determined in 5 reference persons (scientists without exposure to the vapours and aerosols of bitumen). The samples were taken before and after the shift. In the comet assay, no statistically significant differences in "%DNA" in the tail were determined in the samples taken before and after the shift. Similar findings were obtained for all exposure scenarios (asphalt mixes) tested. Therefore, there were no differences between the various modified asphalts. The ratio between pre-shift and post-shift levels in almost all of the exposed workers was within the range of the levels determined in the small reference group. However, the authors found a statistically significant correlation between post-shift DNA damage and urinary concentrations of the PAH metabolites naphthol and 1-hydroxypyrene. However, as this correlation was not found in the pre-shift samples, it cannot be unequivocally attributed to PAH exposure. The authors pointed out that the PAH concentration in the inhaled air of the workers was low in this study (see Table 7) (Lindberg et al. 2008).

DNA strand breaks were determined in the lymphocytes from induced sputum and blood from 42 workers exposed to the vapours and aerosols of bitumen generated during the high-temperature processing of mastic asphalt. The frequency of DNA strand breaks in the lymphocytes from induced sputum was similar before and after the shift and varied to a high extent. No relationship could be found between the levels determined in induced sputum and those in blood. The frequency of DNA strand breaks in the lymphocytes from induced sputum correlated with the total cell count, the neutrophil count and the interleukin-8 concentration before and after the shift. The authors interpreted this as evidence of an increase in the frequency of DNA strand breaks in the lower airways caused by inflammatory effects (Marczynski et al. 2010). As the study did not include reference persons without exposure and the data were not analysed as regards the level of exposure to the vapours and aerosols, no conclusions can be drawn about the effects of exposure to bitumen on the frequency of DNA strand breaks in the lymphocytes from induced sputum.

4.6.3.2 Studies of roofers

In a study carried out in the United States with 26 roofers who handled the relevant bitumen products and a reference group of 15 construction workers who had not been exposed to the vapours and aerosols of bitumen in the preceding 5 years, DNA strand breaks were investigated in leukocytes by comet assay (without FPG). In addition, 19 of the 26 roofers had been exposed to coal tar pitch dust while removing old roof coverings that contained tar. In the group of 7 roofers without additional exposure to coal tar, a slight but significant increase in the frequency of DNA strand breaks, determined as "%DNA" in the tail, was found from the beginning to the end of the working week (from 13.6 ± 1.9 to 16.7 ± 1.4). The levels at the beginning of the working week were approximately equivalent to those of the reference persons without exposure. After 3 or 4 days, the post-shift levels were similar to those of the roofers who had also removed roof coverings containing coal tar (Toraason et al. 2001); an effect of the bitumen applied on the frequency of DNA strand breaks under the described study conditions cannot be excluded.

4.6.3.3 Studies without data for the type of bitumen

In a study with 30 male workers in Turkey who were exposed to bitumen emissions and a reference group of 30 persons without exposure, DNA damage was analysed in the peripheral lymphocytes by alkaline single-cell gel electrophoresis (comet assay). The values for the parameter "%DNA" in the tail determined in the group of exposed persons (24.34 ± 2.72;

mean ± standard deviation) were significantly higher than the values found in the reference group (20.04 ± 2.75) (Bacaksiz et al. 2014). No data were provided for the type of bitumen, the type of work activity or the duration and level of exposure.

4.6.4 Chromosomal changes

A large number of studies that investigated the development of chromosomal damage after exposure to the vapours and aerosols of bitumen or the carcinogenic compounds (including PAHs) these contain have become available since the 2001 documentation (Greim 2002) was published. The primary objective of these studies was to determine the frequency of sister chromatid exchange (SCE), chromosomal aberrations and micronuclei. The findings published by 2001 were inconsistent and subject to criticism by the Commission because of potential co-exposure and insufficient statistical significance (Burgaz et al. 1998; Järvholm et al. 1999). The studies published since the 2001 documentation are described below.

4.6.4.1 Studies of paving workers and mastic asphalt workers

In a study carried out in Australia with the primary objective of comparing genotoxic effects in lymphocytes and exfoliated urothelial cells, the frequency of micronuclei in the lymphocytes of 12 road layers was compared with that of 18 staff members of a hospital store. The workers exposed to bitumen were younger than the persons of the reference group (30.8 ± 2.0 years compared with 39.8 ± 2.8 years). The frequency of micronuclei was higher in the group of persons exposed to bitumen (16.24 ± 0.63 micronuclei/1000 binuclear cells (BNC); 10.65 ± 0.24 cells containing micronuclei/1000 BNC) than in the reference group (9.24 ± 0.29 micronuclei/1000 BNC; 5.93 ± 0.13 cells containing micronuclei/1000 BNC). In an analysis of the data at the group level, this was initially interpreted as an effect induced by exposure to bitumen that went beyond the known effect of aging on micronuclei (Murray and Edwards 2005). However, this study did not provide information on either the duration and level of exposure or the bitumen used, as the primary objective of the investigation was a systematic comparison of the frequency of micronuclei in lymphocytes and urothelial cells and not an investigation of the effects of exposure to bitumen on micronucleus frequency. What is striking about the results of the study is that a relatively large number of cells containing micronuclei had more than 1 micronucleus, as can be determined from the difference between the values for micronuclei/1000 BNC and cells containing micronuclei/1000 BNC included in the study report.

A study carried out in Turkey investigated the frequency of micronuclei and SCE in peripheral lymphocytes in 26 asphalt workers who handled concrete asphalt at temperatures of around 170 °C and a reference group of 26 administrative workers. The asphalt workers were exposed for an average of 11.2 years (standard deviation 8.9 years; range 3–22 years). The authors found a significant ($p < 0.001$) increase in the micronucleus frequency (1.98 ± 0.21‰) in asphalt workers before the shift in comparison with the levels in administrative workers (1.53 ± 0.14‰). Similar findings were reported for the SCE frequency. Therefore, there was a significant increase ($p < 0.001$) in the SCE frequency in the peripheral lymphocytes of asphalt workers (7.22 ± 1.63 per cell) pre-shift in comparison with the SCE levels in administrative workers (5.45 ± 1.09 per cell). In addition, the frequency of micronuclei and SCE increased with the duration of exposure. No significant differences in the frequency of micronuclei or SCE before and after the shift were observed. As a marker of internal exposure to the vapours and aerosols of bitumen, the 1-hydroxypyrene concentration in the urine increased in asphalt workers over the course of the week. However, it was not associated with the frequency of micronuclei on an individual level. Likewise, the SCE frequency was not associated with the 1-hydroxypyrene concentration in urine (Karaman and Pirim 2009). As the duration of exposure and age are usually associated with one another, it cannot be determined whether the increase in micronucleus frequency was actually the result of cumulative exposure or whether it was merely the increase in micronucleus frequency that comes with aging that has been demonstrated in many studies.

A study with 36 paving workers ("mixing and paving", no other details) and 37 control persons in India investigated not only DNA damage (see above), but also micronuclei in peripheral lymphocytes. Urinary 1-hydroxypyrene was determined as a marker of exposure. A significant increase in micronucleus frequency was found in the group of exposed

persons. Stratification of the groups by smoking habits and alcohol consumption revealed that smokers (> 5 cigarettes/day) and persons who consumed alcohol (> 120 g/day) had higher frequencies of micronuclei than non-smokers and persons with lower alcohol consumption, both in the group of exposed persons and in the group of control persons. Persons exposed to bitumen for longer than 10 years were found to have slightly higher frequencies of micronuclei than employees exposed for a shorter period of time (Sellappa et al. 2011).

In the German Human Bitumen Study (Raulf-Heimsoth et al. 2011 c), micronucleus frequencies in peripheral lymphocytes were determined pre-shift and post-shift in 225 mastic asphalt workers and 69 paving workers who were not exposed to bitumen emissions (Welge et al. 2011). The exposure group was exposed to a median concentration of vapours and aerosols of bitumen of 4.41 mg/m^3, related to the bitumen condensate standard (interquartile range: 2.64–7.34 mg/m^3). The 2 groups were similar in age structure and the number of smokers among the participants. In both groups, the median micronucleus frequency was 6.0 micronuclei per 1000 binuclear lymphocytes (MN/1000 BNC; interquartile ranges: reference group 4.0–8.3 MN/1000 BNC, mastic asphalt workers 4.0–8.5 MN/1000 BNC). A median of 6.5 MN/1000 BNC (interquartile ranges: reference group 4.0–9.0 MN/1000 BNC, mastic asphalt workers 4.4–9.3 MN/1000 BNC) was determined in both groups after the shift. To investigate the effects of exposure to bitumen and other possible influencing factors, linear regression models were applied taking into consideration potential confounders such as smoking status and age. In both groups, the age of the persons examined was found to be the strongest influencing factor and confirmed the findings of previous investigations of other research groups (Bonassi et al. 2001). No relationship was found between the micronucleus frequency and the level of exposure to the vapours and aerosols of bitumen. Although both groups had similar micronucleus frequencies, a model of micronucleus frequencies post-shift demonstrated that group membership (mastic asphalt workers compared with reference persons without exposure) had a weak effect on micronucleus frequency ($\exp(\beta) = 1.24$; 95% CI: 1.02–1.51; $p = 0.032$). In spite of the lymphocyte life span, this effect was not observed in a model of micronucleus frequencies pre-shift, even though it should have been (provided that it was biologically relevant and was associated with the group or exposure). Therefore, the authors did not consider this effect relevant for the evaluation of the effects of exposure, also because it was not apparent in the non-modelled data. In summary, no exposure-related effects on micronucleus frequency in the peripheral lymphocytes of mastic asphalt workers can be derived from the data of the German Human Bitumen Study.

Conclusions drawn from micronucleus studies: Two studies carried out in Turkey (Burgaz et al. 1998; Karaman and Pirim 2009) and 1 study from Australia (Murray and Edwards 2005) found increased micronucleus frequencies in the peripheral lymphocytes of workers exposed to bitumen; however, these findings were not confirmed by a smaller study from Sweden (Järvholm et al. 1999) and an extensive study from Germany (Welge et al. 2011). The reason for the disparity in the study findings cannot be conclusively explained. Not all of the studies included all of the necessary information, particularly data for the level of exposure, the types of asphalt used and the processing temperatures, and not all were adjusted for age and smoking status.

In a study from Turkey that investigated 40 paving workers exposed to the vapours and aerosols of bitumen and a reference group of 40 persons working in administrative and academic positions (both groups were made up of 20 smokers and 20 non-smokers), buccal mucosal cells were examined by buccal micronucleus cytome assay. In addition to analysing micronucleus frequencies, this test examines other cytogenetic parameters and parameters for cytotoxicity and apoptosis. On average, a significantly higher number of micronuclei were determined in the group of exposed persons than in the group of reference persons (10.4 ± 0.70‰ compared with 4.48 ± 0.45‰). The mean length of employment was about 9 years. No data were provided for the level and kind of exposure to the vapours and aerosols of bitumen (Çelik et al. 2013).

In an intervention study, 8 workers who were exposed during the manual laying of asphalt paving (hand pavers) and 22 finishers who worked in closed cabins were examined each year from 1996 and 1999. Only for the year 1996 was the scope of the study extended to include 8 refinery workers involved in bitumen production (positive controls) and 24 additional road pavers. The reference groups were made up of a group of 6 office workers who were not exposed to the vapours and aerosols of bitumen (white collar workers) and a group of 87 reference persons working in industry (industrial controls, no other details). Different end points relevant for genotoxicity were investigated in the peripheral lymphocytes: structural and numerical chromosomal aberrations (CA), SCE, high-frequency SCE and HPRT mutation

frequencies and ultraviolet (UV) light-induced DNA repair synthesis (UDS). A questionnaire was used to collect data for tobacco and alcohol consumption, exposure to ionising radiation or genotoxic chemicals, diseases and work history. At the beginning of the study period, significantly increased frequencies of structural chromosomal aberrations were determined in the hand pavers and finishers in comparison with the frequencies found in the reference groups. Over the course of the study period, the frequency decreased and was below the frequencies determined in the 2 reference groups during the final examination in 1999. The decrease in the overall frequency of chromosomal aberrations is attributed to improved working conditions for the road pavers who manually laid asphalt and for the finishers resulting from the replacement of crude oil by detergents as cleaning agents for the equipment. In addition, ventilation in the finisher cabins was improved to prevent the accumulation of diesel exhaust fumes. No changes were made to the type of asphalt applied. The findings for acentric fragments were similar to those for total aberrations and were interpreted in the same manner. The groups did not differ significantly with respect to chromatid breaks, chromatid exchange, dicentric chromosomes, ring chromosomes, and gaps (Major et al. 2001). In summary, no association was found between the increased frequencies of chromosomal aberrations determined in the hand pavers at the beginning of the study period and exposure to the vapours and aerosols of bitumen. No increase in the frequency of chromosomal aberrations was determined in either group after the implementation of better working conditions and the replacement of other potentially genotoxic substances (in this case: crude oil). The significantly higher overall frequency of chromosomal aberrations observed in the 8 workers involved in bitumen production who were examined once in 1996 cannot be regarded as evidence of genotoxic effects induced by bitumen as the workers were deliberately chosen as positive controls because they were known to have been exposed to PAHs through their work at an oil refinery.

In a follow-up study carried out by this research group (Tompa et al. 2007), the study described above was extended to include the years 2003 to 2006 and its scope was broadened by adding a group of managers exposed to bitumen and a group of workers from asphalt mixing plants. Overall, the collective was made up of 33 industrial controls, 23 managers, 23 hand pavers, 28 finishers and 15 workers in asphalt mixing plants. The findings essentially mirrored those of the study above, with the following additions: high frequencies of chromosomal aberrations were determined in the managers even though their work history showed them to have had little exposure to bitumen emissions. Lifestyle factors were discussed as a possible cause. After improvements in the working conditions initially reduced the frequency of chromosomal aberrations in hand pavers and finishers to the level of the controls from 1996 to 1999, chromosomal aberrations later increased in frequency once more. This was attributed to the fact that crude oil was again used more often as a cleaning agent and the cabins of the finishers were found to be poorly ventilated. However, these changes can also be interpreted as a normal variation in the occurrence of chromosomal aberrations resulting from lifestyle factors, as this was a purely observational study that lacked a strict study design. The differences in the SCE frequency (which was again analysed concurrently in this study) between the industrial control group and the exposed groups were likewise not statistically significant. Therefore, as in the case of the publication discussed above (Major et al. 2001), the effects observed in this study cannot be attributed to exposure to bitumen emissions.

A study with 19 paving workers and 22 reference persons in Italy investigated SCE in lymphocytes. The paving workers handled concrete asphalt at a processing temperature of 160 °C. No differences were found between exposed and reference persons with respect to the frequency of SCE (Cavallo et al. 2006).

4.6.4.2 Studies of exposure to bitumen emissions in a refinery

In a study that focused on the parameter "premature centromere division" as a possible marker of chromosomal instability, chromosomal aberrations were determined in 9 workers who had been exposed to the vapours and aerosols of bitumen in a refinery for a period lasting between 2 and 26 years. The total number of aberrations (other than gaps) was significantly higher in this small group in comparison with the 87 reference persons living in the vicinity of chemical industry who were not exposed to genotoxic chemicals. However, about 3 quarters of the exposed workers, but only half of the reference persons, were smokers (Major et al. 1999). No data were provided for the level of exposure, the exact work activities and for possible co-exposure to other crude oil products.

Tab. 7 Data for genotoxicity in workers exposed to the vapours and aerosols of bitumen

Workers	Exposure/exposure marker/ method of determination	Effects	Confounders, remarks	References
Oxidized bitumen (roofing)				
oxidative stress (8-oxo-dGuo as a biomarker)				
19 roofers (9 smokers), no control group, USA, Florida, 4 different construction sites	PAH metabolites (geometric mean ng/l pre-shift, ng/l post-shift): 1-OH-naphthalene (992, 2553) 9-OH-phenanthrene (441, 1033) 1-OH-pyrene (361, 692)	urine: statistically significant increase in 8-oxo-dGuo post-shift geometric mean: pre-shift 9 µg/l, after 6-hour shift 40 µg/l strict correlation between 8-oxo-dGuo and PAH metabolites, 2 workers with skin burns who did not use gloves while working: 105.6 µg/l 8-oxo-dGuo	8-oxo-dGuo levels higher in smokers than in non-smokers, concurrent exposure to coal tar pitch dust	Serdar et al. 2012
26 roofers (16 smokers), 15 controls (3 smokers), USA, "hot asphalt", Trumbull, Type III Steep ASTM D-312-95A or Type IV PA-100, 210/225 Extra Steep Roofing Asphalt ASTM DIN 312-84 (ERT=400/450 °C), "asphalt fume" 19 roofers had concurrent exposure to coal tar pitch dust	personal air monitoring: analysis of total particulate matter and PAHs according to NMAM Method 5042 (NIOSH 1998 a) and NMAM Method 5800 (NIOSH 1998 b), mean levels for 1-OH-pyrene determined using the method of Jongeneelen (Tolos et al. 1990) exposed persons (without exposure to coal tar pitch dust), beginning of the week: 0.26 ± 0.13 µmol 1-OH-pyrene/mol creatinine end of the working week: 0.58 ± 0.29 µmol 1-OH-pyrene/mol creatinine (1740 ng/l) controls, beginning of the week: 0.08 ± 0.12 µmol 1-OH-pyrene/mol creatinine end of the working week: 0.12 ± 0.12 µmol 1-OH-pyrene /mol creatinine	urine: no statistically significant increase in 1-OH-pyrene, 8-oxo-dGuo, 8-epi-prostaglandin2$_{\alpha}$, leukocytes: no statistically significant increase in 8-oxo-dGuo	no smoking effect	Toraason et al. 2001
DNA strand breaks in peripheral blood leukocytes (comet assay)				
26 roofers (16 smokers), 15 controls (3 smokers), USA	see above	significantly higher number of DNA strand breaks in 7 roofers exposed to "bitumen fumes" but not to coal tar than in the controls (levels were higher at the end of the working week than at the beginning)	no smoking effect	Toraason et al. 2001
DNA strand breaks (γH2AX)				
20 roofers, no control group, USA, Colorado	personal air monitoring during the shift: ng/m^3; geometric mean ± geometric SD, naphthalene 362 ± 2.9 benzo[*e*]pyrene 3.5 ± 8.9 PAH metabolites (µg/g creatinine; geometric mean ± geometric SD), for example 1-OH-pyrene: Mondays pre-shift: 706.3 ± 3.7 Mondays post-shift: 2100.6 ± 3.0 Thursdays pre-shift: 1032.8 ± 4.5 Thursdays post-shift: 1790.0 ± 3.7	slight increase in γH2AX in lymphocytes during the shift, but a marked increase in 8-oxo-dGuo no relationship between external and internal exposure on the one hand and the 2 investigated effect markers on the other hand	no control group	Serdar et al. 2016

Tab. 7 *(continued)*

Workers	Exposure/exposure marker/ method of determination	Effects	Confounders, remarks	References
Straight-run bitumen / air-rectified bitumen (paving)				
BPDE–DNA adducts (^{32}P-postlabelling)				
49 pavers "hot mix asphalt" (11 smokers), 36 controls (13 smokers), USA	no data	mononuclear blood cells: samples taken during all 4 seasons: no significant differences between exposed persons and controls, increase in DNA adducts during the working week, not in the "off-season", not in the control group; significant differences found in a seasonal comparison, the smallest number of DNA adducts in roller operators, the largest in screedmen	adjusted for smoking habits and body weight	McClean et al. 2007 b
320 mastic asphalt workers (199 smokers), 118 controls (61 smokers), Germany	laying temperature 230–270 °C, mostly work performed inside or in underground car parks, personal air monitoring of vapours/aerosols, bitumen condensate standard, median (interquartile range): exposed persons: 5.08 mg/m^3 (2.64–8.67) controls: 0.29 mg/m^3 (0.1–0.44) exposure according to Pesch et al. (2011): median ΣPAH concentrations; 1-OH-pyrene: exposed persons: 2.47 µg/m^3; 0.7 µg/l urine controls: 0.21 µg/m^3; 0.3 µg/l urine	no differences in BPDE adduct levels between exposed persons and controls, no differences pre-shift and post-shift	adjusted for age, smoking and German nationality	Marczynski et al. 2011
6 tunnel workers (3 smokers), Germany	rolled asphalt (RA), mastic asphalt (MA), laying temperature 180–250 °C bitumen condensate standard (median in mg/m^3, interquartile range): personal air monitoring: RA: 2.64 (1.32–3.53) MA: 11.6 (7.2–17.48) stationary determination: RA: 2.64 MA: 51.26 benzo[*a*]pyrene, stationary determination (ng/m^3): RA: 2.4 MA: 45 PAH metabolites in the urine (1-OH-pyrene, median, ng/g creatinine): RA: 530 (823 ng/l) MA: 299 (466 ng/l)	white blood cells: increase in BPDE–DNA adduct levels during the shift independent of type of asphalt application	"tunnel construction site"	Raulf-Heimsoth et al. 2011 a
8-oxo-dGuo as a biomarker for oxidative DNA damage				
320 mastic asphalt workers (199 smokers), 118 controls (61 smokers), Germany	see above	pre-shift and post-shift: statistically significant increase in 8-oxo-dGuo adducts, no positive association between adduct levels and exposure	see above	Marczynski et al. 2011

Tab. 7 *(continued)*

Workers	Exposure/exposure marker/ method of determination	Effects	Confounders, remarks	References
202 mastic asphalt workers (133 smokers), 55 controls (paving workers without exposure to bitumen) (23 smokers), Germany	laying temperature: 240–260 °C, personal air monitoring vapours/aerosols, bitumen condensate standard median (interquartile range): exposed persons: 5.43 mg/m³ (2.5–10.43) subgroups: 3 high: ≥ 14.7 mg/m³ 172 slight: < 14.7 mg/m³ PAH levels in air: naphthalene: 0.32–1.03 µg/m³ phenanthrene: 0.12–0.4 µg/m³ pyrene: 0.028–0.145 µg/m³ median levels in urine: 1-OH-pyrene (ng/g creatinine): exposed persons: pre-shift: 271.4 post-shift: 482.2 controls: pre-shift: 196.9 post-shift: 193.4 Σ OH-phenanthrene (ng/g creatinine): exposed persons: pre-shift: 961.9 post-shift: 1557.5 controls: pre-shift: 881.5 post-shift: 995.4	exposed persons: 8-oxo-dGuo adducts ↑ post-shift ($p < 0.014$), 8-oxo-dGuo adducts significantly ↑ pre-shift and post-shift in comparison with the controls; (+) anti-BPDE–DNA adducts: no effects	adjusted for age, smoking and German nationality	Marczynski et al. 2007 (subset of Marczynski et al. 2011)
66 mastic asphalt workers (44 smokers), 49 controls (20 smokers), Germany	bitumen condensate standard (median in mg/m³, interquartile range): exposed persons: 7.79 (3.67–23.8) median levels in urine: 1-OH-pyrene (ng/g creatinine): exposed persons: pre-shift: 165 post-shift: 296 controls: pre-shift: 148 post-shift: 179 Σ OH-phenanthrene (ng/g creatinine): exposed persons: pre-shift: 850 post-shift: 1556 controls: pre-shift: 894 post-shift: 945	white blood cells: exposed persons: 8-oxo-dGuo adducts ↑ in a comparison of pre-shift and post-shift levels ($p = 0.01$), post-shift: significant increase only in workers with low levels of exposure ($p = 0.02$), not in those with high levels of exposure, significant increase in adducts post-shift not only in asphalt workers, but also in the control group	adjusted for age, smoking and German nationality	Marczynski et al. 2006 (subset of Marczynski et al. 2011)
6 tunnel workers (3 smokers), Germany	see above	white blood cells: increase in 8-oxo-dGuo adduct levels during the shift irrespective of type of asphalt application	limited relevance because of small number of cases, "tunnel construction site"	Raulf-Heimsoth et al. 2011 a
19 paving workers (9 smokers), 22 controls (11 smokers), Italy	laying temperature 160 °C, personal air monitoring (NIOSH 1998 c), analysis of air samples taken on 3 workdays for 14 different PAH concentrations according to Perico et al. (2001): total PAHs median: 2.843 µg/m³ (0.434–15.858 µg/m³) benzo[*a*]pyrene: 0.005 µg/m³ (0.003–0.020 µg/m³) PAHs with 4–6 rings: 0.150 µg/m³ (0.033–0.945 µg/m³) urinary 1-OH-pyrene: pre-shift: 0.27 µg/g creatinine post-shift: 0.81 µg/g creatinine (1260 ng/l)	lymphocytes: oxidative DNA damage (FPG-modified comet assay) exposed persons: 7/19 (37%), controls: 0/22, statistically significant increase in the frequency of comets in exposed persons in comparison with the controls (both in FPG-treated and untreated cells), urinary 1-OH-pyrene significantly higher after 3 workdays than at the beginning of the working week	adjusted for smoking	Cavallo et al. 2006

Tab. 7 *(continued)*

Workers	Exposure/exposure marker/ method of determination	Effects	Confounders, remarks	References
oxidative stress (disulfide/thiol homeostasis)				
34 asphalt workers (non-smokers, average length of employment 15.2 ± 6.8 years, no other data), 35 controls (healthy non-smokers, "officers"), Turkey	urinary 1-OH-pyrene at the end of the working week (ng/g creatinine: median (range)): exposed persons: 3328 (1000–370 845) controls: 331 (65–1174)	disulfide/thiol ratio in serum and 1-OH-pyrene concentration in urine significantly higher in the exposed group than in the control group, positive correlation between disulfide/thiol ratio and 1-OH-pyrene concentration in urine	no specific data for exposure and activity, no data for the reference group	Yilmaz et al. 2016
DNA strand breaks (comet assay)				
30 asphalt workers (8 smokers), 30 controls (8 smokers), Turkey	no data	statistically significant increase in DNA strand breaks ("%DNA in the tail" in exposed persons 24.34; in controls 20.04) ($p < 0.01$), difference between exposed smokers and exposed non-smokers not significant ($p > 0.05$)	smoking and alcohol consumption considered	Bacaksiz et al. 2014
36 asphalt workers (20 smokers), 37 controls (19 smokers), South India	no data for level of exposure and bitumens used; duration of exposure: 11.08 ± 3.31 years urinary 1-OH-pyrene (median µmol/mol creatinine ± SD): exposed persons: 1.68 ± 0.93 (5040 ng/l) controls: 0.55 ± 0.42	peripheral lymphocytes: significantly longer average comet tail lengths with DNA fragments ($p < 0.05$), statistically significant difference between exposed smokers and exposed non-smokers ($p < 0.05$) and between exposed persons who consumed alcohol and exposed persons who did not ($p < 0.05$)	adjusted for smoking and alcohol consumption, no protective clothing	Sellappa et al. 2011
15 road pavers (10 smokers), 5 controls (no smokers), Finland	conventional asphalt (stone mastic asphalt, asphalt concrete) asphalt modified with waste plastic and tall oil pitch (145–165 °C) inhalable particles: bitumen "fumes": 0.05–0.29 mg/m³ bitumen "vapour": 0.4–1.9 mg/m³ PAHs: 0.5–3.5 µg/m³ benzo[*a*]pyrene: < 0.01 µg/m³ urinary 1-OH-pyrene: see Väänänen et al. (2006)	lymphocytes from the oral mucosa: no statistically significant differences between pre-shift and post-shift levels and between exposed persons and controls; positive correlation between DNA strand breaks and urinary 1-OH-pyrene and 1-naphthol only in post-shift samples but not in pre-shift samples, effects not induced by PAH exposure alone	no effects from smoking; barbecued food and hot beverages: increased DNA damage	Lindberg et al. 2008
6 tunnel workers (3 smokers), Germany	see above	peripheral lymphocytes: higher frequency of DNA strand breaks post-shift than pre-shift, no relationship between frequency of strand breaks and exposure, irrespective of type of asphalt application	"tunnel construction site"	Raulf-Heimsoth et al. 2011 a
66 mastic asphalt workers (44 smokers), 49 controls (20 smokers), Germany	see above	white blood cells: exposed persons: pre-shift and post-shift: DNA strand breaks ↑ ($p < 0.0001$), significant correlation with urinary levels of 1-OH-pyrene and OH-phenanthrene post-shift ($p = 0.001$ and $p = 0.004$)	adjusted for age, smoking and German nationality	Marczynski et al. 2006 (subset Marczynski et al. 2011)

Tab. 7 *(continued)*

Workers	Exposure/exposure marker/ method of determination	Effects	Confounders, remarks	References
202 mastic asphalt workers (133 smokers), 55 controls (paving workers without exposure to bitumen) (23 smokers), Germany	see above	white blood cells: exposed persons: post-shift: DNA strand breaks ↓ ($p < 0.05$) statistically significant increase in number of DNA strand breaks pre-shift and post-shift in exposed persons in comparison with controls, association between number of DNA strand breaks post-shift and 1-OH-pyrene	adjusted for age, smoking and German nationality	Marczynski et al. 2007 (subset of Marczynski et al. 2011)
42 mastic asphalt workers (27 smokers), no control group, Germany	mostly work performed inside, underground car parks, laying temperature 230–260 °C, duration of exposure: 96 months (72–180) exposure: median: 2.4 mg/m³ (1.5–4.1 mineral oil standard); 3.6 (2.25–6.15 bitumen condensate standard)	leukocytes from induced sputum: no changes in number of DNA strand breaks, lymphocytes from the blood: number of DNA strand breaks higher pre-shift than post-shift, statistically significant correlation between DNA strand breaks in sputum leukocytes and IL-8 concentrations pre-shift and post-shift, IL-8 ng/ml (median): pre-shift: 5.6 (1.7–11.3), post-shift: 3.0 (1.5–8.4)	adjusted for age, smoking and German nationality, no control group, no conclusions could be drawn	Marczynski et al. 2010
320 mastic asphalt workers (199 smokers), 118 controls (61 smokers), Germany	see above	higher frequency of DNA strand breaks in exposed persons (pre-shift and post-shift), no positive association between DNA damage and level of exposure in air and internal exposure	increase in strand breaks determined in workers still within the range of the levels determined earlier in persons without exposure	Marczynski et al. 2011
SCE and CA				
24 road pavers, 8 hand pavers, 22 finishers (26 smokers), 6 controls (office workers), 87 industrial controls (46 smokers), Hungary	no data for the method used to determine exposure levels exposure 1996: hand pavers: 4.32–12.99 mg/m³ finishers: 0.15–5.6 mg/m³ controls: no data	peripheral lymphocytes from the blood: 1996/1997: significantly higher levels in exposed persons in comparison with controls ($p < 0.05$) 1999: decrease in CA in exposed persons, levels brought into line with those of the controls, probably because of improved working conditions, no statistically significant differences: chromatid breaks, chromatid exchange, dicentric chromosomes, ring chromosomes, gaps	adjusted for smoking status, alcohol consumption, exposure	Major et al. 2001
19 paving workers (9 smokers), 22 controls (11 smokers), Italy	see above	lymphocytes: SCE frequency: no statistically significant differences	adjusted for smoking	Cavallo et al. 2006

Tab. 7 *(continued)*

Workers	Exposure/exposure marker/ method of determination	Effects	Confounders, remarks	References
26 asphalt workers (1 smoker), 26 controls (1 smoker), Turkey	exposure period: 11.2 years (SD 8.9 years, range 3–22 years) no data for level of exposure, type of bitumen used	peripheral lymphocytes: SCE frequency: significantly increased pre-shift in comparison with the controls, no association between SCE frequency and urinary 1-OH-pyrene	not adjusted for age, smoking status considered	Karaman and Pirim 2009
89 asphalt workers (54 smokers), 33 controls (15 smokers), Hungary	no determinations "mixing asphalt", laying temperature 150 °C	peripheral lymphocytes from the blood: CA: significantly increased in exposed workers ($p < 0.05$) SCE: no statistically significant differences	investigation of different road construction activities	Tompa et al. 2007
28 road pavers (non-smokers), 30 controls (non-smokers), Norway	personal air monitoring: total PAH levels: 0.2–24 µg/m³; coal tar-free asphalt; heating temperature: 150–200 °C	excretion of 1-OH-pyrene in exposed workers: 0.96 µmol/l, controls: 0.60 µmol/l (significant), no significant differences in pre-shift and post-shift levels, peripheral lymphocytes: SCE frequency: no significant differences		Järvholm et al. 1999
MN				
40 road construction workers (20 smokers), 40 controls (20 smokers), Turkey	no data for level of exposure, type of bitumen used, exposure period: 9 years	buccal mucosal cells: statistically significant increase in micronucleus frequency ($p < 0.001$)	no significant differences between exposed smokers and exposed non-smokers	Çelik et al. 2013
225 mastic asphalt workers (139 smokers), 69 controls (41 smokers), Germany	vapours and aerosols, bitumen condensate standard (median in mg/m³, interquartile range): exposed persons: 4.41 (2.64–7.34) controls: 0.29 (0.1–0.44)	peripheral lymphocytes from the blood: no differences between exposed and control persons (pre-shift and post-shift)	adjusted for smoking status and age	Welge et al. 2011
36 paving workers (20 smokers), 37 controls (19 smokers), South India	see above	peripheral lymphocytes: statistically significant increase in micronucleus frequency ($p < 0.05$), increase in micronucleus frequency in the control group/exposed group between smokers and non-smokers and between those who consumed alcohol and those who did not, increase in micronucleus frequency in workers with exposure for more than 10 years	no protective clothing other than safety shoes	Sellappa et al. 2011
202 mastic asphalt workers (133 smokers), 55 controls (23 smokers), Germany	see above	peripheral lymphocytes: no increase in micronucleus frequency		Marczynski et al. 2007 (subset of Marczynski et al. 2011)
12 road layers (6 smokers), 18 controls (6 smokers), Australia	no data for level and duration of exposure or type of bitumen used	lymphocytes and exfoliated cells from the urine: significant increase in micronucleus frequencies in both cell types ($p < 0.01$)	no smoking effect	Murray and Edwards 2005

Tab. 7 *(continued)*

Workers	Exposure/exposure marker/ method of determination	Effects	Confounders, remarks	References
26 asphalt workers (1 smoker), 26 controls (1 smoker), Turkey	exposure period: 11.2 years (SD 8.9 years, range 3–22 years), no data for level of exposure or type of bitumen used	peripheral lymphocytes: micronucleus frequency: significantly increased pre-shift in comparison with the controls, increase in micronucleus frequency with duration of exposure, increased urinary excretion of 1-OH-pyrene	not adjusted for age, smoking status considered	Karaman and Pirim 2009
28 road pavers (non-smokers), 30 controls (non-smokers), Sweden	personal air monitoring: total PAH levels: 0.2–24 µg/m^3; coal tar-free asphalt; heating temperature: 150–200 °C, determination of 1-OH-pyrene, SCE, MN	1-OH-pyrene excretion in exposed persons: 0.96 µmol/l, controls: 0.60 µmol/l (significant), no statistically significant differences between pre-shift and post-shift levels, peripheral lymphocytes: MN: no statistically significant differences		Järvholm et al. 1999
HPRT mutation frequency and UDS				
24 road pavers, 8 hand pavers, 22 finishers (26 smokers), 6 controls (office workers), 87 industrial controls (46 smokers), Hungary	no data for the determination of exposure, exposure 1996: hand pavers: 4.32–12.99 mg/m^3 finishers: 0.15–5.6 mg/m^3 controls: no data	peripheral lymphocytes from the blood: no statistically significant differences		Major et al. 2001

BPDE: benzo[*a*]pyrene-7,8-diol-9,10-epoxide; CA: chromosomal aberrations; HPRT: hypoxanthine-guanine phosphoribosyltransferase; MN: micronuclei; NMAM: NIOSH Manual of Analytical Methods; 1-OH-pyrene: 1-hydroxypyrene; 8-oxo-dGuo: 8-oxo-7,8-dihydro-2′-deoxyguanosine; PAH: polycyclic aromatic hydrocarbons; SCE: sister chromatid exchange; SD: standard deviation; UDS: DNA repair synthesis

4.6.5 Other genotoxicity end points

4.6.5.1 Studies of paving workers

The study by Major (2001) mentioned above investigated UV light-induced UDS in peripheral lymphocytes. No significant differences were found between hand pavers and finishers or the control groups. The HPRT mutation frequencies determined in the 2 groups of exposed persons did not differ significantly from those found in the control persons.

A method-oriented study was carried out to investigate the usefulness of phosphorylated histone (γH2AX) as a short-term biomarker for DNA damage among roofers (Serdar et al. 2016). Exposure to PAHs was determined in 20 roofers by personal sampling and analysis during the shift on Mondays and Thursdays of the working week. Before and after the shift, blood samples were taken to determine γH2AX in lymphocytes and urine samples were collected to determine PAH metabolites (1- and 2-hydroxynaphthalene and 1-hydroxypyrene) and 8-oxo-dGuo (comparison with γH2AX). There was a marked increase in 8-oxo-dGuo concentrations and a slight increase in γH2AX concentrations during the shift. No relationships were found between external and internal exposure on the one hand and the 2 investigated effect markers on the other hand.

4.6.6 Summary

4.6.6.1 Oxidized bitumen

Exposure of workers to the vapours and aerosols of oxidized bitumen yielded evidence of an increased frequency of DNA strand breaks (Toraason et al. 2001). PAH–DNA adducts, determined as benzo[*a*]pyrene–DNA adducts, cannot be unequivocally attributed to exposure to oxidized bitumen, as the workers were concurrently exposed to coal tar pitch dust (Herbert et al. 1990 b).

After exposure to the vapours and aerosols of oxidized bitumen without co-exposure to coal tar pitch dust, the urinary levels of 1-hydroxypyrene were 1740 ng/l (Toraason et al. 2001) and 1500 ng/l (McClean et al. 2007 a) (original data converted to ng/l according to Serdar et al. (2012)).

4.6.6.2 Straight-run bitumen/air-rectified bitumen (paving grade bitumen and mastic asphalt)

New studies published since 2001 did not find consistent evidence of increased genotoxicity or mutagenicity in exposed workers induced by straight-run bitumen and air-rectified bitumen (Lindberg et al. 2008; Tompa et al. 2007). No evidence of increased genotoxicity or mutagenicity induced by the vapours and aerosols of bitumen was found in vivo in humans, particularly in studies that investigated a large number of cases (n > 200) such as the German Human Bitumen Study (Marczynski et al. 2011; Welge et al. 2011) and in studies with a sufficiently large number of different genotoxicity and mutagenicity markers (Major et al. 2001). However, other studies did observe increased levels of individual genotoxicity parameters in exposed persons (Cavallo et al. 2006; Çelik et al. 2013; Karaman and Pirim 2009; Murray and Edwards 2005; Sellappa et al. 2011). The findings of the human case–control studies of Welge et al. (2011), Marczynski et al. (2011) and Major (2001) carry greater weight because of the concurrent investigation of different end points, the size of the study collective and the detailed description of exposure conditions. These studies did not find higher frequencies of micronuclei, chromosomal aberrations, HPRT mutations, SCE, DNA strand breaks or oxidative DNA base damage induced by exposure to bitumen.

The predominantly negative results for genotoxicity and mutagenicity are supported by studies that did not determine an increase in DNA adduct levels (Marczynski et al. 2011; McClean et al. 2007 b). A relationship between exposure and PAH-related DNA adducts of the carcinogen benzo[*a*]pyrene was not found (Marczynski et al. 2011; McClean et al. 2007 b), even though very sensitive methods such as ^{32}P-postlabelling were used in some cases.

Most of the studies used the levels of 1-hydroxypyrene in the urine as a marker of PAH exposure. The levels obtained were below 2.5 µg/l urine, the level set as the threshold by the Biological Exposure Indices (BEI) committee of the American Conference of Governmental Industrial Hygienists (ACGIH 2017). According to current knowledge, no relevant genotoxicity is expected to be induced by PAHs below this threshold. In road paving workers with exposure to bitumen, the mean levels of 1-hydroxypyrene in the urine (where applicable, converted to ng/l according to the method of Serdar et al. (2012) to allow comparison) were 1865 ng/l (McClean et al. 2004 b), 700 ng/l (McClean et al. 2012), 184 ng/l (Campo et al. 2011), 2110 ng/l (Sobus et al. 2009), 870 ng/l (Väänänen et al. 2003), 1980 ng/l (Väänänen et al. 2006), 1260 ng/l (Cavallo et al. 2006), 460 to 820 ng/l (Human Bitumen Study, Pesch et al. (2011)), 5040 ng/l (Sellappa et al. 2011) and 1170 ng/l (Karaman and Pirim 2009). On the basis of these findings, PAHs play only a subordinate role in the development of a theoretical genotoxic risk for workers.

However, in spite of methods that allow bitumens to be processed at lower temperatures, straight-run and air-rectified bitumen, particularly mastic asphalt, may continue to be applied under very unfavourable exposure conditions in the future, for example in tunnels lacking adequate ventilation. Therefore, increased exposure to carcinogenic compounds, including PAHs, cannot be ruled out in exceptional cases.

4.7 Carcinogenicity

This section reviews epidemiological studies that were not described in the 2001 documentation (Greim 2002) and that were published after 2001.

4.7.1 Case–control studies

A number of case–control studies (Jöckel et al. 1998; Richiardi et al. 2004; Watkins et al. 2002) did not observe a significant increase in the odds ratio (OR) for lung cancer resulting from occupational exposure to bitumen. The relevance of these studies is limited for several reasons, including a lack of specificity in the estimation of exposure levels and the inclusion of an insufficient number of cases.

A study carried out in Germany compared 156 men with bladder tumours with 336 control persons with prostate cancer. An OR for bladder tumours of 2.92 (95% CI: 1.32–6.48) was determined for the category "frequent exposure to bitumens". However, concurrent exposure to coal tar or pitch is possible (Geller et al. 2008). No adjustment was made for this by the authors. No quantitative data were provided for the frequency and level of exposure or the nature of the work activities.

In Canada, 1009 persons with malignant brain tumours were compared with 5039 random population controls from Canadian registries. Mailed questionnaires were used to collect data for the study. The analyses were performed by logistic regression and adjusted for age, province of residence, sex, education level, alcohol and tobacco consumption and total energy intake via the diet. The OR for brain tumours after exposure to bitumen ("for more than a year") was 1.29 (95% CI: 1.02–1.62). While the OR for men was 1.20 (95% CI: 0.93–1.54), women were found to have a markedly higher OR of 1.85 (95% CI: 1.03–3.34). The trend ($p = 0.033$) for the length of bitumen exposure was statistically significant; the OR was 1.21 for exposure for 1 to under 10 years (95% CI: 0.92–1.60; 77 cases, 274 controls) and 1.39 for exposure for at least 10 years (95% CI: 0.97–1.99; 43 cases, 162 controls) (Pan et al. 2005). The report did not provide any data for the nature of the work activities or the type of bitumen handled.

A case–control study that was nested in parts of the IARC cohort (38 296 male asphalt workers from Denmark, Germany, Finland, France, Israel, the Netherlands and Norway; see Section 4.7.2) investigated workers who had worked in the bitumen industry for at least 2 seasons, were under 75 years of age on 1 January 1980 and had not developed cancer. The group of cases comprised 433 workers from the cohort who had developed a lung tumour between 1980 and 2002 to 2005 (the end of the follow-up period varied in the different national subcohorts). The control group was made up of 1253 randomly selected persons without respiratory tract cancer. Demographic data as well as information on lifestyle and occupational history were collected by telephone interview. As only 2% of the persons with lung cancer could be reached for an interview, in almost all of the cases the interviews were held with relatives and work colleagues, while 66% of the workers of the control group were available for an interview. The number of participants varied greatly, not only between the individual countries, but also between cases (65%) and controls (58%). The individual exposure levels to the vapours and aerosols of bitumen, organic vapours and 4-ring to 6-ring PAHs were modelled semi-quantitatively. In addition, dermal exposure to bitumen vapour condensate was estimated and adjusted for working hours and hygiene habits. Possible confounders (asbestos, tar, quartz, diesel exhaust fumes) were divided into the categories "no", "low" and "high" according to estimates by experts. The data were analysed by logistic regression after adjustment for age, country and smoking pack-years. The ORs for lung cancer were 1.12 (95% CI: 0.84–1.49) for general exposure to bitumen, 1.20 (95% CI: 0.93–1.55) for organic vapours, 1.20 (95% CI: 0.85–1.69) for PAHs in the inhaled air, and 1.17 (95% CI: 0.88–1.56) for dermal exposure to bitumen vapour condensate. The adjusted ORs for the duration of exposure to bitumen, average level of exposure and cumulative exposure to the vapours and aerosols of bitumen were slightly increased in comparison with those of the category "never exposed" and were in the order of 1.1 and 1.3, which was not statistically significant. A linear trend analysis did not demonstrate a significant association between these levels of exposure and the incidence of lung cancer. This applies to both routes of exposure, dermal absorption and inhalation. Conversely, an OR of 1.60 (95% CI: 1.09–2.36) was determined for cumulative exposure to tar (Olsson et al. 2010). The varying number of participants and the necessity of relying almost exclusively on relatives and colleagues in the interviews for the group of cases may have introduced some bias of unknown direction. In spite of considerable efforts on the part of the authors, some of the exposure data for the vapours and aerosols of bitumen and possible confounders are based solely on estimates.

The study of Olsson et al. (2010) was re-evaluated with a detailed estimation of the exposure levels to bitumen and confounders. The adjusted OR for the duration of exposure, cumulative exposure and average exposure to bitumen was

between 0.6 and 1.2. A linear trend was not found between exposure levels and the incidence of lung cancer. In this respect, the re-analysis was not able to establish a relationship between bitumen vapour and lung cancer (Agostini et al. 2013).

4.7.2 Cohort studies

The multicentric cohort study carried out by the IARC comprised a collective of just under 80 000 workers from the asphalt industry, roofers and workers from related trades who handled or did not handle bitumen in Denmark, Germany, Finland, France, Israel, the Netherlands, Norway and Sweden (Boffetta et al. 2001, 2003 a, b). Depending upon the subcohort, the first year of employment was between 1910 and 1965 and the last year of employment was between 1992 and 1999. On the basis of a job category system, the investigated workers were divided into groups of workers who were exposed to the vapours and aerosols of bitumen (n = 29 820) and workers in building and ground construction without exposure to bitumen (n = 32 250). The remaining workers formed a group of participants who were not classifiable in terms of their exposure to bitumen. Using information provided by the companies, a "coal tar-free" subcohort was formed comprising 17 443 bitumen workers and 30 273 building and ground construction workers. The mortality data were collected for the time period from 1953 to 1979 (beginning) and 1995 to 2000 (end) and relative risks were calculated by matching with the "WHO Mortality Data Base" (specified according to age, calendar period, country and sex). Overlapping between the IARC multicentre study and the epidemiological studies previously described in the 2001 documentation (Greim 2002) was documented (Pukkala 1995) or, in some cases, probable (Engholm et al. 1991; Hansen 1989 a, b).

Overall, both total mortality and mortality caused by malignant neoplasms were slightly reduced in bitumen workers in the total cohort (SMR (standardized mortality ratio) overall 0.96 (95% CI: 0.93–0.99); malignant neoplasms SMR 0.95 (95% CI: 0.90–1.01); Boffetta et al. 2001). In the pooled analysis of Boffetta et al. (2003 a), a trend of increased SMRs was observed for lung cancer; however, this trend was statistically significant only for bitumen workers overall (1.17; 95% CI: 1.04–1.3) and paving workers (1.17; 95% CI: 1.01–1.35) (see Table 8). An analysis within the cohort using Poisson regression and the building and ground construction workers as a reference group did not find a statistically significant increase in lung cancer risk for the individual occupational groups.

Tab. 8 Standardized mortality ratios (SMR) for lung cancer (IARC multicentre study) (Boffetta et al. 2001, 2003 a)

Subcohorts	SMR	95% CI	Number of deaths
bitumen workers (total)	1.17	1.04–1.30	330
paving workers	1.17	1.01–1.35	189
mastic asphalt workers	2.39	0.78–5.57	5
workers in asphalt mixing plants	1.12	0.73–1.66	25
roofers and waterproofers	1.33	0.73–2.23	14
building and ground construction workers without exposure to bitumen (reference group)	1.01	0.89–1.15	no data
other occupational groups	1.01	0.88–1.15	no data

A job exposure matrix with semi-quantitative estimates of the level of exposure to the vapours and aerosols of bitumen, organic vapours, PAHs, diesel exhaust fumes, asbestos, quartz and coal tar was developed on the basis of questionnaires (Burstyn et al. 2000, 2003). The SMR for lung cancer of workers exposed to bitumen (1.08; 95% CI: 0.99–1.18) was almost at the same level as that of workers without exposure to bitumen (1.05; 95% CI: 0.92–1.19). An SMR of about 1 was determined for the groups with exposure to coal tar, asbestos or quartz, which may, however, have been caused by cumulative exposure levels that were, on average, low. Conversely, the SMR for lung cancer was significantly increased (1.23; 95% CI: 1.02–1.48) in the "coal tar-free" subcohort, but without any significant association with the duration of exposure, cumulative exposure or average exposure level (Boffetta et al. 2003 b). The analyses of the cohorts of the IARC multicentre study (Boffetta et al. 2003 a, b) were not adjusted for cigarette smoking.

In the Swedish (Bergdahl and Järvholm 2003) and Dutch subcohorts (Hooiveld et al. 2003) of the IARC multicentre study, the data were adjusted for smoking habits on the basis of secondary data for smoking prevalence in the respective countries. In the Swedish cohort, this had no influence on the estimated risk of lung cancer in construction workers with exposure to bitumen. In comparison with construction workers without exposure to bitumen, the SMR was 1.03 (95% CI: 0.70–1.45) after adjusting for tobacco smoking. The authors concluded that bitumen workers do not have an increased risk of lung cancer from exposure to the vapours and aerosols of bitumen at levels similar to those found in Sweden in the 1960s and 1970s. After adjusting for smoking habits, all relative lung cancer risks from exposure to bitumen were decreased in the Dutch cohort, which was interpreted as evidence of confounding through smoking.

To facilitate the collection of data for tumours with a good survival prognosis, the cancer incidence for various tumours was investigated in the IARC subcohorts from Denmark, Finland, Norway and Sweden. The increase in the standardized incidence ratio (SIR) for lung cancer was statistically significant in the overall subcohort (SIR 1.21; 95% CI: 1.07–1.36) and was particularly marked in paving workers (SIR 1.26; 95% CI: 1.08–1.47) in comparison with the lung tumour incidence determined on the basis of data from the national cancer registries. No trend was detected with regard to the time since the first exposure. Significant increases in SIRs were not found in the other subgroups; roofers, for example, had an SIR of 1.06 (95% CI: 0.53–1.90). No excess cancer of the bladder was noted, only a trend that was dependent on the time since the first exposure. However, this trend was not statistically significant (Randem et al. 2004 a). The results were not adjusted for smoking.

A study that investigated another subset of the cohort (male asphalt paving workers from Denmark, Finland, Norway and Israel) for a possible relationship between the incidence of bladder cancer and exposure to PAHs used benzo[*a*]pyrene as the marker of exposure. Trends were observed; however, these were not found to be statistically significant (Burstyn et al. 2007). The calculated risks were not adjusted for smoking or exposure to coal tar.

A follow-up study up to 2004 is available for the German subcohort of the IARC multicentre study. The study found significantly increased SMRs for neoplasms of the lungs (1.77; 95% CI: 1.46–2.16), larynx, oesophagus and the bladder and for oral-pharyngeal tumours. However, at the same time the study found an excess of deaths from alcoholism, liver cirrhosis and non-malignant respiratory deaths (indicating an increased prevalence of smoking). According to the authors, the higher incidence of mortality from lung cancer observed among the asphalt workers was probably caused by a higher prevalence of smoking or exposure to coal tar (Behrens et al. 2009).

4.7.3 Meta-analyses

A meta-analysis reviewed all epidemiological sources that were available at that time. Only peer-reviewed publications were included and, if several articles had been published on a study, the latest updated version was used. Studies that calculated the relative risk (RR) based on an internal control population were preferred to those with results based on external control groups. The analysis included 16 country-specific studies of roofers and 11 studies of paving workers. After adjusting for coal tar, the overall relative risk of lung cancer in roofers declined from 1.67 (95% CI: 1.39–2.02) to 1.10 (95% CI: 0.91–1.33). However, adjusting for coal tar had hardly any effect on the results for paving workers (0.98, 95% CI: 0.81–1.18 to 0.96, 95% CI: 0.80–1.16) (Fayerweather 2007). An over-adjustment may have been made for roofers, particularly because the meta-analysis included studies that had already been adjusted for tar, which may have weakened the RR to almost 1.

Another meta-analysis of all studies that fulfilled the inclusion criteria revealed a slightly increased RR of lung cancer of 1.33 (95% CI: 1.20–1.47) (Mundt et al. 2018). The RR was slightly higher in roofers (1.79; 95% CI: 1.46–2.19) than in paving workers (1.12; 95% CI: 1.04–1.21). After the exclusion of studies that were of lower quality, for example because of poorly estimated exposure levels, insufficient data for occupation, duration of exposure or co-exposure to coal tar, the authors did not find an overall higher risk for lung cancer in workers exposed to bitumen (RR 0.94; 95% CI: 0.74–1.20). The results of the 8 lung cancer studies included in the meta-analysis were adjusted for such confounders as smoking and, with one exception, also for exposure to tar. An overall higher risk was determined for head and neck tumours in the oral cavity, oesophagus, pharynx or larynx, both when all analysed studies were taken into consideration (RR overall 1.48; 95% CI: 1.22–1.81; roofers: RR 1.86; 95% CI: 1.22–3.83; paving workers: RR 1.37; 95% CI: 0.97–1.93) and

after the exclusion of lower-quality studies (RR 1.31; 95% CI: 1.03–1.67; data not differentiated by occupation). The risk for oesophageal tumours was increased (all studies 1.30; 95% CI: 1.06–1.59; after the exclusion of lower-quality studies RR 1.48; 95% CI: 1.0–2.19). When the studies of paving workers and roofers were evaluated separately, significantly higher risks were determined among roofers (RR 1.34; 95% CI: 1.07–1.67 for all studies). The findings of the studies included in the meta-analysis that investigated head and neck tumours were almost exclusively based on external comparisons for cancer incidence and mortality and had not been adjusted for smoking and coal tar. After exclusion of the lower-quality studies, a slightly increased risk of stomach cancer (RR 1.29; 95% CI: 1.03–1.62) was determined. However, this finding was largely based on a single study; the other studies did not report any noteworthy findings. An increased risk caused by exposure to bitumen was not observed for bladder and kidney cancers and for skin cancer (melanomas and non-melanomas). After systematically analysing the quality of the findings of the meta-analysis, the authors concluded that the higher-quality studies provided strong evidence against an increased risk of lung cancer; that is, the level of evidence was rated as high. The authors assigned a moderate level of confidence to the evidence against an increased risk of kidney cancer. The level of confidence for the findings relating to lung cancer and for all other tumour localizations—irrespective of whether the meta-analysis found an increased risk or not—was low for all studies; in other words, the evidence was not convincing.

4.7.4 Summary

Overall, no evidence of increased mortality from cancer (all forms of cancer) was found in bitumen workers. However, a slightly increased risk of lung cancer was observed, but not consistently; it is not possible to conclusively attribute this effect to a particular type of bitumen or to a particular activity. The reliability of the findings of the available studies is limited; therefore, both an overestimation and an underestimation of the potential lung cancer risk is possible. However, a markedly higher lung cancer risk can largely be ruled out on the basis of the overall findings of the studies. In comparison with other studies, greater significance can be accorded to the results of the nested case–control studies of Olsson et al. (2010) and Agostini et al. (2013) because of the relatively good estimation of the level of exposure; neither found a significant increase in lung cancer risk among a relatively large number of cases (433 and 393, respectively).

The existing studies, including the IARC multicentre study, have considerable shortcomings:

- a lack, particularly of quantitative data for the estimated level of exposure to the vapours and aerosols of bitumen;
- a lack, particularly of quantitative data for the estimated level of exposure to significant potential occupational and non-occupational confounders, primarily coal tar and smoking;
- a lack of data for the levels of occupational exposure before or after handling bitumen at work;
- with respect to the IARC multicentre study: heterogeneity of exposure across countries, representativeness of bitumen workers, comparability of the national SMRs.

4.8 Other effects

Since 2000, 3 studies have investigated the potential tumour-promoting properties of the vapours and aerosols of bitumen (Fenga et al. 2000; Loreto et al. 2007; Rapisarda et al. 2009) by analysing via immunohistochemistry the expression of specific proteins that may influence the cellular response to stress signals or play a role in cell cycle regulation/apoptosis. The investigations were performed using skin biopsies from paving workers with long-term exposure to bitumen and from control persons without exposure. By comparing the findings with those from control samples, it was possible to determine changes in the expression of the heat shock protein HSP 27 (Fenga et al. 2000), the proteins Bax and Bcl-2, which are involved in the regulation of apoptosis (Loreto et al. 2007), TRAIL (Tumor Necrosis Factor Related Apoptosis Inducing Ligand), DR5 (Death Receptor) and Caspase-3 (Rapisarda et al. 2009). Also, a significantly larger number of nuclei of apoptotic cells were determined by the TUNEL method (Terminal Deoxynucleotidyl Transferase Mediated dUTP Nick End Labelling) in the samples collected from exposed workers (Rapisarda et al. 2009).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

There are no new data available.

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

5.2.1.1 Straight-run bitumen, air-rectified bitumen

In a study carried out in the United States, female Sprague Dawley rats (Hla:(SD)CVF) were exposed whole-body by inhalation to the vapours and aerosols of a paving grade bitumen heated to 150 °C. No further data were provided for the paving grade bitumen (Ma et al. 2003 b). The duration of exposure varied from group to group and ranged from a single exposure for 1 hour to exposure for 6 hours a day on 5 consecutive days. The number of animals per group was not reported in more detail. The method used to produce and to determine exposure levels was described in the report (Wang et al. 2001). Cumulative exposure levels were in the range from 53 to 1734 mg × h/m^3; the concentrations of TOM in air were between 10.4 and 57.8 mg/m^3. The study investigated the acute responses in the lungs (differential cell count, acellular lactate dehydrogenase activity and protein content of bronchoalveolar lavage fluid), alveolar macrophage activity and changes in the activities of xenobiotic-metabolizing enzymes in the lungs. No signs of damage or inflammation were determined in the lungs. However, cytochrome-P450 (CYP) 1A1 and cytosolic quinone oxidoreductase levels were increased and CYP2B1 levels reduced. The induction of CYP1A1 was localized in undamaged bronchiolar epithelial cells (club cells), alveolar septa and endothelial cells by immunofluorescence microscopy. These changes alter PAH metabolism and, according to the authors, may lead to mutagenic or carcinogenic effects in the lungs.

The lung tissue of groups of 5 male Fischer 344 rats was examined after nose-only exposure to the vapours and aerosols of bitumen by inhalation for 6 hours a day on 5 consecutive days (Gate et al. 2006). To produce the vapours and aerosols, 50/70 bitumen from Venezuela (CAS number 8052-42-4) was heated to 170 °C. The total particulate matter concentration was 114.6 ± 16.8 mg/m^3; reference was made to an earlier publication with respect to the PAH levels (benzo[*a*]pyrene: 200 ng/m^3; sum of 4–6 ring PAHs: 15–16 µg/m^3) (Binet et al. 2002). The PAH profile was comparable with the PAH profile determined in paving workers. The lung tissue of the 5 rats of the exposure group and the 5 control animals was prepared immediately after the last exposure. In the bronchoalveolar lavage (BAL) fluid of the exposed animals, the total number of cells was double the control value and the number of macrophages, neutrophilic granulocytes and lymphocytes were significantly increased. In addition, there was a marked increase in the expression of inflammation-promoting genes in the BAL cells (TNFα, IL-1β, MIP2). The altered expression of 26 genes involved in inflammatory and immune responses could be determined by microarray analysis. The gene expressions of cytokines IL-6 and IL-18 and chemokines CCL2/MCP1, CXCL1/CINC1 and CXCL2/MIP2 were among the most upregulated in the lung tissue. The gene expression of the PAH-metabolizing enzymes CYP1A1 and CYP1B1 was increased 800 and 27 times, respectively; by contrast, CYP2F2 (naphthalene metabolism) was 3.4 times lower. Other genes, for example NQO1, ALDH3A1 and GSTA5, with an Ah-receptor binding site in their promotor, were likewise significantly upregulated in exposed lung tissue. Exposure to bitumen also led to the increased expression of genes involved in the cellular response to oxidative stress (SOD2, HSP1A1, HMOX1, GPX1, MT1A). Overall, exposure to the vapours and aerosols of bitumen altered the expression of 363 genes or EST (Expressed Sequence Tags).

Groups of 50 to 86 male and female Wistar rats were exposed nose-only to the vapours and aerosols of bitumen for 24 months (Fuhst et al. 2007). The bitumen vapour and aerosol atmosphere was generated according to the process developed by Fraunhofer ITEM (Pohlmann et al. 2006 a, b; Preiss et al. 2006). This process involved heating a condensate that was generated prior to the beginning of the study from the vapour and aerosol phase of a paving grade bitumen (B 50/70) heated in a tank to a temperature of 175 °C. This type of bitumen finds widespread use in Germany.

The benzo[*a*]pyrene concentration in the condensate was about 0.2 µg/g. The bitumen vapour and aerosol atmosphere was diluted to the desired exposure concentration by adding air and then fed to the inhalation units; the volumetric flow rate, temperature and humidity were monitored continuously. The vapours and aerosols tested were generated based on typical scenarios from the workplace; qualitatively, their constituents were equivalent to those found with actual workplace exposure.

Regular analytical monitoring of the exposure atmosphere ensured that the concentration in the air remained constant and identical for the animals (Pohlmann et al. 2006 a). The rats were exposed for 6 hours a day to the vapours and aerosols of bitumen at mean total hydrocarbon concentrations (THCs) in air of 0, 4.1, 20.7 or 103.9 mg/m^3 (related to mineral oil as the reference standard), which are equivalent to 0, 6.0, 30.4 or 152.6 mg/m^3 related to bitumen condensate as the reference standard. In the 3 concentration groups, the vapour-to-particle ratios were 15% to 85% at 6 mg/m^3, 27% to 73% at 30.4 mg/m^3 and 50% to 50% at 152.6 mg/m^3, respectively (bitumen condensate standard). In the low concentration group, the benzo[*a*]pyrene concentrations were below the limit of detection, in the middle and high concentration groups, the concentrations were 5 and 30 ng/m^3, respectively. The total number of 272 male and 272 female SPF Wistar rats (CRL:WI(WU)BR) were divided into groups of 86 animals of each sex for the control and high concentration groups and groups of 50 animals of each sex for the low and middle concentration groups. All animals were examined daily for clinical signs; body weights and feed consumption were monitored regularly. No signs of toxicity were determined in the rats during the study period. A comparison of the groups did not yield significant differences in mortality. The decrease in body weights of the rats of the middle and high concentration groups in comparison with those of the control group was statistically significant (♂/♀: –3%/–8% and –7%/–8%). During the study period, BAL was carried out in 6 male and 6 female rats of the control and high concentration groups after 7 and 90 days and after 12 months. The rats of the high concentration group exhibited signs of slight inflammatory effects in the bronchoalveolar region of the lungs. These were manifest as an increase in the concentrations of lactate dehydrogenase and γ-glutamyltransferase in the BAL fluid and were statistically significant. Examinations for cell proliferation (nasal cavity, terminal bronchioles and lung parenchyma) and for histopathological changes in the respiratory tract were carried out in an additional 6 male and 6 female rats after the same periods of time. Increased cell proliferation in the transition zone from the respiratory to the olfactory epithelium was found only in male rats (see Table 9).

Tab. 9 Time-dependent histopathological findings after exposure of SPF Wistar rats to the vapours and aerosols of bitumen (152.6 mg/m^3 bitumen condensate standard) (Fuhst et al. 2007)

	7 days	90 days	12 months
Main nasal cavity and paranasal sinuses			
basal cell hyperplasia	weak to moderate HC: 6/6 ♂, 6/6 ♀ C: 0/6 ♂, 0/6 ♀ (mainly in the transition zone between respiratory and olfactory epithelium)	weak HC: 6/6 ♂, 6/6 ♀ C: 0/6 ♂, 0/6 ♀ (mainly in the transition zone between respiratory and olfactory epithelium)	focal/multifocal very weak to weak HC: 2/6 ♂, 2/6 ♀ C: 0/6 ♂, 0/6 ♀
multifocal goblet cell hyperplasia	no findings	very weak to moderate HC: "trimming levels" 1 to 3 C: "trimming level" 1 statistically significant difference in severity	very weak to moderate HC: 6/6 ♂, 6/6 ♀ C: 1/6 ♂, 2/6 ♀
eosinophilic cytoplasmic inclusions	no findings	very weak to weak HC: 6/6 ♂, 6/6 ♀ C: 0/6 ♂, 0/6 ♀ (multifocal; mainly in the transition zone between respiratory and olfactory epithelium)	very weak to moderate HC: 4/6 ♂, 6/6 ♀ C: 2/6 ♂, 1/6 ♀ (hyaline degeneration; in epithelial cells)
focal/multifocal hyperplasia of the respiratory epithelium	no findings	no findings	very weak to weak HC: 4/6 ♂, 1/6 ♀ C: 0/6 ♂, 0/6 ♀

Tab. 9 *(continued)*

	7 days	90 days	12 months
multifocal inflammatory cell infiltrates of the respiratory and/or olfactory epithelium	no findings	no findings	very weak to weak HC: 5/6 ♂, 6/6 ♀ C: 1/6 ♂, 0/6 ♀
Lungs			
focal/multifocal bronchoalveolar hyperplasia of the bronchiolar type (alveolar bronchiolization)	no findings	no findings	very weak to weak HC: 5/6 ♂, 4/6 ♀ C: 0/6 ♂, 0/6 ♀
multifocal bronchiolar goblet cell hyperplasia	very weak HC: 1/6 ♂, 0/6 ♀ C: 0/6 ♂, 0/6 ♀	no data	very weak HC: 4/6 ♂, 2/6 ♀ C: 0/6 ♂, 0/6 ♀
multifocal alveolar histiocytosis (accumulation of macrophages)	very weak to weak HC: 2/6 ♂, 2/6 ♀ C: 0/6 ♂, 0/6 ♀	weak to moderate HC: 6/6 ♂, 6/6 ♀ C: 5/6 ♂, 1/6 ♀	very weak to weak HC: 6/6 ♂, 6/6 ♀ C: 2/6 ♂, 0/6 ♀
bronchiolar/alveolar hypertrophy	no findings	HC: 3/6 ♂, 3/6 ♀ C: 1/6 ♂, 0/6 ♀	no data
alveolar/interstitial (mixed) inflammatory, mononuclear cell infiltrates	no findings	no findings	very weak HC: 2/6 ♂, 2/6 ♀ C: 0/6 ♂, 0/6 ♀
multifocal interstitial fibrosis	no findings	no findings	weak HC: 0/6 ♂, 1/6 ♀ C: 0/6 ♂, 0/6 ♀

C: control group; HC: high concentration group

At concentrations of 30.4 mg/m^3 (bitumen condensate standard) and above, a significant, concentration-dependent increase in very slight (38% of the animals of the exposure group) to slight (6%) bronchiolar-type bronchoalveolar hyperplasia was found in the lungs of animals of both sexes. In the high concentration group, the fraction of very slight to slight hyperplasia was 66% and 26%. The increase in low-grade effects was significant only at concentrations of 152.6 mg/m^3 (bitumen condensate standard) and above. In addition, a significant increase in the incidence of primarily very slight to slight mononuclear inflammatory cell infiltrates was observed in both sexes at this concentration and above. The only moderate case occurred in the control group. A significant increase in the incidence of very slight to slight alveolar histiocytosis was observed at concentrations of 30.4 mg/m^3 and above in the males and at concentrations of 152.6 mg/m^3 and above in the females; the background incidence was 64%.

The incidence of goblet cell hyperplasia in the olfactory epithelium was significantly increased in male rats at concentrations of 6 mg/m^3 (bitumen condensate standard) and above. This effect occurred with comparable frequency in the females at 6 mg/m^3; however, the increase was not significant because of the high incidence determined in the control group. This effect is regarded as adaptive and not adverse because it was not found together with degenerative changes or signs of inflammation and is therefore not included in the derivation of the MAK value. For the same reason, the small number of cytoplasmic inclusions was not regarded as adverse. They were significantly increased at concentrations of 6 mg/m^3 and above in the males and, because of the higher incidence found in the control group, at concentrations of 30.4 mg/m^3 and above in the females. At concentrations of 30.4 mg/m^3 and above, a significant increase in the number of inflammatory cell infiltrates was found in animals of both sexes (see Table 10).

On the basis of the increased incidence of bronchoalveolar hyperplasia in the lungs and of inflammatory cells in the olfactory epithelium, a NOAEC (no observed adverse effect concentration) of 6 mg/m^3 and a LOAEC (lowest observed adverse effect concentration) of 30.4 mg/m^3 (bitumen condensate standard) can be derived from this study.

Tab. 10 Non-neoplastic effects after 24 months of exposure of SPF Wistar rats to the vapours and aerosols of bitumen (Fuhst et al. 2007)

	Concentration [bitumen condensate standard/m³]							
	0 mg/m³		6 mg/m³		30.4 mg/m³		152.6 mg/m³	
	♂[a)]	♀[a)]	♂[a)]	♀[a)]	♂[a)]	♀[a)]	♂[a)]	♀[a)]
Main nasal cavity and paranasal sinuses								
proliferative lesions								
multifocal basal cell hyperplasia of the olfactory epithelium (mainly very weak to weak)	0	0	1	0	1	3	20*	27*
hyperplasia of the respiratory epithelium (very weak to weak)	0	0	3	0	3	2	13*	20*
adaptive goblet cell hyperplasia (very weak to moderate)	1	7	11*	10	25*	37*	46*	47*
degenerative and inflammatory lesions								
multifocal to diffuse eosinophilic cytoplasmic inclusions (hyaline degeneration) (weak to severe)	1	12	13*	11	16*	27*	31*	38*
multifocal respiratory eosinophilic cytoplasmic inclusions (hyaline degeneration) (very weak to moderate)	2	7	5	3	7	21*	22*	24*
multifocal mononuclear/inflammatory (mixed) cell infiltrates of the respiratory and/or olfactory epithelium (very weak to weak)	2	11	8	5	18*	22*	27*	34*
Lungs								
multifocal bronchoalveolar (adaptive) hyperplasia of the bronchiolar type (alveolar bronchiolization) (very weak to weak)	4	6	1	7	22*	21*	46*	44*
multifocal histiocytosis (accumulation of intraalveolar macrophages) (very weak to weak)	32	39	31	34	47*	44	50*	50*
multifocal inflammatory mononuclear cell infiltrates (mainly very weak to weak)	7	2	2	3	9	8	37*	39*
lung-associated lymph nodes								
accumulation of foam cells (histiocytosis) (very weak to weak)	1	1	1	2	0	5	12*	26*

[a)] exposure of groups of 50 animals

*$p < 0.05$

5.2.1.2 Oxidized bitumen

A subacute toxicity study was carried out with repeated inhalation exposure of Wistar rats (Crl:WU) to the vapours and aerosols of a condensate (Parker et al. 2011). This was collected from the vapour phase from a storage tank for roofing asphalt (type III "built-up roofing asphalt", BURA) at a temperature of 201 °C using the method developed by Fraunhofer ITEM (Pohlmann et al. 2006 a, b; Preiss et al. 2006) (see above). The benzo[*a*]pyrene concentration of the condensate was 4.1 µg/g. The exposure system consisted of an evaporator to generate a defined exposure atmosphere of vapours and aerosols and special exposure units in which the animals were exposed to a flow of air from the generated atmosphere. The bitumen vapour and aerosol atmosphere was generated by heating the condensate. The

bitumen vapour and aerosol atmosphere was diluted with air to achieve the desired exposure concentration and then directed to the exposure units. The volumetric flow rate, temperature and humidity were monitored. The exposure units were made up of cylinders and 1 animal was placed in each cylinder in such a way that exposure was only via the nose. Regular analytical monitoring of the exposure atmosphere ensured that the concentration in the air remained constant and identical in each exposure unit. The exposure levels had been determined during a preliminary study (range-finding study with exposure to the vapours and aerosols of bitumen at concentrations of 100, 300 and 1000 mg/m^3). The groups were exposed to preset total hydrocarbon concentrations in the vapours and aerosols of bitumen of 30, 100 and 297 mg/m^3 (equivalent to about 35, 117 or 350 mg/m^3 related to bitumen condensate as the reference standard: converted according to the Fraunhofer ITEM method (ITEM 2017)). The exposure levels were monitored by collecting samples using a combination of glass fibre filter followed by an XAD adsorbent tube. The samples were analysed by IR spectroscopy and related to a reference standard that was not described further. The rats were exposed on 7 days a week for 6 hours a day. The control group and the 3 concentration groups were made up of groups of 12 male and 12 female rats. The male and female rats were exposed for 28 days. During the first 14 days of the study, body weight gains and feed consumption were reduced in the male rats of the high concentration group in comparison with the levels determined in the control group; the same findings were observed in male rats exposed to a total hydrocarbon concentration of 300 mg/m^3 and in all rats of the range-finding study at 1000 mg/m^3. As regards subacute toxicity, significant increases in the absolute and relative lung weights were found in the females of the high and medium concentration groups. A concentration-dependent increase in the absolute liver weights was determined in female rats (significant at a total hydrocarbon concentration of 297 mg/m^3). In the rats of the range-finding study, the reduction in thymus weights was statistically significant at a total hydrocarbon concentration of 1000 mg/m^3. The histopathological examination of the nasal cavity yielded contradictory findings: while the incidence of inflammatory cell infiltrates was reduced in the rats of the high concentration group, in the range-finding study, an increase was observed in all animals including those of the control group. Slight effects were found in the lungs of the rats of the high exposure group. These were described as a slightly increased accumulation of alveolar macrophages in combination with minimal mononuclear/inflammatory cell infiltrates and minimal to slight bronchiolar hyperplasia (alveolar bronchiolization); the effects were regarded as adaptive. On the basis of these findings, the authors derived a systemic NOAEC for the total hydrocarbon concentration of 100 mg/m^3 for male rats (increased absolute and relative lung weights, reduced feed consumption and body weight gains and slight effects in the lungs) and of 30 mg/m^3 for female rats (increased relative lung weights and slight effects in the lungs).

5.2.2 Summary

A number of studies investigated the effects of inhalation exposure of rats to vapours and aerosols generated from the condensates of emissions from the relevant types of bitumen.

5.2.2.1 Straight-run bitumen, air-rectified bitumen

In studies of subacute inhalation toxicity in rats, a large number of gene expression changes were observed in the lung cells that are, for example, linked with PAH metabolism (also after intratracheal instillation of bitumen condensates), the immune response or oxidative stress. There was a significant increase in the total cell count, the number of macrophages, neutrophilic granulocytes and lymphocytes in BAL fluids. Long-term exposure led to histopathological, non-neoplastic changes in the main nasal cavity and paranasal sinuses, lungs and associated lymph nodes; these are considered a sign of irritant effects induced by the vapours and aerosols of bitumen condensates. The NOAEC after long-term exposure was 6 mg/m^3, the LOAEC was 30.4 mg/m^3 (bitumen condensate standard).

5.2.2.2 Oxidized bitumen

Subacute inhalation exposure of rats to vapours and aerosols from the condensates of oxidized bitumen led to an increase in lung and liver weights and mononuclear/inflammatory cell infiltrates in the nose and lungs. The effects in the lungs, such as the accumulation of alveolar macrophages and bronchiolar hyperplasia, may be considered adaptive

effects. The NOAEC was 30 mg THC/m³ and the LOAEC was 100 mg/m³ (equivalent to 35 and 117 mg/m³ related to bitumen condensate as the reference standard: conversion according to the method developed by Fraunhofer ITEM) (ITEM 2017).

5.2.3 Oral administration

There are no new data available.

5.2.4 Dermal application

There are no new data available.

5.2.5 Intratracheal instillation

In a study with male Sprague Dawley rats (Hla:(SD)CVF), bitumen condensate was administered by intratracheal instillation at dose levels of 0, 0.45, 2.22 or 8.88 mg/kg body weight on 3 consecutive days (Ma et al. 2002). The condensate was generated from the vapours and aerosols of paving grade bitumen PG 64-22 heated to a temperature of 160 °C. An examination of lung microsomes revealed a significant, dose-dependent increase in the activity of CYP1A1, but not of CYP2B1. An increase in the formation of micronuclei in the polychromatic erythrocytes of the bone marrow was determined in rats of the high dose group (see Section 5.6.2), which the authors attributed to changes in CYP metabolism.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

There are no new data available.

5.3.2 Eyes

There are no new data available.

5.4 Allergenic effects

There are no new data available.

5.5 Reproductive and developmental toxicity

Oxidized bitumen

The study of Parker et al. (2011) described in Section 5.2.1.2 investigated also reproductive and developmental toxicity. Groups of 12 female and 12 male rats were first exposed separately to a built-up roofing asphalt condensate (BURA) containing THC of 0, 30, 100 and 297 mg/m³ (equivalent to 0, 35, 117 or 350 mg/m³ related to bitumen condensate as the reference standard: conversion according to the method developed by Fraunhofer ITEM (ITEM 2017)) for 6 hours a day, on 7 days a week. They were subsequently mated under the same exposure conditions. Pregnant female rats were exposed up to gestation day 20; male rats were exposed for another 21 days. The exposure period lasted between 35 and 42 days. The fertility of the male parent animals was not impaired; all matings occurred within the first 8 days. The examination of the reproductive organs (not described in more detail) did not provide evidence of adverse histopathological changes. With increasing concentration, a continuous trend towards lower sperm counts (0 mg/m³: 38 125 million/epididymis; 297 mg THC/m³: 24 542 million/epididymis) was observed, which the authors did not interpret as significant after taking the data from historical controls into account. Significant changes to sperm motility and in the

percentage of abnormal sperm that could be associated with exposure to BURA were not observed in the individual exposure groups. Overall, the authors found that the NOAEC for male fertility was 297 mg THC/m^3. In the female parent animals, the only effects observed were reduced body weight gains and feed consumption during gestation; these effects were observed only in the high exposure group (297 mg/m^3). These effects were not observed in the 2-week exposure period prior to mating. In summary, the authors of the study did not find that exposure to BURA induced toxic effects on reproduction or development (including the number of pregnant animals, offspring, corpus luteum, implantation sites and post-implantation losses, or on mating or fertility indices, the length of gestation and the sex ratio in the offspring). Overall, the authors determined a NOAEC of 297 mg/m^3 for female fertility.

5.6 Genotoxicity

5.6.1 In vitro

5.6.1.1 Bacteria

Bitumen extracts and the aerosols and vapours from different sources were investigated in Salmonella mutagenicity tests (see Table 11).

Direct comparison of straight-run/air-rectified bitumen and oxidized bitumen

Studies investigated the effects of oxidation during the production of oxidized bitumen on its mutagenic potential. Dimethyl sulfoxide (DMSO) extracts from samples of bitumen made from different crude oils were used as test materials; air was injected to convert the extracts into products with different softening points and penetration indices similar to those used in paving and roofing applications. The products were analysed using the Salmonella mutagenicity test carried out according to ASTM Standard Method E1687 (ASTM: American Society for Testing and Materials). The mutagenicity index (MI) was 41% to 50% lower than that of the input bitumen. The authors attributed the weaker mutagenic properties of oxidized bitumen to the lower PAH levels (39%–71%) determined in comparison with the levels in the input samples (Trumbore et al. 2011).

Modified Salmonella mutagenicity tests carried out according to ASTM Standard Method E1687-04 were performed with condensates of the vapours and aerosols of 4 different types of paving grade bitumen (straight-run bitumen PG 64-22, 147 °C) and 4 types of roofing bitumen (type III, 201 °C bitumen). The bitumen samples were collected using the Fraunhofer method after heating in tanks (Pohlmann et al. 2006 b). Tests were also carried out with an additional condensate sample generated in the laboratory from 1 of the oxidized roofing bitumens at a temperature of 232 °C according to the method of Sivak et al. (1989). The mutagenicity indices of the 4 paving grade bitumen samples were below 1, those of the 4 samples of roofing bitumen were between 0.9 and 1.6. In comparison with the corresponding sample from the storage tank (MI: 1.2), the mutagenicity index of the laboratory sample of roofing bitumen was markedly higher at 3.3. The sample of roofing bitumen generated at a higher temperature (232 °C) in the laboratory was thus more highly mutagenic than the sample of the same material collected from the storage tank at 201 °C (Kriech et al. 2007).

Straight-run bitumen, air-rectified bitumen

In a Salmonella mutagenicity test with the strain TA98 and activation using hamster enzymes, DMSO extracts of "Asphalt Cement 20" (AC-20, asphalt concrete, CAS number 8052-42-4) and "Coastal Residuum" (CR, CAS number 64741-56-6) were tested at concentrations up to the toxicity threshold. Exposure to the AC-20 samples at various concentrations did not result in an increase in revertants; the mutagenic effects induced by exposure to CR were relatively slight in comparison with those induced by the positive control ("heavy catalytically cracked gas oil" containing a high level of PAHs, CAS number 64741-62-4) (average number of revertants with the negative control: 22.7; number of revertants at the highest concentration tested of 35 µl/plate: 42.7; number of revertants with the positive control: 291.7) (Goyak et al. 2011).

Possible toxic effects of emissions that may be generated by recycled asphalt were investigated using samples of bitumen emissions produced in the laboratory and collected from the workplace. The laboratory samples were collected on Teflon filters (aerosols) followed by an XAD-2 adsorbent tube (vapours); only Teflon filters were used to collect the samples from the workplace. Following extraction and elution with DMSO, the samples were tested in the Salmonella mutagenicity test at 5 different concentrations. Although the laboratory samples were available in sufficient quantities to perform the tests with Salmonella strains TA98 and YG1024 both with and without the addition of metabolic activation by an S9 fraction from rat liver, only some of the workplace samples were available in sufficient amounts to be tested in the strains both with and without metabolic activation. The laboratory samples were generated from type B120 bitumen, B80 bitumen with coal fly ash and B120 bitumen containing waste plastics, all of which were heated to about 170 °C. The workplace samples were collected in the breathing zone of the workers during the laying (stone mastic asphalt (SMA) samples, SMA with B80 bitumen, with lime or with coal fly ash as a filler) and renewal (by remixing (REM)) of asphalt surfaces (REM-SMA samples, remixing of SMA with lime or with coal fly ash as a filler; REM-AC samples, remixing of asphalt concrete (AC)). All laboratory vapour samples yielded negative results in the Salmonella mutagenicity test; by contrast, positive results were obtained when the aerosol fractions were tested with the test strain TA98 in the presence of metabolic activation, and when the samples with plastic components were tested with the strains TA98 and YG1024 in the absence of metabolic activation. All workplace samples yielded positive results with and without the addition of metabolic activation. The additive coal fly ash did not lead to increased mutagenicity in tests. The REM-AC fraction had a very high mutagenicity index value in tests with the Salmonella strain TA98 and metabolic activation (Heikkilä et al. 2003).

Other studies also investigated the potential effects of recycled additives on mutagenicity in bacteria using laboratory and workplace samples of bitumen emissions. Vapours and aerosols were generated in the laboratory by heating SMA containing B80 bitumen and SMA-WPT (SMA modified with waste plastic and tall oil pitch: 70% B200 bitumen, 12% waste plastic (90% polyethylene, 10% polypropylene), 18% tall oil pitch). Samples were collected on Teflon filters at a temperature of 150 °C. The extraction of 3 pooled filters in each case was carried out with 200 µl DMSO and 10 ml dichloromethane in an ultrasonic bath. The dichloromethane was then evaporated from 2 combined extracts. The workplace samples were collected in the breathing zone of the workers during paving operations (laying temperatures between 154 °C and 165 °C) with AC and AC-WPT (asphalt concrete modified with waste plastic and tall oil pitch) and the application of SMA (consisting of 0.6% natural asphalt) and SMA-WPT. The samples were treated using the same method as the laboratory samples. The laboratory SMA and SMA-WPT samples did not induce a significant increase in the number of revertants when tested using the Salmonella mutagenicity test with the strains TA98 and YG1024 both in the presence and in the absence of metabolic activation (rat liver S9 fraction). No mutagenic activity was determined with the workplace samples of AC and AC-WPT. The SMA samples from the workplace were tested only with the TA98 strain and with metabolic activation because of an insufficient amount of sample material. These tests also yielded negative results (Lindberg et al. 2008).

5.6.1.2 Mammalian cells (see also Table 11)

Straight-run bitumen/air-rectified bitumen

The laboratory samples of bitumen emissions from SMA and SMA-WPT and workplace samples from SMA, SMA-WPT, AC and AC-WPT were not only analysed using the Salmonella mutagenicity test (see Section 5.6.1.1) but also with the comet assay and micronucleus test in mammalian cells (Lindberg et al. 2008). Cultured human bronchial epithelial cells (BEAS 2B) were incubated with each sample for 6 hours at 6 different concentrations (1.25–40 µg/ml in the absence of metabolic activation; 5–80 µg/ml in the presence of metabolic activation). Following the comet assay, a special computer programme was used to count damaged and undamaged nuclei. DNA damage was detected only in the laboratory-generated SMA-WPT samples (stone mastic asphalt with waste plastic and tall oil pitch) in the absence of metabolic activation. In a micronucleus test carried out to investigate damage to chromosomes and the spindle apparatus, cells were exposed to each sample for 6 hours at 8 different concentration levels in the presence of S9 activation (1.25–80 µg/ml) or 6 different concentrations in the absence of metabolic activation (1.25–40 µg/ml). The samples

were analysed by light microscope after the addition of cytochalasin B to inhibit cell division, 48 hours of additional culturing and subsequent staining of only binucleated cells that had not undergone complete cell division. A significant increase ($p = 0.002$, Fisher's exact test) in the formation of micronuclei was induced by the laboratory-generated SMA samples (stone mastic asphalt with lime or coal fly ash) at the highest concentration tested of 40 µg/ml and in the absence of metabolic activation. Increased micronuclei formation was not observed in the SMA-WPT samples in the absence of metabolic activation; however, toxicity was clearly higher in these samples than in the SMA samples and samples with concentrations above 10 µg/ml could no longer be analysed. Increased micronuclei formation was not observed in samples of either SMA or SMA-WPT with metabolic activation. The SMA samples collected from the workplace caused a significant increase in the frequency of micronuclei ($p = 0.047$, Fisher's exact test) at 20 µg/ml and in the absence of metabolic activation. The next-higher concentration of 40 µg/ml was again so toxic that the number of cells was too small to be counted. The SMA-WPT samples from the workplace induced a statistically significant increase in the frequency of micronuclei (10, 20 and 40 µg/ml; $p = 0.006$, 0.014, 0.026, Fisher's exact test) at 3 different concentrations without metabolic activation, but the increase was not dependent on the concentration. In the presence of metabolic activation, no significant effects were induced by SMA or SMA-WPT samples from the workplace. A detectable level of genotoxic changes was not found in either unmodified (AC) or modified (AC-WPT) samples of asphalt concrete (Lindberg et al. 2008).

Bitumens without further characterization

Human osteosarcoma cells (HOS) were treated in vitro with bitumen extract at different concentrations (25, 50 and 100 µl/ml culture medium, no concentration data, 3 ml culture medium/dish). The test substance was generated by extracting 5 g of bitumen with 200 ml of dichloromethane. The sample was freeze-dried and dissolved in 5 ml of dichloromethane and then used for in vitro and in vivo assays. No data were provided for the source of the bitumen tested. After the end of the exposure period of 12 days, 90% of the treated cells had died; about 50% died within the first 96 hours. Additional toxicity data were not provided for the different quantities applied. No data were given for the percentage cell death in the untreated cells. After 3 exposures to bitumen on days 3, 7 and 10 after culturing with 25 and 50 µl/ml, respectively, and further culturing of the cells, the surviving proliferating cells exhibited characteristics of tumour cells such as growth in soft agar and proliferation in several layers. After incubation for 3 weeks, proliferating cells were no longer observed in the cell culture treated with 100 µl/ml and the sample was not tested further. Proliferation was normal in parental cells. A comparison of the karyotypes revealed 47 chromosomes in the isolated transformed clones of the treated cells because of trisomy in chromosome 8; the untreated cells had 46 chromosomes. Two-dimensional gel electrophoresis and mass spectrometric analysis revealed changes in the proteomic profiles in comparison with those of the control group. Both the increased and reduced expression of specific proteins with a known potential for transforming or tumour-promoting properties was observed. Dorsal injection of the transformed cells in mice with 6×10^6 cells did not induce tumours either in normal mice or in mice with immunodeficiency (SCID) within 120 days of injection. SCID mice injected with the positive control (KHOS cells of a known tumourigenic cell line) developed primary tumours within 2 weeks of injection (Dhondge et al. 2012).

5.6.2 In vivo

The studies of genotoxicity in vivo that were published since the 2001 documentation (Greim 2002) are shown in Table 12.

5.6.2.1 Oxidized bitumen

A subacute toxicity study investigated the bone marrow from the thigh bones of Wistar rats by means of the micronucleus test. Groups of 5 male and 5 female rats were randomly selected for the concentration groups. The high exposure group was exposed to vapours and aerosols of bitumen with a total hydrocarbon concentration of 297 mg/m^3 (IR spectroscopic analysis related to an unnamed standard) for 6 hours a day, on 7 days a week, for a total of 28 days. An additional positive control group of 5 male and 5 female rats was given an oral dose of cyclophosphamide dissolved

Tab. 11 Genotoxicity of bitumen in vitro

Test system	Test sample	Concentration	Effective conc.	Cytotoxicity	Results		Comments	References
					–m. a.	+m. a.		
Bacteria								
Direct comparison of straight run/air-rectified bitumen with oxidized bitumen								
gene mutations								
ASTM Standard Method E 1687	"made from diverse asphalt crude oil sources widely used for oxidized roofing products": extracts: 4 different bitumen samples before and after oxidation (1–4, 3 and 4 with catalyst), extraction with DMSO	12–60 µl/plate	no data	no data	n. t.	before oxidation: + after oxidation: – (1) MI 1.3; $MI_{oxid.}$ 0.66 (decreased by 49.2%) (2) MI 1; $MI_{oxid.}$ 0.59 (decreased by 41%) (3) MI 0.36; $MI_{oxid.}$ 0.18 (decreased by 50%) (4) MI 0.25; $MI_{oxid.}$ 0.13 (decreased by 48%)	mutagenicity significantly ↓ after oxidation (41%–50%) significant difference in absolute values between samples 1 and 2 and samples 3 and 4; samples 3 and 4 markedly higher flash points; data for PAH fractions with 4–6 rings (fluorescence method) determined in the samples: fluorescence significantly ↓ after oxidation in the laboratory (39%–71%)	Trumbore et al. 2011
ASTM Standard Method E 1687-04	"bitumen fumes" – filter extract storage tank samples: 4 different "paving bitumens" (collected at an average temperature of 147 °C, TP-A–TP-D) and 4 different "roofing bitumens" (collected at an average temperature of 201 °C, TR-A–TR-D) above the opening of the respective storage tank using the Fraunhofer ITEM method (Pohlmann et al. 2006 a, b) laboratory sample: "laboratory fume generation" with oxidized bitumen at 232 °C according to Sivak et al. (1989) (LR-A)	no data	no data	no data	n. t.	storage tank samples "paving bitumens": – storage tank samples TP-A–TP-D: + m. a., MI: TP-A 0.89 TP-B 0.98 TP-C 1.0 TP-D 0.69 storage tank samples "roofing bitumens": – and + storage tank samples TR-A–TR-D: + m. a., MI: TR-A 1.2 TR-B 1.6 TR-C 1.0 TR-D 0.9 laboratory sample LR-A: + MI in comparison with TR-A/LR-A: 1.2/3.3 laboratory sample: + m. a., MI: LR-A 3.3	benzo[*a*]pyrene concentration [mg/m³]: storage tank samples TP-A–TP-D: TP-A < 0.08 TP-B < 0.08 TP-C 0.55 TP-D < 0.08 storage tank samples TR-A–TR-D: TR-A 3.1 TR-B 7 TR-C 2.6 TR-D 1.2 laboratory sample: LR-A 5.8	Kriech et al. 2007

Tab. 11 *(continued)*

Test system	Test sample	Concentration	Effective conc.	Cytotoxicity	Results		Comments	References
					–m.a.	+m.a.		
Straight run/air-rectified bitumen								
gene mutations								
Salmonella typhimurium TA98	extracts (extraction with DMSO, dilution series with DMSO): (1) AC-20 asphalt (CAS number 8052-42-4) (2) Coastal Residuum (CAS number 64741-56-6)	(1) 0.005–1 µl/plate (2) 1–50 µl/plate		(1) 1 µl/plate (2) ≥ 25 µl/plate	n.t.	– (1) – (average number of revertants at 1 µl/plate: 33.3; negative control: 33.7; positive control: 327.8) (2) – (average number of revertants at the highest concentration tested of 35 µl/plate: 42.7; negative control: 22.7; positive control: 291.7)	no concentration data, activation with "hamster metabolic enzymes"	Goyak et al. 2011
Salmonella typhimurium TA98, YG1024 (mutagenicity test for nitroaromatics contained in diesel exhaust fumes)	C_2Cl_4 filter extracts laboratory samples generated at 170–180 °C: (1) Bitumen BI20 ($BI20_{vapour}$, $BI20_{particles}$) (2) bitumen mix with "waste plastic" ($BI20\text{-}WP_{vapour}$, $BI20\text{-}WP_{particles}$) (3) bitumen mix with "pulverized coal fly ash" ($B80\text{-}CFA_{vapour}$, $B80\text{-}CFA_{particles}$) workplace samples (paved asphalt 160–210 °C, remixed asphalt 150–350 °C) (4) stone mastic asphalt + lime (10%) ($SMA\text{-}L_{particles}$) (5) stone mastic asphalt + coal fly ash (10%) ($SMA\text{-}CFA_{particles}$) (6) remixing of SMA + lime (10%) ($REM\text{-}SMA\text{-}L_{particles}$) (7) remixing of SMA + coal fly ash (10%) ($REM\text{-}SMA\text{-}CFA_{particles}$) (8) remixing of asphalt cement ($REM\text{-}AC_{particles}$)	0.031–0.5 mg/plate	no data	no data	vapour fraction: – particle fractions: + SMA and REM: + laboratory samples: vapour: BI20: – B80-CFA: – BI20-WP: – particles: BI20: – B80-CFA: weak + (TA98) BI20-WP: + (TA98, YG1024) workplace samples: particles: SMA-L: + (TA98, YG1024) SMA-CFA: + (TA98, YG1024) REM-SMA-L: + (TA98, YG1024) REM-SMA-CFA: + (TA98, YG1024) REM-AC: n.t.	vapour fraction: – particle fractions: + SMA and REM: + laboratory samples: vapour: BI20: – B80-CFA: – BI20-WP: – particles: BI20: + (TA98) B80-CFA: + (TA98, YG1024) BI20-WP: + (TA98) workplace samples: particles: SMA-L: + (TA98, YG1024 n.t.) SMA-CFA: + (TA98, YG1024 n.t.) REM-SMA-L: + (TA98, YG1024) REM-SMA-CFA: + (TA98, YG1024 n.t.) REM-AC: ++ (TA98)	data for PAH fractions determined in the samples	Heikkilä et al. 2003

Tab. 11 *(continued)*

Test system	Test sample	Concentration	Effective conc.	Cytotoxicity	Results		Comments	References
					–m.a.	+m.a.		
Salmonella typhimurium TA98, YG1024	"fume" condensates generated in the laboratory (about 150 °C): (1) SMA (6.5% Bitumen B80) (2) SMA-WPT (6.5% bitumen (70% Bitumen 200, 18% tall oil pitch, 12% plastic)) at the workplace (145–165 °C): (3) AC (5.3% Bitumen B80) (4) AC-WPT (5.3% bitumen (70% Bitumen 200, 18% tall oil pitch, 12% plastic)) (5) SMA (5.9% bitumen (B80), 0.6% natural asphalt)	0.03–0.5 mg/plate		no data	laboratory: SMA: – (TA98, YG1024) SMA-WPT: – (TA98, YG1024) workplace: AC and AC-WPT: – (TA98, YG1024)	laboratory SMA: – (TA98, YG1024) SMA-WPT: – (TA98, YG1024) workplace: AC and AC-WPT: – (TA98, YG1024) SMA: – (TA98)	testing could not be performed because sample quantities too small: workplace SMA, TA98 –m.a. workplace SMA, YG1024 +/–m.a. workplace SMA-WPT with both strains (results of the determinations of exposure or analysis of emissions, (Väänänen et al. 2006): inhalable particles: 0.05–0.29 mg/m³ bitumen "fumes": 0.05–0.29 mg/m³ bitumen "vapour": 0.4–1.9 mg/m³ PAH: 0.5–3.5 µg/m³ benzo[*a*]pyrene: < 0.01 µg/m³)	Lindberg et al. 2008
Mammalian cells								
Straight-run/air-rectified bitumen								
comet assay, indicator test								
BEAS-2B cells (human bronchial epithelial cells)	Bitumen B80 and B200 test of mixtures generated in the laboratory with the bitumens above: (1) SMA (2) SMA-WPT at the workplace: (3) AC (4) AC-WPT (5) SMA (6) SMA-WPT	–m.a.: 1.25–40 µg/ml +m.a.: 5–80 µg/ml	no data	laboratory: SMA at 10 µg/ml workplace: SMA 40 µg/ml	laboratory: SMA-WPT: + SMA: – workplace: –	laboratory: SMA-WPT: – SMA: – workplace: –		Lindberg et al. 2008

Tab. 11 *(continued)*

Test system	Test sample	Concentration	Effective conc.	Cytotoxicity	Results		Comments	References
					–m.a.	+m.a.		
micronucleus test								
BEAS-2B cells	Bitumen B80 and B200 test of mixtures generated in the laboratory with the bitumens above: (1) SMA (2) SMA-WPT at the workplace: (3) AC (4) AC-WPT (5) SMA (6) SMA-WPT	–m.a.: 1.25–40 µg/ml +m.a.: 1.25–80 µg/ml	no data	laboratory: SMA-WPT at >10 µg/ml workplace: SMA 40 µg/ml	laboratory: SMA: + SMA-WPT: – workplace: SMA: + SMA-WPT: + AC and AC-WPT: –	laboratory: SMA: – SMA-WPT: – workplace: SMA: – SMA-WPT: – AC and AC-WPT: –		Lindberg et al. 2008
Bitumens without further characterization								
chromosomal aberrations, aneuploidy								
HOS cells (human osteosarcoma cells)	bitumen extract (no additional data)	0, 25, 50, 100 µl/ml	25, 50 µl/ml	90% cytotoxicity after 12 days, 50% within the first 96 hours	changes in proteomic profile, characteristics of transformed cells, trisomy 8	n.t.	no tumour formation after injection of treated cells in mice	Dhondge et al. 2012

AC: asphalt cement; CFA: coal fly ash; L: lime; –m.a./+m.a.: without a metabolic activation system/with a metabolic activation system; MI: mutagenicity index; n.t.: not tested; PI3K: phosphatidylinositol 3-kinase; REM: remixing; SMA: stone mastic asphalt; WP: waste plastic; WPT: waste plastic tall oil pitch; –: negative; +: positive; ++: markedly positive

in water at a dose level of 60 mg/kg body weight for 24 hours prior to examination. Similar results were obtained in the micronucleus test for the exposure groups and the control group. The group with exposure to cyclophosphamide yielded positive results. In a range-finding study, the formation of blood cells in the bone marrow of female rats was impaired at 1000 mg/m^3 (Parker et al. 2011).

5.6.2.2 Straight-run/air-rectified bitumen

Groups of 3 rats (Sprague Dawley BD6; no data for sex) were exposed nose-only for 6 hours a day, on 5 consecutive days, to vapours and aerosols with TPM concentrations of 5 mg/m^3 and 50 mg/m^3 that were produced by heating a 45/60 bitumen of Venezuelan origin to 200 °C and with a TPM concentration of 5 mg/m^3 that were produced by heating coal tar to 110 °C. The vapour and aerosol condensates contained benzo[*a*]pyrene at concentrations of 3.2 mg/kg in the 5 mg/m^3 bitumen group, 12 mg/kg in the 50 mg/m^3 bitumen group and 2.6 mg/kg in the coal tar group. The sum of the analysed PAHs with 4–6 rings was 66 mg/kg, 2103 mg/kg and 31 mg/kg, respectively. An additional rat per group was used as the negative control. No unusual findings were reported after exposure to TPM concentrations of 5 mg/m^3 from emissions from both bitumen and coal tar; the authors attributed this to insufficient PAH levels in the emissions. For this reason, an additional experiment was carried out with 3 rats at a TPM concentration of 50 mg/m^3 (bitumen emissions); evidence of a DNA adduct (no other data) in the lungs was revealed by ^{32}P-postlabelling. In the same publication, the authors described a skin painting test with the application of a 45/60 bitumen condensate in a single rat (Sprague Dawley BD6). ^{32}P-Postlabelling revealed evidence of similar DNA adducts in the skin and lungs (Genevois-Charmeau et al. 2001).

Earlier studies carried out by the same research group (Genevois et al. 1996) with dermal application observed these DNA adducts in the same tissues of BD4 rats.

The mutagenic effects induced by vapours and aerosols generated by heating straight-run bitumen/air-rectified bitumen (50/70, Venezuela, CAS number 8052-42-4) to 170 °C were investigated in the lungs of male transgenic Big Blue® rats. Twelve rats (and 12 controls) were exposed nose-only to a total particulate matter concentration of 112 ± 13 mg/m^3 for 6 hours a day on 5 consecutive days. Six rats from the exposed group and 6 rats from the control group were examined 3 and 30 days after the last exposure. There were no signs of inflammatory responses in the lungs. A DNA adduct was identified in the lung tissues by ^{32}P-postlabelling both 3 and 30 days after the end of exposure (Bottin et al. 2006; Gate et al. 2007); the adduct was similar to the one determined by an earlier study with topical application of the same bituminous substance (Genevois-Charmeau et al. 2001). However, it differed from the adducts found during a round robin test with standard DNA samples and benzo[*a*]pyrene (Phillips and Castegnaro 1999).

Rats were exposed by inhalation to the vapours and aerosols of bitumen for 5 days, 1 month and 12 months. The study was carried out in the same laboratories and under the same conditions as the study of Fuhst et al. (2007) (for the study design, see Section 5.2.1.1). The total hydrocarbon concentrations in the air were 0, 4.1, 20.7 or 103.9 mg/m^3 (related to the mineral oil standard, equivalent to 0, 6.0, 30.4 or 152.6 mg/m^3 related to the bitumen condensate standard). The groups of 24 male and 24 female SPF Wistar rats (Crl:WI(WU)BR) of the control group and the exposed groups were exposed for 6 hours a day. The analysis of the urine samples for PAHs and their metabolites revealed increasing concentrations of naphthols, phenanthrene, "premercapturic acid" from phenanthrene, 1-hydroxyphenanthrene and phenanthrene-1,2-dihydrodiol in male and female rats of the medium and high concentration groups over the duration of the study. DNA adducts that formed dependent on time and concentration in the lungs and in the nasal and alveolar epithelium were identified by ^{32}P-postlabelling; the highest adduct levels were detected in the nasal epithelium (Halter et al. 2007).

In a study carried out to identify DNA adducts, 32 female B6C3F1 mice were exposed for 4 hours a day, on 10 consecutive days, to vapours and aerosols generated by heating PG 64-22 paving grade bitumen to between 150 °C and 170 °C (representative for the midwestern United States). The concentrations of paving grade bitumen in the air were between 152 mg/m^3 and 198 mg/m^3. Samples were collected by PTFE filter and then fed through an XAD-2 adsorbent tube; the samples were analysed by GC/MS calibrated with 16 reference PAHs against an internal standard. Three different DNA adducts of benzo[*a*]pyrene could be identified in the lung tissues: N^2-deoxyguanosinebenzo[*a*]pyrene-7,8-dihydrodiol-

9,10-epoxide, N^6-deoxyadenosinebenzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide and N^4-deoxycytidinebenzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (Wang et al. 2003 b).

Groups of 5 male transgenic Big Blue® mice (C57BL/6[LIZ]) were exposed nose-only for 6 hours a day, on 5 consecutive days, to TPM concentrations of 0 or 98.7 ± 6.1 mg/m³ produced by heating paving grade bitumen (50/70 pen, Venezuela, CAS number8052-42-4) to 170 °C. The benzo[*a*]pyrene concentration was 198 ± 50 ng/m³. The animals were examined 30 days after the end of exposure and the DNA was extracted from the lungs and examined for DNA adducts. No differences were found in comparison with the findings from the control group (Micillino et al. 2002).

As described above (Halter et al. 2007), groups of 24 male and 24 female SPF Wistar rats (Crl:WI(WU)BR) were exposed to total hydrocarbon concentrations of 0, 4.1, 20.7 or 103.9 mg/m³ (related to the mineral oil standard, equivalent to 0, 6.0, 30.4 or 152.6 mg/m³ related to the bitumen condensate standard) for 6 hours a day. The levels of 8-oxo-dGuo adducts in the nasal, lung and alveolar epithelium and in the peripheral blood were not increased.

As described by Wang et al. (2001), female Sprague Dawley rats (Hla:(SD)CVF; number not specified) were exposed to the vapours and aerosols generated by heating a paving grade bitumen to 150 °C; the bitumen was not characterized further. The rats were exposed either once for 1 hour or once for 6 hours (cumulative doses: 53 mg × hour/m³ or 353 mg × hour/m³, respectively) or on 5 days for 6 hours a day (cumulative doses: 641 mg × hour/m³ or 1150 mg × hour/m³) (Zhao et al. 2004). The examination of the alveolar macrophages from these animals by comet assay revealed dose-dependent DNA damage caused by exposure to bitumen. Significant levels of DNA damage were found even after a single exposure lasting 6 hours or after exposure for 5 days (641 mg × h/m³). In the latter dose group, the comet assay revealed also DNA damage in the lung tissue.

Groups of 5 male transgenic Big Blue® mice (C57BL/6[LIZ]) were exposed nose-only for 6 hours a day, on 5 consecutive days, to vapours and aerosols with TPM concentrations of 0 or 98.7 ± 6.1 mg/m³ that were generated by heating paving grade bitumen (50/70 pen, Venezuela, CAS number 8052-42-4) to 170 °C. The benzo[*a*]pyrene concentration was 198 ± 50 ng/m³. The animals were examined 30 days after the end of exposure and DNA was extracted from the lungs and examined for mutation frequencies at the gene loci cII and lacI. No differences were found in comparison with the findings from the control group (Micillino et al. 2002).

As described above, the mutagenic effects of exposure to vapours and aerosols generated by heating paving grade bitumen (50/70, Venezuela, CAS number 8052-42-4) to 170 °C was investigated in the lungs of male transgenic Big Blue® rats (Bottin et al. 2006; Gate et al. 2007). A modification of the mutation spectrum in the "neutral reporter gene cII" of the transgenic rat genome was expressed by an increase in G:C to T:A and A:T to C:G transversions, but was not significant.

As described above (Halter et al. 2007), groups of 24 male and 24 female SPF Wistar rats (Crl:WI(WU)BR) were exposed to total hydrocarbon concentrations of 0, 4.1, 20.7 or 103.9 mg/m³ (related to the mineral oil standard, equivalent to 0, 6.0, 30.4 or 152.6 mg/m³ related to the bitumen condensate standard) for 6 hours a day. The number of micronuclei in the erythrocytes or polychromatic erythrocytes was not found to be increased, but the number of red blood cells in the bone marrow was slightly reduced in 4 of 6 animals of the high concentration group after 12 months.

As described by Wang et al. (2001), female Sprague Dawley rats (Hla:(SD)CVF) (number not specified) were exposed for 6 hours a day, on 5 days, to the vapours and aerosols generated by heating a paving grade bitumen to 150 °C (cumulative dose: 1734 mg × hour/m³) (Zhao et al. 2004). The bitumen was not characterized further. The induction of micronuclei was not found in the polychromatic erythrocytes extracted from the bone marrow samples from these animals. The intratracheal application of a condensate of a paving grade bitumen (PG 64-22; 160 °C) in Sprague Dawley rats at dose levels of 0, 0.45, 2.22 or 8.88 mg/kg body weight and day for 3 days resulted in the induction of micronuclei in the polychromatic erythrocytes of the bone marrow (Ma et al. 2002). Cytotoxicity was determined at doses of 8.88 mg/kg body weight and day and above on the basis of a decrease in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE).

5.6.2.3 Other studies

As described in Wang et al. (2001), female Sprague Dawley rats (Hla:(SD)CVF) (number not specified) were exposed for 6 hours a day, on 5 days, to the vapours and aerosols generated by heating a paving grade bitumen to 150 °C (cumulative doses: 641 mg × hour/m^3 and 1150 mg × hour/m^3). The bitumen was not characterized further (Zhao et al. 2004). A mutagenicity test with Salmonella typhimurium (YG1024 and Y1029) was carried out to examine the mutagenic activity of 2-aminoanthracene and benzo[*a*]pyrene in a microsomal fraction extracted from the lung cells of exposed rats. The increase in the mutagenic activity of 2-aminoanthracene was statistically significant after metabolic activation by the lung microsomes of the rats of the high dose group; no such increase was found in the mutagenic activity of benzo[*a*]pyrene.

As described above (Halter et al. 2007), groups of 24 male and 24 female SPF Wistar rats (Crl:WI(WU)BR) were exposed to total hydrocarbon concentrations of 0, 4.1, 20.7 or 103.9 mg/m^3 (related to the mineral oil standard, equivalent to 0, 6.0, 30.4 or 152.6 mg/m^3 related to the bitumen condensate standard) for 6 hours a day. Changes in the expression of genes associated with respiratory disorders were investigated in extracts from the lungs, the nasal epithelium and leukocytes by reverse transcriptase polymerase chain reactions. A concentration-dependent increase in the expression of CYP1A1 and CYP2G1 (nasal and lung tissues) was observed and also changes in the expression of genes in the nasal tissue of male rats (cathepsin K and D, cadherin 22, platelet activating factor acetylhydrolase isoform 1b alpha subunit, and the regulator of signal transduction 4 by G-protein) that were not dependent on the concentration but could potentially induce adverse health effects.

Tab. 12 Genotoxicity of bitumen in vivo

End point	Test system	Test sample, temperature	Course of exposure	Results		Comments	References
				Comment			
Oxidized bitumen (roofing)							
micronuclei, PCE, bone marrow	Wistar rats	"BURA fume condensate" from type III "built-up roofing asphalt" at 201 °C (bitumen condensate)	nose-only exposure, 0, 30, 100, 300 mg THC/m^3 6 hours/day, 7 days/week, 28 days	no significant differences	–	range-finding study: after 14 days, significant suppression of erythrocyte formation in ♀ at 1000 mg/m^3	Parker et al. 2011
Straight-run/air-rectified bitumen (paving)							
DNA adducts lungs, liver, kidneys and lymphocytes; ^{32}P-postlabelling	Sprague Dawley BD6 rats	bitumen condensate from 45/60 bitumen (Venezuela) 200 °C	nose-only exposure, 0, 5, 50 mg/m^3 6 hours/day, 5 days	DNA adduct (no other data) in the lung in 3/3 rats at 50 mg/m^3	+	DNA adduct with the same chromatographic properties as found in skin painting studies (Genevois et al. 1996; Genevois-Charmeau et al. 2001)	Genevois-Charmeau et al. 2001
DNA adducts lungs; ^{32}P-postlabelling	transgenic Big Blue® rats	"50/70 pen batch bitumen" (CAS number 8052-42-4) of Venezuelan origin "bitumen fume" 170 °C	nose-only exposure, 0, 112 ± 13 mg TPM/m^3 4.6 µm Ø 6 hours/day, 5 days	detectable DNA adducts both 3 and 30 days after exposure, transversions	+	no increase in inflammatory parameters; bitumen-specific DNA adduct: similar to that which developed after dermal exposure in rats (Genevois-Charmeau et al. 2001), but not identical with benzo[*a*]pyrene–DNA adduct	Bottin et al. 2006; Gate et al. 2007

Tab. 12 *(continued)*

End point	Test system	Test sample, temperature	Course of exposure	Results		Comments	References
				Comment			
DNA adducts nasal, lung and alveolar epithelium, peripheral blood; ^{32}P-postlabelling	SPF Wistar rats, groups of 24 ♂ and 24 ♀	condensate from bitumen 50/70 (B65) 170 °C (Preiss et al. 2006) as in Fuhst et al. (2007)	nose-only exposure, 0, 4, 20, 100 mg THC/m^3 equivalent to 0, 6.0, 30.4 or 152.6 mg/m^3 related to the bitumen condensate standard, 6 hours/day, 5 days/week, 5 days, 1 month, 12 months	significant increase in the number of DNA adducts in the nasal, lung and alveolar epithelium	+		Halter et al. 2007
				no adducts in white blood cells	–		
DNA adducts lungs; GC/MS and Q-TOF-MS and ^{32}P-postlabelling	B6C3F1 mice, ♀	vapours and aerosols from PG 64–22 "paving" bitumen 150 °C–170 °C	whole-body exposure, 0, 152–198 mg/m^3, 4 hours/day, 10 days	3 different DNA adducts of benzo[*a*]pyrene	+		Wang et al. 2003 a
DNA adducts lungs; ^{32}P-postlabelling	transgenic Big Blue® mice, ♂	vapours and aerosols from "paving" bitumen 50/70 (CAS number 8052-42-4) of Venezuelan origin 170 °C	nose-only exposure, 0, 100 mg/m^3, 6 hours/day, 5 days, examination 30 days after exposure	no differences in adducts	–		Micillino et al. 2002
8-oxo-dGuo adducts nasal, lung and alveolar epithelium, peripheral blood	SPF Wistar rats, groups of 24 ♂ and 24 ♀	condensate from bitumen 50/70 (B65) 170 °C (Preiss et al. 2006) as in Fuhst et al. (2007)	nose-only exposure, 0, 4, 20, 100 mg THC/m^3 equivalent to 0, 6.0, 30.4 or 152.6 mg/m^3 related to the bitumen condensate standard, 6 hours/day, 5 days/week, 5 days, 1 month, 12 months	no induction of 8-oxo-dGuo adducts	–		Halter et al. 2007
DNA fragmentation, alveolar macrophages, lungs; comet assay	Sprague Dawley rats, ♀	Wang et al. (2001) "paving" bitumen, vapours and aerosols 150 °C–170 °C	whole-body exposure, cumulative dose: 0, 353, 641, 1150 mg × hour/m^3, 6 hours/day, 5 days; 53 mg × hour/m^3, single for 1 hour	concentration-dependent induction of DNA damage	+		Zhao et al. 2004
gene mutation in the cII and lacI gene, lungs	transgenic Big Blue® mice, ♂	vapours and aerosols from "paving" bitumen 50/70 (CAS number 8052-42-4) of Venezuelan origin 170 °C	nose-only exposure, 0, 100 mg/m^3, 6 hours/day, 5 days, examination 30 days after exposure	no significant differences in the mutation frequency in cII and lacI genes	–		Micillino et al. 2002
gene mutation in the cII gene, lungs	transgenic Big Blue® rats	"50/70 pen batch bitumen" (CAS number 8052-42-4) of Venezuelan origin "bitumen fume" 170 °C	nose-only exposure, 0, 112 ± 13 mg TPM/m^3, 4.6 µm Ø 6 hours/day, 5 days	after 30 days, the same mutation frequency in cII as in the controls, slight but not significant increase in transversions	–	no increase in inflammatory parameters	Bottin et al. 2006; Gate et al. 2007

Tab. 12 *(continued)*

End point	Test system	Test sample, temperature	Course of exposure	Results		Comments	References
				Comment			
micronuclei, peripheral blood and bone marrow	SPF Wistar rats, groups of 24 ♂ and 24 ♀	condensate from bitumen 50/70 (B65) 170 °C (Preiss et al. 2006) as in Fuhst et al. (2007)	nose-only exposure, 0, 4, 20, 100 mg THC/m³ equivalent to 0, 6.0, 30.4 or 152.6 mg/m³ related to the bitumen condensate standard, 6 hours/day, 5 days/week, 5 days, 1 month, 12 months	no significant increase in micronuclei in the peripheral blood (examination: 5 days, 1 month, 12 months) and bone marrow (examination: 12 months)	–	at 100 mg THC/m³, 12 months: reduced erythrocyte formation in the bone marrow in 4/6 animals and increased erythrocyte formation in the bone marrow in 2/6 animals	Halter et al. 2007
micronuclei, PCE, bone marrow	Sprague Dawley rats, ♀	Wang et al. (2001) "paving" bitumen, vapours and aerosols 150 °C–170 °C	whole-body exposure, cumulative dose: 0, 1733 mg × hour/m³ 6 hours/day, 5 days	no induction of micronuclei in PCE	–	no systemic mutagenicity	Zhao et al. 2004
micronuclei, PCE, bone marrow	Sprague Dawley rats	"asphalt fume condensate" from PG 64–22 "paving" bitumen 160 °C	intratracheal instillation, 0, 0.45, 2.22, 8.88 mg/kg body weight/day, 3 days, examination 24 hours after exposure	significant increase in micronuclei in PCE	+	significant increase in CYP1A1, but not CYP2B1 activity; cytotoxicity: significant decrease in PCE at 8.88 mg/kg body weight/day	Ma et al. 2002
other studies							
Salmonella mutagenicity test +m.a. using lung microsomes from exposed rats; test for mutagenicity of 2-aminoanthracene and benzo[*a*]pyrene	Sprague Dawley rats, ♀ Salmonella typhimurium YG1024, YG1029	Wang et al. (2001) "paving" bitumen, vapours and aerosols 150 °C–170 °C	whole-body exposure, cumulative dose: 0, 641, 1150 mg × hour/m³, 6 hours/day, 5 days	2-aminoanthracene mutagenicity dependent on metabolic activation at 38.3 mg/m³;	+		Zhao et al. 2004
				no benzo[*a*]pyrene mutagenicity that was dependent on metabolic activation	–		
regulation of 16 genes, lung and nasal epithelium, peripheral blood	SPF Wistar rats, groups of 24 ♂ and 24 ♀	condensate from bitumen 50/70 (B65) 170 °C (Preiss et al. 2006) as in Fuhst et al. (2007)	nose-only exposure, 0, 4, 20, 100 mg THC/m³ equivalent to 0, 6.0. 30.4 or 152.6 mg/m³ related to the bitumen condensate standard, 6 hours/day, 5 days/week, 5 days, 1 month, 12 months	change in the expression of candidate genes that are involved in the inflammatory/immune response and pulmonary disorders; example: concentration-dependent increase in the expression of CYP1A1 and CYP2G1 (nasal and lung tissues)	+		Halter et al. 2007

GC/MS: gas chromatography/mass spectrometry; 8-oxo-dGuo: 8-oxo-7,8-dihydro-2′-deoxyguanosine; Q-TOF-MS: quadrupole time-of-flight mass spectrometry; PCE: polychromatic erythrocytes; THC: total hydrocarbon concentration; TPM: total particulate matter

5.6.2.4 Summary

The evaluation does not include studies in which exposure cannot be clearly attributed to either oxidized bitumen or straight-run bitumen. In addition, the assessment does not take findings from studies of modified bitumen into account.

In vitro

In the 2001 documentation (Greim 2002), the results obtained from Salmonella mutagenicity tests with bitumen solutions and extracts were primarily negative (straight-run bitumen: Bittighofer et al. 1983; Blackburn et al. 1986; Gage et al. 1991; McGowan et al. 1992; Monarca et al. 1987; Pasquini et al. 1989; Sonntag and Erdinger 1989, oxidized bitumen: Sonntag and Erdinger 1989) or not clearly positive (straight-run bitumen: Booth et al. 1998; Machado et al. 1993). Conversely, in most cases, bitumen vapour condensates increased the incidence of mutagenicity in Salmonella typhimurium, particularly after metabolic activation (straight-run bitumen: De Méo et al. 1996, oxidized bitumen: Machado et al. 1993).

In $TK^{+/-}$ mutation assays using the mouse lymphoma cell line L5178Y, the frequency of mutations was significantly increased after the addition of a metabolic activation system, but not in the absence of metabolic activation (straight-run bitumen: Akkineni et al. 2001; Genevois et al. 1998, oxidized bitumen: Akkineni et al. 2001). In the presence of metabolic activation, bitumen vapour condensates led to the formation of adducts in dissolved DNA and DNA strand breaks (straight-run bitumen: Hong and Lee 1999). Solutions containing bitumen induced DNA adducts and DNA-protein cross-links in cultured eukaryotic cells (straight-run bitumen: De Méo et al. 1996; Hong and Lee 1999). Bitumen vapour condensates increased the number of micronuclei in V79 cells (oxidized bitumen: Qian et al. 1996, 1999), but did not induce chromosomal aberrations in CHO cultures (straight-run bitumen: Reinke et al. 2000).

In recent studies using a modified Salmonella mutagenicity test, DMSO extracts from **straight-run bitumen** induced mutations. After **oxidation** in the laboratory, the mutagenicity indices of the DMSO extracts were 41% to 50% lower than those of straight-run bitumen. According to the authors, the biologically active PAH fraction decreased during the production of oxidized bitumen, with the result that only few or no mutations could be detected (Trumbore et al. 2011).

In the modified Salmonella mutagenicity test, mutations were induced by **oxidized bitumen** in only 2 of 4 samples collected from storage tanks and from a laboratory sample, while no mutations were induced by **straight-run bitumen** (Kriech et al. 2007) (mutagenicity index > 1: mutations, < 1: no mutations).

Straight-run bitumen (aerosol and vapour samples of AC, unmodified and modified, see Section 5.6.1.1) did not cause genotoxic changes in the Salmonella mutagenicity test, comet assay or micronucleus test (Lindberg et al. 2008).

In vivo

In the 2001 documentation (Greim 2002), local and systemic DNA adducts were either induced in animal studies after exposure to bitumen solutions, extracts and condensates (straight-run bitumen: Genevois et al. 1996; oxidized bitumen: Qian et al. 1998) or not induced (straight-run bitumen: Pasquini et al. 1989, 1992). Studies carried out by Genevois et al. (1996) in BD4 rats found that the adduct patterns detected could not be explained solely on the basis of the unsubstituted PAHs found in the epicutaneously applied bitumen vapour condensates. Repeated oral exposure of rats to larger quantities of a crude oil vacuum distillation residue did not increase structural chromosomal aberrations in the bone marrow of the animals (straight-run bitumen: API 1984 a, b).

Oxidized bitumen

A micronucleus test in the bone marrow of rats yielded negative results up to a total hydrocarbon concentration of 297 mg/m^3 (equivalent to 350 mg/m^3 related to the bitumen condensate standard: conversion according to the method of Fraunhofer ITEM (ITEM 2017; 1200 ng BaP/m^3). In the range-finding study, blood formation in the bone marrow was impaired at a total hydrocarbon concentration of 1000 mg/m^3 (1170 mg/m^3 related to the bitumen condensate standard) (Parker et al. 2011).

Straight-run bitumen, air-rectified bitumen

In the 2001 documentation (Greim 2002), the DNA adducts determined by ^{32}P-postlabelling after inhalation exposure to straight-run bitumen were different from those induced by PAHs. After oral exposure, DNA adduct frequencies were similar in both the exposure and control groups.

Studies found increased frequencies of DNA adducts in the nasal epithelium and in the lungs at a total hydrocarbon concentration of 20 mg/m^3, but not in the white blood cells of rats after nose-only inhalation exposure to total hydrocarbon concentrations of up to 100 mg/m^3 (30 ng BaP/m^3) for 5 weeks (Halter et al. 2007). In addition, neither an increase in 8-oxo-dGuo adducts was determined in rats as evidence of oxidative stress (Halter et al. 2007), nor an increased frequency of mutations in *cII* genes in the lungs of transgenic Big Blue® rats and cII and lacI genes in the lungs of transgenic Big Blue® mice (100 mg/m^3 with 300 ng BaP/m^3; Bottin et al. 2006; Gate et al. 2007; Micillino et al. 2002). The induction of micronuclei was observed in a study with intratracheal instillation only at a cytotoxic dose of 8.88 mg/kg body weight (Ma et al. 2002), but not in 2 studies after exposure by inhalation (Halter et al. 2007; Zhao et al. 2004).

Changes in the expression of genes involved in the inflammatory and immune response and in pulmonary diseases and an increase in the expression of CYP1A1 and CYP2G1 in nasal and lung tissues were determined after exposure by inhalation (Halter et al. 2007).

Accessibility of the germ cells

In the case of **oxidized bitumen,** accessibility of the germ cells is assumed because systemic genotoxic or mutagenic effects are evidence of the systemic availability of genotoxic or mutagenic constituents or metabolic products of vapours and aerosols.

In the case of **oxidized bitumen** and **straight-run bitumen/air-rectified bitumen**, systemic availability can be inferred from the impaired formation of blood cells in the bone marrow of rats after exposure by inhalation (Halter et al. 2007; Parker et al. 2011). Thus, the accessibility of the germ cells is assumed.

5.7 Carcinogenicity

5.7.1 Short-term studies

A study used specially generated samples to investigate whether changes in signal transduction mechanisms associated with tumour formation can be induced by exposure to bitumen emissions. Vapours and aerosols of bitumen were generated at a temperature of 150 °C using a "dynamic asphalt fume generation system" and collected on glass fibre filters. The system did not include an adsorbent for vapours. Following extraction and elution with dichloromethane, the samples were used in cell culture tests for exposure with the stable transfectants of the mouse skin epidermal JB6P+Cl41 cell line expressing the AP-1 luciferase reporter gene (with 20 µg/ml) and primary mouse epidermal keratinocytes from the epidermis of newborn AP-1 transgenic mice (with 10 µg/ml, 6 hours). In addition, skin samples were collected from the mice before and after painting with "asphalt fumes" (24 hours). The AP-1 activity in the cells and skin samples was then quantified on the basis of the expressed luciferase reporter gene. A statistically significant increase in AP-1 activity was determined in the JB6P+Cl41 cells 12 to 24 hours after treatment. A statistically significant, marked increase in AP-1 activity was observed also in the skin samples and keratinocytes of transgenic mice after exposure. According to the authors, AP-1 activation is an essential mechanism that plays an important role in the promotion of skin tumours. In addition, the study investigated the signal transduction pathways that lead to the activation of AP-1. Immunoblots were used to demonstrate that the activation of AP-1 resulting from exposure probably occurred via the PI3K/Akt signal transduction pathway and not via MAP kinases. The authors carried out an additional assay to demonstrate that the growth properties of JB6P+Cl41 cells changed after exposure. Their growth became anchorage independent, which is another characteristic of tumour cells. Therefore, tumourigenic properties were detected in the cells of the JB6P+CI41 mouse cell line and in the keratin and skin samples of transgenic mice after exposure (Ma et al. 2003 a).

A study investigated the initiation and promotion of tumours after exposure to BURA bitumen (type III "built-up roofing asphalt"). Groups of 30 male mice (Crl:CD1) were exposed via the skin to 37.5 µl of a solution containing 25 mg of "field-matched" condensate in mineral oil, equivalent to 67%. The test substance was identical to that used by Clark et al. (2011) (see Section 5.7.2). To test the substance for tumour initiation, the "field-matched" condensate solution was applied to the shaved dorsal skin twice a week for 2 weeks, followed by the application of 5 µg of tetradecanoylphorbol acetate (TPA) in acetone, equivalent to 0.01%, twice weekly for 25 weeks. Squamous cell papillomas were found in 5 of 30 mice, but only in 1 of 30 animals of the negative control group, which were administered mineral oil instead of "field-matched" condensate. To test the substance for tumour-promoting effects, tumour induction was initiated by a single epicutaneous application of 50 µg of 7,12-dimethylbenzo[*a*]anthracene (DMBA) at the beginning of the initiation phase, followed by the application of 37.5 µl of a solution containing 25 mg of "field-matched" condensate in mineral oil (equivalent to 67%) twice weekly from weeks 3 to 31. Squamous cell carcinomas were observed in 2 mice. In comparison, tumours developed in 29 of 30 mice of the positive control group. These animals had been given DMBA for initiation and TPA for promotion. The authors concluded that the carcinogenic effects induced by the "field-matched" condensate (see Clark et al. 2011) were more likely caused by a genotoxic mechanism than tumour-promoting effects (Freeman et al. 2011; see Table 13).

5.7.2 Long-term studies

5.7.2.1 Inhalation

Groups of 50 male and 50 female SPF Wistar rats were exposed nose-only to a bitumen vapour condensate generated at 175 °C (for a detailed description, see Section 5.2.1.1). Commercially available paving grade bitumen B65 (= B50/70) was used as the feedstock. This type of bitumen contains about 70% "air-rectified" bitumen (CAS number 64742-93-4) with the remainder made up of "straight-run vacuum residue" (CAS number 64741-56-6). The animals were exposed to total hydrocarbon concentrations of 0, 4.1, 20.7 and 103.9 mg/m^3 (related to the mineral oil standard, equivalent to 0, 6.0, 30.4 and 152.6 mg/m^3 related to the bitumen condensate standard) for 6 hours a day, on 5 days a week, for up to 24 months. Mortality was similar in all groups, but slightly higher in females. The incidence of bronchoalveolar hyperplasia was significantly higher in both sexes in the groups exposed to the medium concentration and above. A significant increase in the overall tumour incidence or in individual organs was not recorded. A male rat exposed to a concentration of 152.6 mg/m^3 developed a poorly differentiated adenocarcinoma in the nasal cavity (Fuhst et al. 2007). This type of tumour is comparatively rare in rats and may possibly have been caused by exposure to bitumen.

5.7.2.2 Dermal application

The studies below are summarized in Table 13.

A study with the dermal application of saturated solutions of "Asphalt Cement 20" (AC-20, asphalt concrete, CAS number 8052-42-4) or "Coastal Residuum" (CR, CAS number 64741-56-6) in mineral oil to the skin of male C3H/HeN-CrlBR mice did not yield evidence of carcinogenic effects. Groups of 50 mice were exposed via the shaved dorsal skin to 37.5 µl of a 30% solution of AC-20 and a 70% solution of CR in mineral oil twice weekly for 24 months. The mice of the 3 control groups (50 mice per group) were treated using the same procedure, but with toluene and mineral oil for the negative control and a 0.05% solution of benzo[*a*]pyrene in toluene for the positive control. Tumours of the skin were induced in 92% of the mice of the positive control group (45 squamous cell carcinomas and a papilloma), but not in any of the animals of the other exposure groups (Goyak et al. 2011).

In a study carried out in the United States, the effects induced by the application of 3 different bitumen condensates to the skin of groups of 80 male C3H/HeNCrl mice were investigated over a period of 104 weeks. One of the condensates was generated from the vapour of paving grade bitumen ("field-matched-paving" condensate) at a temperature of 148 °C, the 2 other condensates were produced from the vapour of bitumen used for roofing sheets (type III BURA "built-up roofing asphalt") at temperatures of 199 °C ("field-matched" BURA condensate) and 232 °C ("lab" BURA condensate). The conditions under which the condensates were generated were modified in such a way that the chemical profiles of the

condensates were as similar as possible to actual samples from the workplace. More information about the procedures used to produce the bitumen condensates can be found in the publication of Kriech et al. (2007). The benzo[*a*]pyrene concentration in the condensate of paving grade bitumen was below the limit of detection of 0.08 mg/kg; the BURA condensates contained 3 and 6 mg/kg benzo[*a*]pyrene, respectively. The bitumen condensates were applied in mineral oil at a dose volume of 37.5 µl non-occlusively to the shaved dorsal skin of mice using a pipette. On the basis of the findings of a pilot study, the intervals of application were chosen in such a way that irritation of the skin was not induced. As the irritant effects varied in severity, the study was divided into 2 parts to evaluate the paving grade bitumen and BURA separately. Both parts of the study included a positive and a negative control group with 50 and 80 male mice, respectively. The paving grade bitumen condensate was applied daily at a dose level of 7.14 mg in mineral oil, equivalent to 50 mg of condensate per week. The BURA condensates were applied twice weekly at a dose level of 25 mg in mineral oil, which was also equivalent to 50 mg of condensate per week. All control groups were given 0.05% benzo[*a*]pyrene in 37.5 µl of toluene or pure mineral oil, respectively, twice weekly. Dermal application of paving grade bitumen did not have any significant effects on survival, body weights, feed consumption or clinico-chemical parameters. Tests of skin irritation did not reveal any unusual findings over the entire study period. Squamous cell carcinomas were determined in 37 of 50 mice of the benzo[*a*]pyrene control group, but not in the mineral oil control group or the paving grade bitumen group. Only 1 squamous cell papilloma was found in the group exposed to paving grade bitumen. In the dose groups of the part of the study investigating exposure to BURA, 4 mice of the "field-matched" condensate group and 10 of the "lab" condensate group died in the first 6 months. The examinations of these mice did not link the deaths to exposure. The incidence of mortality was similar in the "field-matched" condensate group and the mineral oil group, but was significantly increased in the "lab" condensate group as from week 60 of the study. The irritation index scores (numerical system for the evaluation of the severity of skin irritation) in the "field-matched" condensate group were significantly elevated in comparison with the scores of the control group as from week 53; in the "lab" condensate group, this increase was already observed as from week 46. The irritation index scores of the "field-matched" condensate group gradually increased to a little over half the maximum levels, while in the "lab" condensate group, the scores increased rapidly to near-maximum levels. Skin irritation was found to have a tumour-promoting effect by comparing the irritation index scores of tumour-bearing and non-tumour-bearing mice: squamous cell carcinomas developed in 8 of 62 mice of the "field-matched" condensate group, in which proliferation of skin tissues was observed from week 80, and in 35 of 64 mice of the "lab" condensate group, in which the proliferation of skin tissues was first detected in week 50. In the benzo[*a*]pyrene group, tumours developed in 34 of 49 mice, while none developed in the mice of the mineral oil group (Clark et al. 2011).

5.7.2.3 Summary

Straight-run bitumen, air-rectified bitumen

A significantly increased tumour risk cannot be derived from the findings of the chronic inhalation study in rats. However, a poorly differentiated adenocarcinoma formed in the nasal cavity of 1 animal. The skin painting study did not yield evidence of a carcinogenic effect. The test condensate was generated at a temperature of 148 °C, it contained lower levels of PAHs than the roofing condensates that were investigated at the same time.

Oxidized bitumen

A long-term study with inhalation exposure is not available. Roofing condensates were found to be carcinogenic in skin painting studies. Their potency increased with an increase in the temperature at which the bitumen condensate was generated of 199 °C to 232 °C and a concurrent increase in PAH levels. A study that investigated tumour initiation and promotion found that the carcinogenic effects of these condensates were caused by a genotoxic mechanism.

Tab. 13 Skin painting studies with bitumen condensates in mice

Strain; number of animals	Test substance	CAS number	Temperature	Dose; duration	Squamous cell carcinomas/ keratoacanthomas/ papillomas/ Σ squamous cell carcinomas + papillomas (number of test animals)[a)]	References
C3H/HeNCrl; 80 ♂	"field-matched" built-up roofing asphalt type III (class 2 bitumen), vapour condensate	64742-93-4	199 °C and higher	25 mg in mineral oil, 37.5 µl, 2× per week, weekly dose: 50 mg; 104 weeks	DG: 8/1/4/11 (62) PC: 34/3/2/35 (49)	Clark et al. 2011
C3H/HeNCrl; 80 ♂	"laboratory-generated" built-up roofing asphalt type III (class 2 bitumen), vapour condensate	64742-93-4	232 °C	25 mg in mineral oil, 37.5 µl, 2× per week, weekly dose: 50 mg; 104 weeks	DG: 35/2/3/37 (64) PC: 34/3/2/35 (49)	Clark et al. 2011
C3H/HeNCrl; 80 ♂	"field-matched" paving asphalt (class 1 bitumen), vapour condensate	8052-42-4	148 °C	7.14 mg in mineral oil, 37.5 µl, daily, weekly dose: 50 mg; 104 weeks	DG: 0/0/0/no data (80) PC: 37/3/1/no data (50) negative control group: 1 basal cell carcinoma	Clark et al. 2011
C3H/HeNCrlBR; 50 ♂	Asphalt Cement 20, class 1 bitumen from "napthenic crude"	8052-42-4	no data	30% in mineral oil, 37.5 µl, 2× per week; 24 months	DG: 0/0/0/no data (50) PC: 45/no data/no data/no data (50)	Goyak et al. 2011
C3H/HeNCrlBR; 50 ♂	"Coastal Residuum", class 1 bitumen from "napthenic crude"	64741-56-6	no data	75% in mineral oil, 37.5 µl, 2× per week; 24 months	DG: 0/0/0/no data (50) PC: 45/no data/no data/no data (50)	Goyak et al. 2011
Crl:CD1; 30 ♂	"field-matched" built-up roofing asphalt type III (class 2 bitumen), vapour condensate	64742-93-4	199 °C	25 mg in mineral oil, 37.5 µl, 2 x per week, weekly dose: 50 mg; for 2 weeks; then 5 µg TPA (0.01% in acetone), 2× per week; 25 weeks	DG: 0/0/5/no data (30) PC: 3/3/27/no data (30)	Freeman et al. 2011
Crl:CD1; 30 ♂	"field-matched" built-up roofing asphalt type III (class 2 bitumen), vapour condensate	64742-93-4	199 °C	single application of 50 µg DMBA, 25 mg in mineral oil, 37.5 µl, 2× per week, weekly dose: 50 mg; 28 weeks	DG: 0/1/2/no data (30) PC: 3/3/27/no data (30)	Freeman et al. 2011

[a)] no findings in negative control groups except where noted
DG: dose group; DMBA: 7,12-dimethylbenzo[*a*]anthracene; PC: positive control; TPA: tetradecanoylphorbol acetate

6 Manifesto (MAK value/classification)

The most sensitive end points are carcinogenic effects and effects in the upper and lower respiratory tract.

Carcinogenicity. The vapours and aerosols of bitumen were assigned to Carcinogen Category 2 in 2001 after animal studies revealed genotoxic and carcinogenic properties (Greim 2002). New findings have in the meantime been published; however, some of the data are contradictory (Kriech et al. 2018).

Overall, the epidemiological studies published since 2001 did not provide clear evidence of a general increase in mortality from all cancer types in workers handling bitumen. A slightly increased risk was found for lung cancer, but this effect cannot be clearly attributed to a specific type of bitumen or to a certain type of activity. A marked increase in the risk of lung cancer was primarily observed in earlier studies of roofing activities. However, bias from co-exposure to coal tar is likely, particularly in the case of these studies, and must be taken into consideration. It remains unclear whether the increased risk can be solely attributed to this. By contrast, analyses that examined only data from road paving operations did not find any or only a slight increase in lung cancer risk. The studies available are of limited validity; both an overestimation and an underestimation of the potential lung cancer risk is possible. The nested case–control studies of Olsson et al. (2010) and Agostini et al. (2013) are of greater significance for the evaluation because of the relatively good assessment of exposure. The studies did not find a significant increase in lung cancer risk after examining a relatively large number of cases (433 and 393, respectively).

Straight-run/air-rectified bitumen

Animal studies with dermal application carried out since 2001 to investigate exposure to straight-run bitumens obtained only negative results (Clark et al. 2011; Goyak et al. 2011); also studies of carcinogenic effects carried out up to 2001 yielded primarily negative findings. A carcinogenicity study in rats carried out according to OECD Test Guideline 451 with inhalation exposure to the vapours and aerosols of a mixture of straight-run bitumens containing at least 70% air-rectified bitumen did not reveal a significant increase in tumour incidences in the exposed groups up to the highest concentration tested of 152.6 mg/m^3 (bitumen condensate standard) (Fuhst et al. 2007). Recent studies in humans did not find consistent evidence of increased genotoxicity and mutagenicity in exposed workers (Lindberg et al. 2008; Tompa et al. 2007). Studies, particularly those that examined a large number of cases (n > 200) such as the Human Bitumen Study (Marczynski et al. 2011; Welge et al. 2011), did not find an increased frequency of micronuclei, chromosomal aberrations, HPRT mutations, SCE, DNA strand breaks or oxidative DNA base damage in vivo in humans that could be linked to exposure to bitumen. The results of other studies that described increases in individual genotoxicity parameters (Cavallo et al. 2006; Çelik et al. 2013; Karaman and Pirim 2009; Murray and Edwards 2005; Sellappa et al. 2011) are difficult to interpret because of a lack of exposure data.

An association could not be made between exposure and PAH-associated DNA adducts of the carcinogenic benzo[*a*]-pyrene (Marczynski et al. 2011; McClean et al. 2007 b) in spite of the use, in some cases, of very sensitive methods such as ^{32}P-postlabelling.

Evidence of carcinogenic effects induced by exposure to defined straight-run bitumens was not found. However, because the chemical composition of straight-run bitumens varies widely, a carcinogenic potential cannot be ruled out completely. As a result, harmful emissions of carcinogenic and mutagenic constituents may be produced.

Therefore, the vapours and aerosols emitted during the high-temperature processing of straight-run bitumen and air-rectified bitumen are classified in Carcinogen Category 3 B. Aggregates and emissions from aggregates that may make a relevant contribution to the health risk have to be assessed separately.

Oxidized bitumen

Recent animal studies of oxidized bitumen (roofing bitumen) that were published since 2001 have demonstrated—as did the majority of earlier studies—that condensates of the vapours and aerosols of oxidized bitumen induce carcinogenic effects in skin painting tests in mice (Clark et al. 2011; Freeman et al. 2011). Therefore, a significant number of the oxidized bitumens tested have to be considered carcinogenic. There are no studies that investigated the carcinogenicity of oxidized bitumen after inhalation exposure. The positive results obtained in animal studies of carcinogenicity, including skin painting tests, are consistent with the results of a genotoxicity study that found an increased frequency of DNA strand breaks in a group of exposed workers (Toraason et al. 2001).

Overall, the vapours and aerosols produced during the high-temperature processing of oxidized bitumen, as commonly used for roofing work, have been classified in Carcinogen Category 2 for carcinogenic substances.

MAK value. A 2-year inhalation study in rats (Fuhst et al. 2007) that was carried out according to OECD Test Guideline 451 can be used for the derivation of a MAK value for **straight-run and air-rectified bitumen**. In this study, rats were exposed by inhalation to the vapours and aerosols produced from a mixture of straight-run and air-rectified bitumens similar to those used at the workplace for 6 hours a day, on 5 days per week, for 104 weeks. The exposure concentrations were 0, 6.0, 30.4 and 152.6 mg/m^3 using bitumen condensate as the reference standard. The most sensitive end points were the effects in the upper and lower respiratory tract. On the basis of the increased incidence of bronchoalveolar hyperplasia in the lungs and of inflammatory cells in the nasal epithelium, a NOAEC of 6 mg/m^3 and a LOAEC of 30.4 mg/m^3 (bitumen condensate standard) was derived from this study.

The results of this study can be compared with the findings of the Human Bitumen Study, which observed effects in the lower respiratory tract of workers after exposure to the vapours and aerosols produced from mastic asphalt at a median concentration of 5.08 mg/m^3 (related to the bitumen condensate standard, interquartile range of 2.64–8.67 mg/m^3). On a group basis, 3 of the 12 parameters investigated in the sputum (total protein, interleukin 8 and matrix metalloproteinase 9) were increased in exposed workers. However, no association could be made with the prevailing concentrations of the vapours and aerosols of bitumen during the work shift. The concentrations of inflammatory markers were not dependent on the duration of exposure or on the "job tasks". The findings can be interpreted as the initial signs of inflammatory changes. The severity of the changes was very slight in comparison with those determined in smokers. The markers have not been evaluated with regard to their clinical relevance. As no association could be made with the work shift, the changes were probably subchronic or chronic. Lung function was not impaired. No evidence of systemic effects was found in the examination of the blood. No differences between the exposed and control groups were observed in the analysis of inflammatory parameters in the nasal lavage (Raulf-Heimsoth et al. 2011 b). This shows that the lower respiratory tract is the most sensitive target organ in humans.

On the basis of the NOAEC of 6 mg/m^3 that was determined in the inhalation study in rats and taking into consideration that the value was derived from an animal study (1:2) and the increased respiratory volume at the workplace (1:2) in comparison with the exposure of animals at rest, a MAK value of 1.5 mg/m^3 (sum of the vapour and inhalable fraction) has been determined for the vapours and aerosols from **straight-run and air-rectified bitumen** using bitumen condensate as the reference standard (1 mg/m^3 as related to the mineral oil standard). Therefore, the MAK value is one third of the mean concentration at which 3 of 12 inflammatory parameters were increased in the sputum of exposed workers. However, the clinical relevance of these parameters is unclear. This margin is considered sufficient. The MAK value was derived from a study of rodents for an additive-free mixture of straight-run and air-rectified bitumen and is also supported by the findings of the Human Bitumen Study of workers who were exposed to vapours and aerosols while handling mastic asphalt. The MAK value is therefore valid also for the vapours and aerosols of the bitumen contained in mastic asphalt as a binding agent. The workers investigated by the Human Bitumen Study also handled lower temperature asphalts enriched with viscosity-changing additives. Therefore, the MAK value is valid also for these types of lower temperature asphalts containing these kinds of aggregates. However, the aggregates themselves and their emissions, which may have a relevant impact on the health risk, have to be evaluated separately.

Peak limitation. The critical effects are the effects in the lungs. The effects are assumed to be cumulative. The substance is therefore classified in Peak Limitation Category II. After exposure to the vapours and aerosols of mastic asphalt in the work area at concentrations of ≤ 15 mg/m^3 (n = 46) and > 15 mg/m^3 (n = 18) (8-hour time-weighted average values, related to bitumen condensate as the reference standard), a concentration-dependent increase in the prevalence of symptoms in the lower respiratory tract and of eye irritation was found during the shift in comparison with the levels determined in the 48 control persons (Raulf-Heimsoth et al. 2007). Concentration peaks are assumed to have an impact on the symptoms observed. No subjective or objective symptoms were reported following exposure of 2 volunteers to the vapours and aerosols of bitumen at a concentration of about 20 mg/m^3 (bitumen condensate standard) for 8 hours (Walter and Knecht 2007). Therefore, at an excursion factor of 2 resulting in a permissible peak concentration of 3 mg/m^3, no local effects are to be expected.

Prenatal toxicity.

Straight-run/air-rectified bitumen

As studies of developmental toxicity are not available, **straight-run and air-rectified bitumen** have been classified in Pregnancy Risk Group D.

Oxidized bitumen

In a 1-generation study with exposure up to gestation day 20, condensates of the vapours and aerosols of oxidized bitumen did not induce foetotoxic effects at concentrations up to 300 mg/m^3. No studies of teratogenicity are available. As a MAK value was not derived for oxidized bitumen, the substance has not been classified in a pregnancy risk group.

Germ cell mutagenicity. Only results that could clearly be attributed to either oxidized bitumen or straight-run bitumen were taken into consideration for the evaluation of germ cell mutagenicity.

Straight-run/air-rectified bitumen

No data relating to germ cells are available for the vapours and aerosols of straight-run bitumen. Benzo[*a*]pyrene adducts were not detected in the blood of exposed persons even by the most sensitive methods. No evidence of increased genotoxicity or mutagenicity (frequency of micronuclei, chromosomal aberrations, HPRT mutations, SCE, DNA strand breaks or oxidative DNA base damage) in humans was found in studies that investigated sufficiently large collectives and provided a detailed description of the exposure conditions. In vitro studies that investigated the effects of extracts or vapours on bacteria in the Salmonella mutagenicity test and on mammalian cells in the comet assay or micronucleus test yielded primarily negative results with and without the addition of a metabolic activation system. A number of animal studies observed DNA adducts in the nose, lungs and alveolar macrophages after exposure by inhalation, but tests for mutations in the lungs at the cII locus in rats and mice and at the lacI locus in mice yielded negative results at concentrations of about 100 mg/m^3 (with 300 ng benzo[*a*]pyrene/m^3). Micronuclei were induced in SD rats after 3 intratracheal injections of straight-run bitumen at the cytotoxic dose of 8.88 mg/kg body weight and day, but not after inhalation of concentrations up to 100 mg/m^3 (with 30 ng benzo[*a*]pyrene/m^3) or 641 mg/m^3. The vapours and aerosols of straight-run bitumen are systemically available after inhalation exposure. Overall, the data for genotoxicity and the estimated PAH levels (see comparative analysis) do not suggest germ cell mutagenic effects, and the vapours and aerosols of **straight-run and air-rectified bitumen** have not been classified in one of the categories for germ cell mutagens.

Oxidized bitumen

No data relating to germ cells are available for the vapours and aerosols of oxidized bitumen. In workers, exposure to the vapours and aerosols of oxidized bitumen yielded evidence of an increased frequency of strand breaks (Toraason et al. 2001). PAH–DNA adducts, determined as benzo[*a*]pyrene–DNA adducts, could not be clearly attributed to oxidized bitumen because of co-exposure to coal tar (Herbert et al. 1990 b). Oxidized bitumen induced mutations in bacteria. No other in vitro studies with oxidized bitumen are available. Furthermore, a valid micronucleus test in rats with inhalation exposure at concentrations up to 300 mg/m^3 (with 1200 ng benzo[*a*]pyrene/m^3) yielded negative results. The vapours and aerosols of oxidized bitumen are systemically available. Overall, there are not enough data to classify them as germ cell mutagens. However, based on the mutations observed in bacteria and the higher level of benzo[*a*]pyrene in the condensates of oxidized bitumen (Parker et al. 2011) in comparison with the level found in the condensates of paving grade bitumen (Fuhst et al. 2007) and the systemic availability of the inhaled vapours and aerosols of oxidized bitumens, a germ cell mutagenic effect is possible and oxidized bitumens have been classified in Category 3 B for germ cell mutagens.

Absorption through the skin. Epicutaneous application of bitumen vapour condensates in rats led to the formation of systemic DNA adducts (Genevois et al. 1996; Greim 2002). A study in volunteers (Walter and Knecht 2007) found that bitumen emissions were readily absorbed from the gas phase. The "H" designation (for substances which can be absorbed through the skin in toxicologically relevant amounts) has thus been retained.

Sensitization. No positive findings were obtained for sensitization in animals. There are no findings in humans. For this reason, the "Sh" or "Sa" designations (for substances which cause sensitization of the skin or airways) have not been given.

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

References

ACGIH (American Conference of Governmental Industrial Hygienists) (2017) Polycyclic aromatic hydrocarbons (PAHs). Documentation of TLVs and BEIs. Cincinnati, OH: ACGIH

Agostini M, Ferro G, Burstyn I, de Vocht F, Portengen L, Olsson A, Boffetta P, Kromhout H, IARC European Asphalt Workers Study consortium (2013) Does a more refined assessment of exposure to bitumen fume and confounders alter risk estimates from a nested case-control study of lung cancer among European asphalt workers? Occup Environ Med 70(3): 195–202. https://doi.org/10.1136/oemed-2012-100839

AGS (Ausschuss für Gefahrstoffe) (2011) Begründung zur ERB zu Benzo[a]pyren in TRGS 910. Dortmund: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin. https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/910/910-benzo-a-pyren.pdf?__blob=publicationFile&v=2, accessed 22 Mar 2017

AGS (Ausschuss für Gefahrstoffe) (2015) Begründung zu Vanadiumverbindungen in TRGS 900. Dortmund: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin. https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/900/900-vanadium-verbindungen.pdf?__blob=publicationFile&v=2, accessed 22 Mar 2017

AGS (Ausschuss für Gefahrstoffe) (2017) Begründung zu Nickelverbindungen in TRGS 910. Dortmund: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin. https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/910/910-nickel.pdf?__blob=publicationFile&v=2, accessed 22 Mar 2017

Akkineni LK, Zeisig M, Baranczewski P, Ekström L-G, Möller L (2001) Formation of DNA adducts from oil-derived products analyzed by 32P-HPLC. Arch Toxicol 74(11): 720–731. https://doi.org/10.1007/s002040000172

API (American Petroleum Institute) (1984 a) Mutagenicity evaluation studies in the rat bone marrow cytogenetic assay and in the mouse lymphoma forward mutation assay. Vacuum residuum-sample 81-13. Study carried out by Litton Bionetics Inc. 31–30614. Washington, DC: API

API (American Petroleum Institute) (1984 b) Mutagenicity evaluation studies in the rat bone marrow cytogenetic assay and in the mouse lymphoma forward mutation assay. Vacuum residuum-sample 81-14. Study carried out by Litton Bionetics Inc. 31–30615. Washington, DC: API

Asphalt Institute and Eurobitume (2015) The bitumen industry - a global perspective. 3rd ed, No. IS-230. Brussels: Asphalt Institute Inc. and European Bitumen Association-Eurobitume. https://www.eurobitume.eu/public_downloads/General/The%20Bitumen%20Industry%203rd%20edition.pdf, accessed 22 Mar 2017

Bacaksiz A, Kayaalti Z, Soylemez E, Tutkun E, Soylemezoglu T (2014) Lymphocyte DNA damage in Turkish asphalt workers detected by the comet assay. Int J Environ Health Res 24(1): 11–17. https://doi.org/10.1080/09603123.2013.773586

BAFA (Bundesamt für Wirtschaft und Ausfuhrkontrolle) (2018) Entwicklung der Mineralölausfuhr (1995–2017). Eschborn: BAFA. http://www.bafa.de/SharedDocs/Downloads/DE/Energie/Mineraloel/moel_entw_mineraloelausfuhr_1995_2020.xlsx?__blob=publicationFile&v=11ww.ecetoc.org/wp-content/uploads/2014/08/JACC-038.pdf, accessed 22 Mar 2017

Behrens T, Schill W, Ahrens W (2009) Elevated cancer mortality in a German cohort of bitumen workers: extended follow-up through 2004. J Occup Environ Hyg 6(9): 555–561. https://doi.org/10.1080/15459620903077682

Bergdahl IA, Järvholm B (2003) Cancer morbidity in Swedish asphalt workers. Am J Ind Med 43(1): 104–108. https://doi.org/10.1002/ajim.10157

Binet S, Bonnet P, Brandt H, Castegnaro M, Delsaut P, Fabries JF, Huynh CK, Lafontaine M, Morel G, Nunge H, Rihn B, Vu Duc T, Wrobel R (2002) Development and validation of a new bitumen fume generation system which generates polycyclic aromatic hydrocarbon concentrations proportional to fume concentrations. Ann Occup Hyg 46(7): 617–628. https://doi.org/10.1093/annhyg/mef081

Bittighofer P, Zinser D, Fliedner TM, Reichenbach K (1983) Belastung und Beanspruchung von Arbeitern im Straßenbau durch Asphalt-Bitumen. In: . Stuttgart: Genter Verlag. p. 439–444

Blackburn GR, Deitch RA, Schreiner CA, Mackerer CR (1986) Predicting carcinogenicity of petroleum distillation fractions using a modified Salmonella mutagenicity assay. Cell Biol Toxicol 2(1): 63–84. https://doi.org/10.1007/BF00117708

Boczkaj G, Przyjazny A, Kamiński M (2014) Characteristics of volatile organic compounds emission profiles from hot road bitumens. Chemosphere 107: 23–30. https://doi.org/10.1016/j.chemosphere.2014.02.070

Boffetta P, Burstyn I, Partanen T, Kromhout H, Svane O, Langard S, Järvholm B, Frentzel-Beyme R, Kauppinen T, Stücker I, Shaham J, Heederik D, Ahrens W, Bergdahl I, Cenée S, Ferro G, Heikkilä P, Hooiveld M, Johansen C, Randem B, Schill W (2001) IARC epidemiological study of cancer mortality among European asphalt workers. IARC Internal Report No. 01/003, 2001, Lyon: IARC, unpublished

Boffetta P, Burstyn I, Partanen T, Kromhout H, Svane O, Langård S, Järvholm B, Frentzel-Beyme R, Kauppinen T, Stücker I, Shaham J, Heederik D, Ahrens W, Bergdahl IA, Cenée S, Ferro G, Heikkilä P, Hooiveld M, Johansen C, Randem BG, Schill W (2003 a) Cancer mortality among European asphalt workers: an international epidemiological study. I. Results of the analysis based on job titles. Am J Ind Med 43(1): 18–27. https://doi.org/10.1002/ajim.10181

Boffetta P, Burstyn I, Partanen T, Kromhout H, Svane O, Langård S, Järvholm B, Frentzel-Beyme R, Kauppinen T, Stücker I, Shaham J, Heederik D, Ahrens W, Bergdahl IA, Cenée S, Ferro G, Heikkilä P, Hooiveld M, Johansen C, Randem BG, Schill W (2003 b) Cancer mortality among European asphalt workers: an international epidemiological study. II. Exposure to bitumen fume and other agents. Am J Ind Med 43(1): 28–39. https://doi.org/10.1002/ajim.10182

Bolliet C, Juery C, Thiebaut B (2013) Impact of oxidation process on polycyclic aromatic hydrocarbon (PAH) content in bitumen. J Occup Environ Hyg 10(8): 435–445. https://doi.org/10.1080/15459624.2013.801820

Bonassi S, Fenech M, Lando C, Lin YP, Ceppi M, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, Barale R, Bigatti MP, Bolognesi C, Jia C, Di Giorgio M, Ferguson LR, Fucic A, Lima OG, Hrelia P, Krishnaja AP, Lee TK, Migliore L, Mikhalevich L, Mirkova E, Mosesso P, Müller WU, Odagiri Y, Scarffi MR, Szabova E, Vorobtsova I, Vral A, Zijno A (2001) HUman MicroNucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. Environ Mol Mutagen 37(1): 31–45. https:/doi.org/10.1002/1098-2280(2001)37:1<31::AID-EM1004>3.0.CO;2-P

Booth ED, Brandt HCA, Loose RW, Watson WP (1998) Correlation of 32P-postlabelling-detection of DNA adducts in mouse skin in vivo with the polycyclic aromatic compound content and mutagenicity in Salmonella typhimurium of a range of oil products. Arch Toxicol 72(8): 505–513. https://doi.org/10.1007/s002040050535

Bottin MC, Gate L, Rihn B, Micillino JC, Nathalie M, Martin A, Nunge H, Morel G, Wrobel R, Ayi-Fanou L, Champmartin C, Keith G, Binet S (2006) Genotoxic effects of bitumen fumes in Big Blue® transgenic rat lung. Mutat Res 596(1–2): 91–105. https://doi.org/10.1016/j.mrfmmm.2005.12.005

Breuer D (2008 a) Methode 6305/1, Bitumen (Dämpfe und Aerosole, Mineralölstandard). Berlin: Erich-Schmidt-Verlag. https://www.ifa-arbeitsmappedigital.de/IFA-AM_6305-1-1, accessed 22 Mar 2017

Breuer D (2008 b) Methode 6305/2, Bitumen (Dämpfe und Aerosole, Bitumenkondensat-Standard). Berlin: Erich-Schmidt-Verlag. https://www.ifa-arbeitsmappedigital.de/IFA-AM_6305-2-2, accessed 22 Mar 2017

Breuer D, Hahn J-U, Höber D, Emmel C, Musanke U, Rühl R, Spickenheuer A, Raulf-Heimsoth M, Bramer R, Seidel A, Schilling B, Heinze E, Kendzia B, Marczynski B, Welge P, Angerer J, Brüning T, Pesch B (2011) Air sampling and determination of vapours and aerosols of bitumen and polycyclic aromatic hydrocarbons in the Human Bitumen Study. Arch Toxicol 85(Suppl 1): S11–S20. https://doi.org/10.1007/s00204-011-0678-1

Burgaz S, Erdem O, Karahalil B, Karakaya AE (1998) Cytogenetic biomonitoring of workers exposed to bitumen fumes. Mutat Res 419(1–3): 123–130. https://doi.org/10.1016/s1383-5718(98)00136-3

Burstyn I, Kromhout H, Kauppinen T, Heikkilä P, Boffetta P (2000) Statistical modelling of the determinants of historical exposure to bitumen and polycyclic aromatic hydrocarbons among paving workers. Ann Occup Hyg 44(1): 43–56. https://doi.org/10.1016/S0003-4878(99)00101-5

Burstyn I, Boffetta P, Kauppinen T, Heikkilä P, Svane O, Partanen T, Stücker I, Frentzel-Beyme R, Ahrens W, Merzenich H, Heederik D, Hooiveld M, Langård S, Randem BG, Järvholm B, Bergdahl I, Shaham J, Ribak J, Kromhout H (2003) Estimating exposures in the asphalt industry for an international epidemiological cohort study of cancer risk. Am J Ind Med 43(1): 3–17. https://doi.org/10.1002/ajim.10183

Burstyn I, Kromhout H, Johansen C, Langard S, Kauppinen T, Shaham J, Ferro G, Boffetta P (2007) Bladder cancer incidence and exposure to polycyclic aromatic hydrocarbons among asphalt pavers. Occup Environ Med 64(8): 520–526. https://doi.org/10.1136/oem.2006.029801

Campo L, Calisti R, Polledri E, Barretta F, Stopponi R, Massacesi S, Bertazzi PA, Fustinoni S (2011) Valutazione dell'esposizione a idrocarburi policiclici aromatici in addetti ad opere di asfaltatura autostra-dale mediante misura di 1-idrossipirene urinario [Assessment of exposure to polycyclic aromatic hydrocarbons in asphalt workers by measurement of urinary 1-hydroxypyrene]. Med Lav 102(6): 484–493

Cavallari JM, Osborn LV, Snawder JE, Kriech AJ, Olsen LD, Herrick RF, McClean MD (2012) Predictors of airborne exposures to polycyclic aromatic compounds and total organic matter among hot-mix asphalt paving workers and influence of work conditions and practices. Ann Occup Hyg 56(2): 138–147. https://doi.org/10.1093/annhyg/mer088

Cavallo D, Ursini CL, Bavazzano P, Cassinelli C, Frattini A, Perniconi B, Di Francesco A, Ciervo A, Rondinone B, Iavicoli S (2006) Sister chromatid exchange and oxidative DNA damage in paving workers exposed to PAHs. Ann Occup Hyg 50(3): 211–218. https://doi.org/10.1093/annhyg/mei072

Çelik A, Yildirim S, Ekinci SY, Taşdelen B (2013) Bio-monitoring for the genotoxic assessment in road construction workers as determined by the buccal micronucleus cytome assay. Ecotoxicol Environ Saf 92: 265–270. https://doi.org/10.1016/j.ecoenv.2013.01.030

Clark CR, Burnett DM, Parker CM, Arp EW, Swanson MS, Minsavage GD, Kriech AJ, Osborn LV, Freeman JJ, Barter RA, Newton PE, Beazley SL, Stewart CW (2011) Asphalt fume dermal carcinogenicity potential: I. dermal carcinogenicity evaluation of asphalt (bitumen) fume condensates. Regul Toxicol Pharmacol 61(1): 9–16. https://doi.org/10.1016/j.yrtph.2011.04.003

Davies MG (1996) A large outbreak of bitumen-induced phototoxicity in a dockyard. Contact Dermatitis 35(3): 188–189. https://doi.org/10.1111/j.1600-0536.1996.tb02351.x

De Méo M, Genevois C, Brandt H, Laget M, Bartsch H, Castegnaro M (1996) In vitro studies of the genotoxic effects of bitumen and coal-tar fume condensates: comparison of data obtained by mutagenicity testing and DNA adduct analysis by 32P-postlabelling. Chem Biol Interact 101(2): 73–88. https://doi.org/10.1016/0009-2797(96)03705-2

Dhondge A, Surendran S, Seralathan MV, Naoghare PK, Krishnamurthi K, Devi SS, Chakrabarti T (2012) Cellular alterations and modulation of protein expression in bitumen-challenged human osteoblast cells. Environ Sci Pollut Res Int 19(9): 4030–4041. https://doi.org/10.1007/s11356-012-0879-z

Ellingsen DG, Ulvestad B, Andersson L, Barregard L (2010) Pneumoproteins and inflammatory biomarkers in asphalt pavers. Biomarkers 15(6): 498–507. https://doi.org/10.3109/1354750X.2010.490305

Engholm G, Englund A, Linder B (1991) Mortality and cancer incidence in Swedish road paving asphalt workers and roofers. Eur Asphalt Mag 2: 62–67

Eurobitume (2018) Angaben zum Produktionsvolumen. Email, 04 May 2018

Fayerweather WE (2007) Meta-analysis of lung cancer in asphalt roofing and paving workers with external adjustment for confounding by coal tar. J Occup Environ Hyg 4(S1): 175–200. https://doi.org/10.1080/15459620701335035

Fenga C, Loreto C, Caltabiano C, Germanò D (2000) Heat Shock Protein 27 is overexpressed in the skin of bitumen exposed workers. Early observations. Boll Soc Ital Biol Sper 76(11–12): 81–86

Freeman JJ, Schreiner CA, Beazley S, Burnett DM, Clark CR, Mahagaokar S, Parker CM, Stewart CW, Swanson MS, Arp EW (2011) Asphalt fume dermal carcinogenicity potential: II. Initiation-promotion assay of Type III built-up roofing asphalt. Regul Toxicol Pharmacol 61(1): 17–22. https://doi.org/10.1016/j.yrtph.2011.05.008

Fuhst R, Creutzenberg O, Ernst H, Hansen T, Pohlmann G, Preiss A, Rittinghausen S (2007) 24 Months inhalation carcinogenicity study of bitumen fumes in Wistar (WU) rats. J Occup Environ Hyg 4(S1): 20–43. https://doi.org/10.1080/15459620701326257

Gage SL, Robertson JM, Donnelly KC, Hagen AP (1991) Qualitative assessment of the mutagenicity of road coating asphalt. Bull Environ Contam Toxicol 47(4): 617–622. https://doi.org/10.1007/BF01700954

Gate L, Langlais C, Micillino J-C, Nunge H, Bottin M-C, Wrobel R, Binet S (2006) Bitumen fume-induced gene expression profile in rat lung. Toxicol Appl Pharmacol 215(1): 83–92. https://doi.org/10.1016/j.taap.2006.01.012

Gate L, Bottin M-C, Rihn B, Micillino JC, Monhoven N, Nunge H, Morel G, Wrobel R, Champmartin C, Keith G, Binet S (2007) Assessment of the pulmonary genotoxicity of bitumen fumes in Big Blue® transgenic rats. J Occup Environ Hyg 4(S1): 217–219. https://doi.org/10.1080/15459620701334889

Geller F, Urfer W, Golka K (2008) Bladder cancer and occupational exposures in North Rhine-Westphalia, Germany. J Toxicol Environ Health A 71(13–14): 856–858. https://doi.org/10.1080/15287390801987956

Genevois C, Brandt HCA, Bartsch H, Obrecht-pflumio S, Wild CP, Castegnaro M (1996) Formation of DNA adducts in skin, lung and lymphocytes after skin painting of rats with undiluted bitumen or coal-tar fume condensates. Polycyclic Aromat Compd 8(2–3): 75–92. https://doi.org/10.1080/10406639608048337

Genevois C, Pfohl-Leszkowicz A, Boillot K, Brandt H, Castegnaro M (1998) Implication of cytochrome P-450 1A isoforms and the AH receptor in the genotoxicity of coal-tar fume condensate and bitumen fume condensates. Environ Toxicol Pharmacol 5(4): 283–294. https://doi.org/10.1016/s1382-6689(98)00013-1

Genevois-Charmeau C, Binet S, Bonnet P, Lafontaine M, Brandt H, Kriech A, Groot PCD, Wissel H, Garren L, Morele Y, Nunge H, Castegnaro M (2001) Inhalation study on exposure to bitumen fumes: formation of DNA adducts in various rat tissues following nose-only inhalation. Polycyclic Aromat Compd 18(4): 427–449. https://doi.org/10.1080/10406630108233819

Gesprächskreis Bitumen (2009) Temperaturabgesenkte Asphalte. Berlin: Berufsgenossenschaft Bau. https://www.bgbau.de/fileadmin/Gisbau/BitumenBroschuere.pdf, accessed 22 Mar 2017

Goodrich J, Goodrich J, Kari W (1986) Asphalt analysis, sulfur, mixes, and seal coats. In: Transportation Research Record 1096. Volume Asphalt composition tests: their application and relation to field performance. Washington, DC: Transportation Research Board, National Research Council. p. 146–167. https://onlinepubs.trb.org/Onlinepubs/trr/1986/1096/1096-017.pdf, accessed 22 Mar 2017

Goyak KO, McKee RH, Minsavage GD, McGowan C, Daughtrey WC, Freeman JJ (2011) Paving asphalt products exhibit a lack of carcinogenic and mutagenic activity. Int J Toxicol 30(5): 492–497. https://doi.org/10.1177/1091581811415700

Greim H, editor (2002) Bitumen (vapour and aerosol). MAK Value Documentation, 2001. In: Occupational Toxicants. Volume 17. Weinheim: Wiley-VCH. p. 37–118. Also available from https://doi.org/10.1002/3527600418.mb805242e0017

Halter R, Hansen T, Seidel A, Ziemann C, Borlak J (2007) Importance of DNA-adduct formation and gene expression profiling of disease candidate genes in rats exposed to bitumen fumes. J Occup Environ Hyg 4(S1): 44–64. https://doi.org/10.1080/15459620701337528

Hansen ES (1989 a) Cancer incidence in an occupational cohort exposed to bitumen fumes. Scand J Work Environ Health 15(2): 101–105. https://doi.org/10.5271/sjweh.1875

Hansen ES (1989 b) Cancer mortality in the asphalt industry: a ten year follow up of an occupational cohort. Br J Ind Med 46(8): 582–585. https://doi.org/10.1136/oem.46.8.582

Heikkilä PR, Väänänen V, Hämeilä M, Linnainmaa K (2003) Mutagenicity of bitumen and asphalt fumes. Toxicol In Vitro 17(4): 403–412. https://doi.org/10.1016/s0887-2333(03)00045-6

Herbert R, Marcus M, Wolff MS, Perera FP, Andrews L, Godbold JH, Rivera M, Stefanidis M, Lu XQ, Landrigan PJ (1990 a) A pilot study of detection of DNA adducts in white blood cells of roofers by 32P-postlabelling. In: Vainio H, Sorsa M, McMichael AJ, editors. Complex mixtures and cancer risk. IARC Scientific Publications, No. 104. Lyon: IARC. p. 205–214. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Scientific-Publications/Complex-Mixtures-And-Cancer-Risk-1990

Herbert R, Marcus M, Wolff MS, Perera FP, Andrews L, Godbold JH, Rivera M, Stefanidis M, Lu XQ, Landrigan PJ (1990 b) Detection of adducts of deoxyribonucleic acid in white blood cells of roofers by 32P-postlabeling. Relationship of adduct levels to measures of exposure to polycyclic aromatic hydrocarbons. Scand J Work Environ Health 16(2): 135–143. https://doi.org/10.5271/sjweh.1806

Hong Y-C, Lee K-H (1999) Enhancement of DNA damage and involvement of reactive oxygen species after exposure to bitumen with UVA irradiation. Mutat Res 426(1): 63–69. https://doi.org/10.1016/s0027-5107(99)00076-7

Hooiveld M, Spee T, Burstyn I, Kromhout H, Heederik D (2003) Lung cancer mortality in a Dutch cohort of asphalt workers: evaluation of possible confounding by smoking. Am J Ind Med 43(1): 79–87. https://doi.org/10.1002/ajim.10141

IARC (International Agency for Research on Cancer) (2013) Bitumens and bitumen emissions, and some N- and S-heterocyclic polycyclic aromatic hydrocarbons. In: IARC monographs on the evaluation of carcinogenic risks to humans. Volume 103. Lyon: IARC. p. 9–303. https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono103.pdf, accessed 22 Mar 2017

IFA (Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung) (2018) GESTIS International Limit Values. Limit value: Bitumen. http://limitvalue.ifa.dguv.de, accessed 22 Mar 2017

ITEM (Fraunhofer-Institut für Toxikologie und Experimentelle Medizin) (2017) Angaben zur Umrechnung auf Bitumenkondensatstandard. Email, 11 Oct 2017

Järvholm B, Nordström G, Högstedt B, Levin JO, Wahlström J, Ostman C, Bergendahl C (1999) Exposure to polycyclic aromatic hydrocarbons and genotoxic effects on nonsmoking Swedish road pavement workers. Scand J Work Environ Health 25(2): 131–136. https://doi.org/10.5271/sjweh.415

Jöckel K-H, Ahrens W, Jahn I, Pohlabeln H, Bolm-Audorff U (1998) Occupational risk factors for lung cancer: a case-control study in West Germany. Int J Epidemiol 27(4): 549–560. https://doi.org/10.1093/ije/27.4.549

Karaman A, Pirim I (2009) Exposure to bitumen fumes and genotoxic effects on Turkish asphalt workers. Clin Toxicol (Phila) 47(4): 321–326. https://doi.org/10.1080/15563650902817393

Knecht U, Stahl S, Woitowitz H-J (1999) Handelsübliche Bitumensorten: PAH-Massegehalte und temperaturabhängiges Emissionsverhalten unter standardisierten Bedingungen. Gefahrst Reinhalt Luft 59(11–12): 429–434

Kriech AJ, Osborn LV, Wissel HL, Redman AP, Smith LA, Dobbs TE (2007) Generation of bitumen fumes using two fume generation protocols and comparison to worker industrial hygiene exposures. J Occup Environ Hyg 4(S1): 6–19. https://doi.org/10.1080/15459620701358102

Kriech AJ, Emmel C, Osborn LV, Breuer D, Redman AP, Hoeber D, Bochmann F, Ruehl R (2010) Side-by-side comparison of field monitoring methods for hot bitumen emission exposures: the German IFA Method 6305, U.S. NIOSH Method 5042, and the Total Organic Matter Method. J Occup Environ Hyg 7(12): 712–725. https://doi.org/10.1080/15459624.2010.529792

Kriech AJ, Schreiner CA, Osborn LV, Riley AJ (2018) Assessing cancer hazards of bitumen emissions – a case study for complex petroleum substances. Crit Rev Toxicol 48(2): 121–142. https://doi.org/10.1080/10408444.2017.1391170

Lee BM, Yin BY, Herbert R, Hemminki K, Perera FP, Santella RM (1991) Immunologic measurement of polycyclic aromatic hydrocarbon-albumin adducts in foundry workers and roofers. Scand J Work Environ Health 17(3): 190–194. https://doi.org/10.5271/sjweh.1711

Lesueur D (2009) The colloidal structure of bitumen: consequences on the rheology and on the mechanisms of bitumen modification. Adv Colloid Interface Sci 145(1–2): 42–82. https://doi.org/10.1016/j.cis.2008.08.011

Lindberg HK, Väänänen V, Järventaus H, Suhonen S, Nygren J, Hämeilä M, Valtonen J, Heikkilä P, Norppa H (2008) Genotoxic effects of fumes from asphalt modified with waste plastic and tall oil pitch. Mutat Res 653(1–2): 82–90. https://doi.org/10.1016/j.mrgentox.2008.03.009

Lindberg S, Agner T, Pedersen E (2015) Phototoxic reaction after exposure to bitumen. J Clin Exp Dermatol Res 6(3): 283. https://doi.org/10.4172/2155-9554.1000283

Loreto C, Rapisarda V, Carnazza ML, Musumeci G, D'Agata V, Valentino M, Martinez G (2007) Bitumen products alter bax, bcl-2 and cytokeratin expression: an in vivo study of chronically exposed road pavers. J Cutan Pathol 34(9): 699–704. https://doi.org/10.1111/j.1600-0560.2006.00687.x

Ma JYC, Yang H-M, Barger MW, Siegel PD, Zhong B-Z, Kriech AJ, Castranova V (2002) Alteration of pulmonary cytochrome P-450 system: effects of asphalt fume condensate exposure. J Toxicol Environ Health A 65(17): 1247–1260. https://doi.org/10.1080/152873902760125732

Ma C, Wang J, Luo J (2003 a) Exposure to asphalt fumes activates activator protein-1 through the phosphatidylinositol 3-kinase/Akt signaling pathway in mouse epidermal cells. J Biol Chem 278(45): 44265–44272. https://doi.org/10.1074/jbc.M309023200

Ma JYC, Rengasamy A, Frazer D, Barger MW, Hubbs AF, Battelli L, Tomblyn S, Stone S, Castranova V (2003 b) Inhalation exposure of rats to asphalt fumes generated at paving temperatures alters pulmonary xenobiotic metabolism pathways without lung injury. Environ Health Perspect 111(9): 1215–1221. https://doi.org/10.1289/ehp.5740

Machado ML, Beatty PW, Fetzer JC, Glickman AH, McGinnis EL (1993) Evaluation of the relationship between PAH content and mutagenic activity of fumes from roofing and paving asphalts and coal tar pitch. Fundam Appl Toxicol 21(4): 492–499. https://doi.org/10.1006/faat.1993.1125

Major J, Jakab MG, Tompa A (1999) The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals. Mutat Res 445(2): 241–249. https://doi.org/10.1016/s1383-5718(99)00129-1

Major J, Jakab MG, Tompa A (2001) Working condition-related improvement in genotoxicological parameters of Hungarian road pavers. J Toxicol Environ Health A 62(5): 319–331. https://doi.org/10.1080/152873901300018039

Marczynski B, Raulf-Heimsoth M, Preuss R, Kappler M, Schott K, Pesch B, Zoubek G, Hahn J-U, Mensing T, Angerer J, Käfferlein HU, Brüning T (2006) Assessment of DNA damage in WBCs of workers occupationally exposed to fumes and aerosols of bitumen. Cancer Epidemiol Biomarkers Prev 15(4): 645–651. https://doi.org/10.1158/1055-9965.EPI-05-0562

Marczynski B, Raulf-Heimsoth M, Spickenheuer A, Mensing T, Welge P, Förster K, Angerer J, Pesch B, Bramer R, Käfferlein HU, Breuer D, Hahn J-U, Brüning T (2007) Ambient and biological monitoring of exposure and genotoxic effects in mastic asphalt workers exposed to fumes of bitumen. J Occup Environ Hyg 4(Suppl 1): 127–136. https://doi.org/10.1080/15459620701296617

Marczynski B, Raulf-Heimsoth M, Pesch B, Kendzia B, Käfferlein HU, Vosshans B, Borowitzki G, Lee E-H, Bramer R, Brüning T (2010) Detection of DNA strand breaks by comet assay in sputum leucocytes of bitumen-exposed workers: a pilot study. Hum Exp Toxicol 29(9): 721–729. https://doi.org/10.1177/0960327109359635

Marczynski B, Raulf-Heimsoth M, Spickenheuer A, Pesch B, Kendzia B, Mensing T, Engelhardt B, Lee E-H, Schindler BK, Heinze E, Welge P, Bramer R, Angerer J, Breuer D, Käfferlein HU, Brüning T (2011) DNA adducts and strand breaks in workers exposed to vapours and aerosols of bitumen: associations between exposure and effect. Arch Toxicol 85(Suppl 1): S53–S64. https://doi.org/10.1007/s00204-011-0682-5

McClean MD, Rinehart RD, Ngo L, Eisen EA, Kelsey KT, Herrick RF (2004 a) Inhalation and dermal exposure among asphalt paving workers. Ann Occup Hyg 48(8): 663–671. https://doi.org/10.1093/annhyg/meh062

McClean MD, Rinehart RD, Ngo L, Eisen EA, Kelsey KT, Wiencke JK, Herrick RF (2004 b) Urinary 1-hydroxypyrene and polycyclic aromatic hydrocarbon exposure among asphalt paving workers. Ann Occup Hyg 48(6): 565–578. https://doi.org/10.1093/annhyg/meh044

McClean MD, Rinehart RD, Sapkota A, Cavallari JM, Herrick RF (2007 a) Dermal exposure and urinary 1-hydroxypyrene among asphalt roofing workers. J Occup Environ Hyg 4(Suppl 1): 118–126. https://doi.org/10.1080/15459620701334756

McClean MD, Wiencke JK, Kelsey KT, Varkonyi A, Ngo L, Eisen EA, Herrick RF (2007 b) DNA adducts among asphalt paving workers. Ann Occup Hyg 51(1): 27–34. https://doi.org/10.1093/annhyg/mel069

McClean MD, Osborn LV, Snawder JE, Olsen LD, Kriech AJ, Sjödin A, Li Z, Smith JP, Sammons DL, Herrick RF, Cavallari JM (2012) Using urinary biomarkers of polycyclic aromatic compound exposure to guide exposure-reduction strategies among asphalt paving workers. Ann Occup Hyg 56(9): 1013–1024. https://doi.org/10.1093/annhyg/mes058

McGowan C, Daughtrey W, Freeman J, McKee R (1992) Lack of carcinogenic and mutagenic activity with asphalt products. Toxicologist 12: 1484A

Micillino JC, Coulais C, Binet S, Bottin M-C, Keith G, Moulin D, Rihn BH (2002) Lack of genotoxicity of bitumen fumes in transgenic mouse lung. Toxicology 170(1–2): 11–20. https://doi.org/10.1016/s0300-483x(01)00507-8

Monarca S, Pasquini R, Scassellati Sforzolini G, Savino A, Bauleo FA, Angeli G (1987) Environmental monitoring of mutagenic/carcinogenic hazards during road paving operations with bitumens. Int Arch Occup Environ Health 59(4): 393–402. https://doi.org/10.1007/BF00405283

Mundt KA, Dell LD, Crawford L, Sax SN, Boffetta P (2018) Cancer risk associated with exposure to bitumen and bitumen fumes: an updated systematic review and meta-analysis. J Occup Environ Med 60(1): e6–e54. https://doi.org/10.1097/JOM.0000000000001202

Murray EB, Edwards JW (2005) Differential induction of micronuclei in peripheral lymphocytes and exfoliated urothelial cells of workers exposed to 4,4′-methylenebis-(2-chloroaniline) (MOCA) and bitumen fumes. Rev Environ Health 20(3): 163–176. https://doi.org/10.1515/reveh.2005.20.3.163

Neghab M, Zare Derisi F, Hassanzadeh J (2015) Respiratory symptoms and lung functional impairments associated with occupational exposure to asphalt fumes. Int J Occup Environ Med 6(2): 113–121. https://doi.org/10.15171/ijoem.2015.473

Neghab M, Derisi FZ, Hassanzadeh J, Dirin V, Heidari S (2017) Toxic responses of different organs following occupational exposure to sub-threshold limit value levels of paving asphalt fumes. Toxicol Environ Chem 99(2): 331–339. https://doi.org/10.1080/02772248.2016.1172581

NIOSH (The National Institute for Occupational Safety and Health) (1998 a) Benzene-soluble fraction and total particulate (asphalt fume). NIOSH Manual of Analytical Methods (NMAM), 4th edition. Method 5042. Cincinnatti, OH: NIOSH. https://www.cdc.gov/niosh/docs/2003-154/pdfs/5042.pdf, accessed 22 Mar 2017

NIOSH (The National Institute for Occupational Safety and Health) (1998 b) Polycyclic aromatic compounds, total (PACs). NIOSH Manual of Analytical Methods (NMAM), 4th edition. Method 5800. Cincinnatti, OH: NIOSH. https://www.cdc.gov/niosh/docs/2003-154/pdfs/5800.pdf, accessed 22 Mar 2017

NIOSH (The National Institute for Occupational Safety and Health) (1998 c) Polynuclear aromatic hydrocarbons by HPLC. NIOSH Manual of Analytical Methods (NMAM), 4th edition. Method 5506. Cincinnatti, OH: NIOSH. https://www.cdc.gov/niosh/docs/2003-154/pdfs/5506.pdf, accessed 22 Mar 2017

Olsson A, Kromhout H, Agostini M, Hansen J, Lassen CF, Johansen C, Kjaerheim K, Langård S, Stücker I, Ahrens W, Behrens T, Lindbohm M-L, Heikkilä P, Heederik D, Portengen L, Shaham J, Ferro G, de Vocht F, Burstyn I, Boffetta P (2010) A case-control study of lung cancer nested in a cohort of European asphalt workers. Environ Health Perspect 118(10): 1418–1424. https://doi.org/10.1289/ehp.0901800

Pan SY, Ugnat A-M, Mao Y, Canadian Cancer Registries Epidemiology Research Group (2005) Occupational risk factors for brain cancer in Canada. J Occup Environ Med 47(7): 704–717. https://doi.org/10.1097/01.jom.0000165747.95801.c5

Parker CM, Schreiner CA, Hallmark N, Kriech AJ, Osborn LV, Fuhst R, Buschmann J, Ernst H, Hansen T, Pohlmann G, Preiss A, Ziemann C (2011) Evaluation of reproductive/developmental and repeated dose (subchronic) toxicity and cytogenetic effects in rats of a roofing asphalt fume condensate by nose-only inhalation. Regul Toxicol Pharmacol 59(3): 445–453. https://doi.org/10.1016/j.yrtph.2011.01.010

Pasquini R, Taningher M, Monarca S, Pala M, Angeli G (1989) Chemical composition and genotoxic activity of petroleum derivatives collected in two working environments. J Toxicol Environ Health 27(2): 225–238. https://doi.org/10.1080/15287398909531293

Pasquini R, Scassellati Sforzolini G, Monarca S, Fatigoni C (1992) In vivo study of genotoxicity markers and enzymatic induction capability of a bitumen sample. J Occup Med Toxicol 1(2): 181–197

Perico A, Gottardi M, Boddi V, Bavazzano P, Lanciotti E (2001) Assessment of exposure to polycyclic aromatic hydrocarbons in police in Florence, Italy, through personal air sampling and biological monitoring of the urinary metabolite 1-hydroxypyrene. Arch Environ Health 56(6): 506–512. https://doi.org/10.1080/00039890109602899

Pesch B, Spickenheuer A, Kendzia B, Schindler BK, Welge P, Marczynski B, Rihs H-P, Raulf-Heimsoth M, Angerer J, Brüning T (2011) Urinary metabolites of polycyclic aromatic hydrocarbons in workers exposed to vapours and aerosols of bitumen. Arch Toxicol 85(Suppl 1): S29–S39. https://doi.org/10.1007/s00204-011-0680-7

Phillips DH, Castegnaro M (1999) Standardization and validation of DNA adduct postlabelling methods: report of interlaboratory trials and production of recommended protocols. Mutagenesis 14(3): 301–315. https://doi.org/10.1093/mutage/14.3.301

Pohlmann G, Preiss A, Koch W, Kock H, Elend M, Raabe M (2006 a) Collection, validation and generation of bitumen fumes for inhalation studies in rats Part 3: Regeneration of bitumen fumes, inhalation setup, validation. Ann Occup Hyg 50(8): 813–819. https://doi.org/10.1093/annhyg/mel055

Pohlmann G, Preiss A, Levsen K, Raabe M, Koch W (2006 b) Collection, validation and generation of bitumen fumes for inhalation studies in rats Part 2: Collection of bitumen fumes from storage tanks. Ann Occup Hyg 50(8): 805–812. https://doi.org/10.1093/annhyg/mel048

Preiss A, Koch W, Kock H, Elend M, Raabe M, Pohlmann G (2006) Collection, validation and generation of bitumen fumes for inhalation studies in rats Part 1: Workplace samples and validation criteria. Ann Occup Hyg 50(8): 789–804. https://doi.org/10.1093/annhyg/mel047

Pukkala EI (1995) Cancer risk by social class and occupation: a survey of 109,000 cancer cases among Finns of working age. Contributions to epidemiology and biostatistics. Volume 7. Wahrendorf J, editor. Basel: Karger

Qian H-W, Ong T, Whong W-Z (1996) Induction of micronuclei in cultured mammalian cells by fume condensates of roofing asphalt. Am J Ind Med 29(5): 554–559. https://doi.org/10.1002/(SICI)1097-0274(199605)29:5<554::AID-AJIM16>3.0.CO;2-#

Qian H-W, Ong T, Nath J, Whong W-Z (1998) Induction of DNA adducts in vivo in rat lung cells by fume condensates of roofing asphalt. Teratog Carcinog Mutagen 18(3): 131–140

Qian H-W, Whong W-Z, Olsen L, Nath J, Ong T (1999) Induction of micronuclei in V79 cells by fractions of roofing asphalt fume condensate. Mutat Res 441(2): 163–170. https://doi.org/10.1016/s1383-5718(99)00045-5

Randem BG, Burstyn I, Langård S, Svane O, Järvholm B, Kauppinen T, Bergdahl IA, Johansen C, Hansen J, Partanen T, Kromhout H, Ferro G, Boffetta P (2004 a) Cancer incidence of Nordic asphalt workers. Scand J Work Environ Health 30(5): 350–355. https://doi.org/10.5271/sjweh.822

Randem BG, Ulvestad B, Burstyn I, Kongerud J (2004 b) Respiratory symptoms and airflow limitation in asphalt workers. Occup Environ Med 61(4): 367–369. https://doi.org/10.1136/oem.2002.006114

Rapisarda V, Carnazza ML, Caltabiano C, Loreto C, Musumeci G, Valentino M, Martinez G (2009) Bitumen products induce skin cell apoptosis in chronically exposed road pavers. J Cutan Pathol 36(7): 781–787. https://doi.org/10.1111/j.1600-0560.2008.01140.x

Raulf-Heimsoth M, Pesch B, Schott K, Kappler M, Preuss R, Marczynski B, Angerer J, Rihs HP, Hahn JU, Merget R, Brüning T (2007) Irritative effects of fumes and aerosols of bitumen on the airways: results of a cross-shift study. Arch Toxicol 81(1): 35–44. https://doi.org/10.1007/s00204-006-0115-z

Raulf-Heimsoth M, Marczynski B, Spickenheuer A, Pesch B, Welge P, Rühl R, Bramer R, Kendzia B, Heinze E, Angerer J, Brüning T (2011 a) Bitumen workers handling mastic versus rolled asphalt in a tunnel: assessment of exposure and biomarkers of irritation and genotoxicity. Arch Toxicol 85(Suppl 1): S81–S87. https://doi.org/10.1007/s00204-011-0685-2

Raulf-Heimsoth M, Pesch B, Kendzia B, Spickenheuer A, Bramer R, Marczynski B, Merget R, Brüning T (2011 b) Irritative effects of vapours and aerosols of bitumen on the airways assessed by non-invasive methods. Arch Toxicol 85(Suppl 1): S41–S52. https://doi.org/10.1007/s00204-011-0681-6

Raulf-Heimsoth M, Pesch B, Rühl R, Brüning T (2011 c) The Human Bitumen Study: executive summary. Arch Toxicol 85(Suppl 1): S3–S9. https://doi.org/10.1007/s00204-011-0679-0

Reinke G, Swanson M, Paustenbach D, Beach J (2000) Chemical and mutagenic properties of asphalt fume condensates generated under laboratory and field conditions. Mutat Res 469(1): 41–50. https://doi.org/10.1016/s1383-5718(00)00068-1

Rhomberg LR, Mayfield DB, Goodman JE, Butler EL, Nascarella MA, Williams DR (2015) Quantitative cancer risk assessment for occupational exposures to asphalt fumes during built-up roofing asphalt (BURA) operations. Crit Rev Toxicol 45(10): 873–918. https://doi.org/10.3109/10408444.2015.1094450

Richiardi L, Boffetta P, Simonato L, Forastiere F, Zambon P, Fortes C, Gaborieau V, Merletti F (2004) Occupational risk factors for lung cancer in men and women: a population-based case-control study in Italy. Cancer Causes Control 15(3): 285–294. https://doi.org/10.1023/B:CACO.0000024223.91059.ed

Rumler R, Rühl R, Nies E, Rode P, Heger M (2007) Health complaints of German mastic asphalt workers. J Occup Environ Hyg 4(Suppl 1): 233–236. https://doi.org/10.1080/15459620701337635

Schaffer A, Shafrir A, Suprun H, Calabrezzi R (1985) Ergebnisse der Gesundheitsüberwachung von Bitumen-Asphalt-Arbeitern. Arbeitsmed Sozialmed Praventivmed 20(9): 205–207

Sellappa S, Mani B, Keyan KS (2011) Cytogenetic biomonitoring of road paving workers occupationally exposed to polycyclic aromatic hydrocarbons. Asian Pac J Cancer Prev 12(3): 713–717

Serdar B, Lee D, Dou Z (2012) Biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs) and DNA damage: a cross-sectional pilot study among roofers in South Florida. BMJ Open 2(4): e001318. https://doi.org/10.1136/bmjopen-2012-001318

Serdar B, Brindley S, Dooley G, Volckens J, Juarez-Colunga E, Gan R (2016) Short-term markers of DNA damage among roofers who work with hot asphalt. Environ Health 15(1): 99. https://doi.org/10.1186/s12940-016-0182-4

Shamsuddin AK, Sinopoli NT, Hemminki K, Boesch RR, Harris CC (1985) Detection of benzo(a)pyrene:DNA adducts in human white blood cells. Cancer Res 45(1): 66–68

Sivak A, Menzies K, Beltis K, Worthington J, Ross A (1989) Assessment of the cocarcinogenic/promoting activity of asphalt fumes. 200-83–2612. Cambridge, MA (USA): Arthur D. Little, Inc. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB91110213.xhtml, accessed 22 Mar 2017

Sobus JR, McClean MD, Herrick RF, Waidyanatha S, Onyemauwa F, Kupper LL, Rappaport SM (2009) Investigation of PAH biomarkers in the urine of workers exposed to hot asphalt. Ann Occup Hyg 53(6): 551–560. https://doi.org/10.1093/annhyg/mep041

Sonntag H-G, Erdinger L (1989) Gutachterliche Stellungnahme zur Frage der gesundheitlichen Relevanz von Emissionen aus Bitumen-Dachbahnen bei Temperaturen von bis zu 80°C. 13 Nov 1989, Heidelberg: Hygiene-Institut der Universität Heidelberg, unpublished

Sörensen A, Wichert B (2009) Asphalt and bitumen. In: Ullmann's encyclopedia of industrial chemistry. Weinheim: Wiley-VCH. https://doi.org/10.1002/14356007.a03_169.pub2, accessed 22 Mar 2017

Tolos WP, Shaw PB, Lowry LK, MacKenzie BA, Deng J-F, Markel HL (1990) 1-Pyrenol: a biomarker for occupational exposure to polycyclic aromatic hydrocarbons. Appl Occup Environ Hyg 5(5): 303–309. https://doi.org/10.1080/1047322X.1990.10389643

Tompa A, Jakab MG, Biró A, Magyar B, Major J (2007) Health, genotoxicology, and immune status of road pavers in Hungary. J Occup Environ Hyg 4(Suppl 1): 154–162. https://doi.org/10.1080/15459620701354481

Toraason M, Hayden C, Marlow D, Rinehart R, Mathias P, Werren D, Olsen LD, Neumeister CE, Mathews ES, Cheever KL, Marlow KL, DeBord DG, Reid TM (2001) DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure. Int Arch Occup Environ Health 74(6): 396–404. https://doi.org/10.1007/s004200100238

Trumbore D, Osborn L, Blackburn G, Niebo R, Kriech A, Maxim LD (2011) Effect of oxidation and extent of oxidation on biologically active PACs in asphalt products. Inhal Toxicol 23(12): 745–761. https://doi.org/10.3109/08958378.2011.608742

Ulvestad B, Randem BG, Skare Ø, Aaløkken TM, Myranek GK, Elihn K, Lund MB (2017) Lung function in asphalt pavers: a longitudinal study. Int Arch Occup Environ Health 90(1): 63–71. https://doi.org/10.1007/s00420-016-1173-z

Väänänen V, Hämeilä M, Kontsas H, Peltonen K, Heikkilä P (2003) Air concentrations and urinary metabolites of polycyclic aromatic hydrocarbons among paving and remixing workers. J Environ Monit 5(5): 739–746. https://doi.org/10.1039/b304096h

Väänänen V, Elovaara E, Nykyri E, Santonen T, Heikkilä P (2006) Road pavers' occupational exposure to asphalt containing waste plastic and tall oil pitch. J Environ Monit 8(1): 89–99. https://doi.org/10.1039/b513505b

Walter D, Knecht U (2007) Standardized investigation of percutaneous absorption of bitumen emission in humans. J Occup Environ Hyg 4(Suppl 1): 144–153. https://doi.org/10.1080/15459620701354556

Wang J, Lewis DM, Castranova V, Frazer DG, Goldsmith T, Tomblyn S, Simpson J, Stone S, Afshari A, Siegel PD (2001) Characterization of asphalt fume composition under simulated road paving conditions by GC/MS and microflow LC/quadrupole time-of-flight MS. Anal Chem 73(15): 3691–3700. https://doi.org/10.1021/ac010334m

Wang JJ, Frazer DG, Law B, Lewis DM (2003 a) Identification and quantification of urinary benzo[a]pyrene and its metabolites from asphalt fume exposed mice by microflow LC coupled to hybrid quadrupole time-of-flight mass spectrometry. Analyst 128(7): 864–870. https://doi.org/10.1039/b302617p

Wang JJ, Marshall WD, Frazer DG, Law B, Lewis DM (2003 b) Characterization of DNA adducts from lung tissue of asphalt fume-exposed mice by nanoflow liquid chromatography quadrupole time-of-flight mass spectrometry. Anal Biochem 322(1): 79–88. https://doi.org/10.1016/j.ab.2003.07.001

Watkins DK, Chiazze L, Fryar CD, Fayerweather W (2002) A case control study of lung cancer and non-malignant respiratory disease among employees in asphalt roofing manufacturing and asphalt production. J Occup Environ Med 44(6): 551–558. https://doi.org/10.1097/00043764-200206000-00018

Welge P, Marczynski B, Raulf-Heimsoth M, Spickenheuer A, Kendzia B, Heinze E, Angerer J, Käfferlein HU, Pesch B, Brüning T (2011) Assessment of micronuclei in lymphocytes from workers exposed to vapours and aerosols of bitumen. Arch Toxicol 85(Suppl 1): S65–S71. https://doi.org/10.1007/s00204-011-0683-4

Yilmaz ÖH, Bal C, Neşelioglu S, Büyükşekerci M, Gündüzöz M, Eren F, Tutkun L, Yilmaz FM (2016) Thiol/disulfide homeostasis in asphalt workers. Arch Environ Occup Health 71(5): 268–272. https://doi.org/10.1080/19338244.2015.1076760

Zhao HW, Yin XJ, Frazer D, Barger MW, Siegel PD, Millecchia L, Zhong BZ, Tomblyn S, Stone S, Ma JKH, Castranova V, Ma JYC (2004) Effects of paving asphalt fume exposure on genotoxic and mutagenic activities in the rat lung. Mutat Res 557(2): 137–149. https://doi.org/10.1016/j.mrgentox.2003.10.006