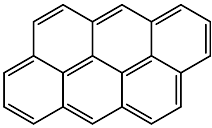
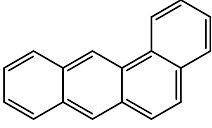
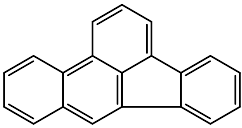
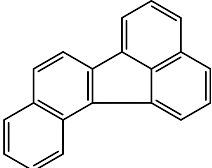
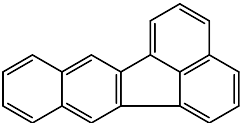
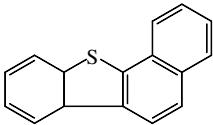
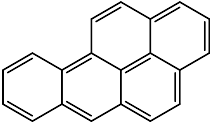
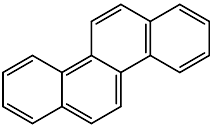
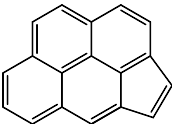
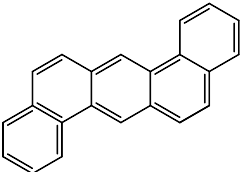


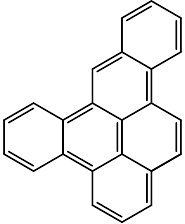
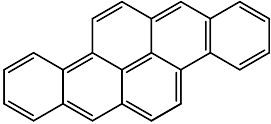
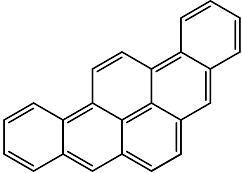
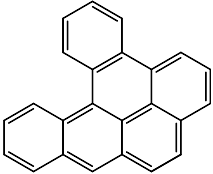
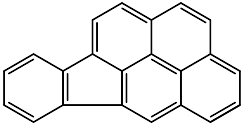
# Polycyclic aromatic hydrocarbons (PAH)

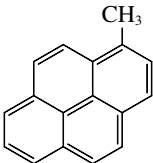
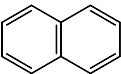
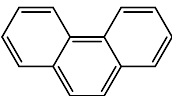
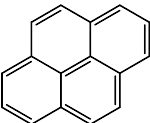
MAK value	–
Peak limitation	–
Absorption through the skin (2007)	H
Sensitization	–
Prenatal toxicity	–

Substance	Structural formula	Carcinogen Category (2007)	Germ Cell Mutagen Category (2007)
anthanthrene		2	– <sup>a</sup>
benzo[ <i>a</i> ]anthracene		2	3A
benzo[ <i>b</i> ]fluoranthene		2	3B
benzo[ <i>j</i> ]fluoranthene		2	3B

## 2 | Polycyclic aromatic hydrocarbons (PAH)

Substance	Structural formula	Carcinogen Category (2007)	Germ Cell Mutagen Category (2007)
benzo[k]fluoranthene		2	3B
benzo[b]naphtho- [2,1-d]thiophene		2	3B
benzo[a]pyrene		2	2
chrysene		2	— <sup>a</sup>
cyclopenta[cd]pyrene		2	3B
dibenzo[a,h]anthracene		2	3A

Substance	Structural formula	Carcinogen Category (2007)	Germ Cell Mutagen Category (2007)
dibenzo[ <i>a,e</i> ]pyrene		2	3B
dibenzo[ <i>a,h</i> ]pyrene		2	3B
dibenzo[ <i>a,i</i> ]pyrene		2	3B
dibenzo[ <i>a,l</i> ]pyrene		2	3B
indeno[1,2,3- <i>cd</i> ]pyrene		2	— <sup>a</sup>

Substance	Structural formula	Carcinogen Category (2007)	Germ Cell Mutagen Category (2007)
1-methylpyrene		2	— <sup>a</sup>
naphthalene		2	3B
phenanthrene		— <sup>b</sup>	— <sup>b</sup>
pyrene		— <sup>b</sup>	— <sup>b</sup>

<sup>a</sup> no classification because of lack of data;<sup>b</sup> no classification on the basis of the available data

Chemical name	see Table 1
CAS number	see Table 1
Molecular formula	see Table 1
Synonyms	see Table 2
Molecular weight	see Table 1
Melting point	see Table 1
Boiling point at hPa	see Table 1
Vapour pressure at 20°C	see Table 1
log K <sub>ow</sub>	see Table 1

This documentation is based mainly on the monograph on selected polycyclic aromatic hydrocarbons by the International Programme on Chemical Safety (WHO 1998) and on extracts from contributions to the research report “Polycyclische Aromatische Kohlenwasserstoffe (PAH)” (DFG 2004) of the *ad hoc* working group “PAH” of the MAK Commission. The tables with individual data taken from WHO (1998) have been compiled in an annex for the sake of clarity. The physico-chemical data of the compounds are listed in Table 1, and the IUPAC names and synonyms are shown in Table 2.

Benz[*a*]anthracene is the name used for this compound in the IUPAC nomenclature of 1979. The publication of the IUPAC nomenclature of 1993 stipulates that an “o” should be included in the name “benz” before the following bracket if there is a vowel in this bracket. However, the IUPAC nomenclature of 1993 also stipulates that the name benzo[*a*]anthracene should be replaced by the name “tetraphene” (Moss 1998). This provision has not been followed in this documentation since documentations are published under the names of the compounds as they are generally used in the fields of occupational medicine and toxicology. This is not the case for the name “tetraphene” for benzo[*a*]anthracene.

However, the IUPAC nomenclature of 1993 prescribes that the name benzo[*a*]pyrene should be replaced by the name “benzo[*pqr*]tetraphene”. It is noted that CAS (Chemical Abstracts Service) does not follow this, but continues to use the name “benzo[*a*]pyrene” as an exception (Moss 1998). Since the compound is known under the name benzo[*a*]pyrene primarily in the field of occupational medicine and toxicology, the provision by IUPAC is not followed in compliance with CAS.

## 1 Toxic Effects and Mode of Action

Polycyclic aromatic hydrocarbons (PAH) are considered to be a class of dangerous substances that is highly relevant on account of its carcinogenicity (IARC 1973, 1983; WHO 1998). After metabolism, PAH may have various toxic effects. Comprehensive and detailed reviews (WHO 1998) and studies (DFG 2004) are available on the effect of individual PAH and their metabolites. Reference is made to the 1995 MAK documentation “Naphthalene” (Volume 11, present series) and the 2001 Supplement in this volume. The carcinogenicity of individual PAH has been clearly demonstrated by animal studies using different exposure models (IARC 1973; WHO 1998). Genotoxic effects of PAH were observed in numerous animal studies; they were also detected in human cells *in vitro* and *in vivo* (WHO 1998; Glatt 2005).

For humans, the main sources of PAH exposure are the inhalation of air polluted with PAH and the ingestion of food contaminated with PAH. Extremely high local exposure is found at certain workplaces, for example in coking plants, tar processing plants, underground mines, etc., where absorption through skin

Table 1 Physico-chemical data and classifications of PAH reviewed in this documentation (WHO 1998); except<sup>a)</sup>

Name	CAS No.	Molecular formula	Molecular mass (g/mol)	Melting point (°C)	Boiling point (°C)	Vapour pressure (Pa at 25 °C)	log K <sub>ow</sub>	Genotoxicity (WHO 1998)	Carcinogenicity (WHO 1998)
anthanthrene	191-26-4	C <sub>22</sub> H <sub>12</sub>	276.3	264	547			(+)	+
benzo[ <i>b</i> ]fluoranthene	205-99-2	C <sub>20</sub> H <sub>12</sub>	252.3	168.3	481	6.7 × 10 <sup>-5</sup>	6.12	+	+
benzo[ <i>a</i> ]anthracene	56-55-3	C <sub>18</sub> H <sub>12</sub>	228.3	160.7	400	2.8 × 10 <sup>-5</sup>	5.61	+	+
benzo[ <i>j</i> ]fluoranthene	205-82-3	C <sub>20</sub> H <sub>12</sub>	252.3	165.4	480	2.0 × 10 <sup>-6</sup>	6.12	+	+
benzo[ <i>k</i> ]fluoranthene	207-08-9	C <sub>20</sub> H <sub>12</sub>	252.3	215.7	480	1.3 × 10 <sup>-8</sup>	6.84	+	+
benzo[ <i>b</i> ]naphtho[2,1- <i>dj</i> ]-thiophene <sup>a</sup>	239-35-0	C <sub>16</sub> H <sub>10</sub> S	234.3	185–188	160–180 (3 torr)	–	–	+	+
benzo[ <i>a</i> ]pyrene	50-32-8	C <sub>20</sub> H <sub>12</sub>	252.3	178.1	496	7.3 × 10 <sup>-7</sup>	6.50	+	+
chrysene	218-01-9	C <sub>18</sub> H <sub>12</sub>	228.3	253.8	448	8.4 × 10 <sup>-5</sup>	5.91	+	+
cyclopenta[ <i>cd</i> ]pyrene	27208-37-3	C <sub>18</sub> H <sub>10</sub>	226.3	170	439	–	–	+	+
dibenzo[ <i>a,h</i> ]anthracene	53-70-3	C <sub>22</sub> H <sub>14</sub>	278.4	266.6	524	1.3 × 10 <sup>-8</sup>	6.50	+	+
dibenzo[ <i>a,l</i> ]pyrene	191-30-0	C <sub>24</sub> H <sub>14</sub>	302.4	162.4	595	–	–	(+)	+
dibenzo[ <i>a,e</i> ]pyrene	192-65-4	C <sub>24</sub> H <sub>14</sub>	302.4	244.4	592	–	–	+	+
dibenzo[ <i>a,h</i> ]pyrene	189-64-0	C <sub>24</sub> H <sub>14</sub>	302.4	317	596	–	–	(+)	+
dibenzo[ <i>a,i</i> ]pyrene	189-55-9	C <sub>24</sub> H <sub>14</sub>	302.4	282	594	3.2 × 10 <sup>-10</sup>	7.3	+	+
indeno[1,2,3- <i>cd</i> ]pyrene	193-39-5	C <sub>22</sub> H <sub>12</sub>	276.3	163.6	536	1.3 × 10 <sup>-8</sup>	6.58	+	+
naphthalene <sup>b</sup>	91-20-3	C <sub>10</sub> H <sub>8</sub>	128.2	81	217.9	10.4	3.4	–	
phenanthrene	85-01-8	C <sub>14</sub> H <sub>10</sub>	178.2	100.5	340	1.6 × 10 <sup>-2</sup>	4.6		
pyrene	129-00-0	C <sub>16</sub> H <sub>10</sub>	202.3	150.4	393	6.0 × 10 <sup>-4</sup>	5.18		
1-methylpyrene <sup>c</sup>	2381-21-7	C <sub>17</sub> H <sub>12</sub>	216.3	70–71	410				

<sup>a</sup> +: positive; -: negative; (+): results are based on a small database<sup>b</sup> not contained in WHO 1998; included because of its carcinogenicity in Osborne-Mendel rats after intratracheal instillation (Wenzel-Hartung et al. 1990; Wenzel-Hartung 1992)<sup>c</sup> The studies with B6C3F<sub>1</sub> mice (NTP 1992) and F344 rats (NTP 2000) showed carcinogenicity; there was a significantly increased incidence of pulmonary alveolar and bronchial adenomas in female mice (NTP 1992) and tumours of the olfactory epithelium in rats (NTP 2000).  
included as a representative of alkylated PAH

**Table 2** IUPAC name and synonyms of PAH (sources: NIST (2007); \*: ChemIDplus database)

Compound	IUPAC name	Synonyms
anthanthrene	dibenzo[def,mno]chrysene	dibenzo[cd,jk]pyrene
benzo[a]anthracene	benzo[a]anthracene	1,2-benzanthracene 1,2-benzanthrene 2,3-benzphenanthrene benzo[b]phenanthrene 2,3-benzophenanthrene tetraphene naphthanthracene
benzo[b]fluoranthene	benz[e]acephenanthrylene	3,4-benz[e]acephenanthrylene benzo[e]fluoranthene 2,3-benzofluoranthene 3,4-benzofluoranthene
benzo[j]fluoranthene	benzo[a]fluoranthene	7,8-benzfluoranthene benzo[l]fluoranthene 10,11-benzofluoranthene dibenzo[a,jk]fluorene benzo-12,13-fluoranthene
benzo[k]fluoranthene	benzo[k]fluoranthene	11,12-benzofluoranthene 8,9-benzofluoranthene 2,3:1',8'-binaphthylene dibenzo[b,jk]fluorene
benzo[b]naphtho- [2,1-d]thiophene*	benzo[b]naphtho- [2,1-d]thiophene	11-thiabenzo[a]fluorene 1,2-benzo-9-thiofluorene benzo[a]dibenzothiophene naphtho[1,2-b]thianaphthene naphtho[1,2-d]benzothiophene 9-thia-1,2-benzofluorene
benzo[a]pyrene	benzo[pqr]tetraphene	benzo[def]chrysene 3,4-benzopyrene 6,7-benzopyrene 1,2-benzopyrene 4,5-benzopyrene
chrysene	chrysene	benzo[a]phenanthrene 1,2-benzophenanthrene 1,2,5,6-dibenzonaphthalene
cyclopenta[cd]pyrene	cyclopenta[cd]pyrene	acepyrene acepyrylene cyclopentenopyrene
dibenzo[a,h]anthracene	dibenzo[a,h]anthracene	1,2:5,6-benz[a]anthracene 1,2:5,6-benzanthracene 1,2,5,6-dibenzoanthracene
dibenzo[a,l]pyrene	dibenzo[def,p]chrysene	2,3:4,5-dibenzopyrene 3,4:8,9-dibenzopyrene 4,5,6,7-dibenzopyrene

Table 2 (Continued)

Compound	IUPAC name	Synonyms
		1,2:9,10-dibenzopyrene 1,2:3,4-dibenzopyrene
dibenzo[ <i>a,e</i> ]pyrene	naphtho[1,2,3,4- <i>def</i> ]chrysene	1,2:4,5-dibenzopyrene
dibenzo[ <i>a,h</i> ]pyrene	dibenzo[ <i>b,def</i> ]chrysene	3,4:8,9-dibenzopyrene
dibenzo[ <i>a,i</i> ]pyrene	benzo[ <i>rst</i> ]pentaphene	dibenzo[ <i>b,h</i> ]pyrene 3,4:9,10-dibenzopyrene 1,2:7,8-dibenzopyrene 4,5,8,9-dibenzopyrene
indeno[1,2,3- <i>cd</i> ]pyrene	indeno[1,2,3- <i>cd</i> ]pyrene	1,10-(1,2-phenylene)pyrene 1,10-( <i>o</i> -phenylene)pyrene <i>o</i> -phenylenepyrene 2,3-( <i>o</i> -phenylene)pyrene 2,3-phenylenepyrene
naphthalene	naphthalene	
phenanthrene	phenanthrene	phenanthrine
pyrene	pyrene	benzo[ <i>def</i> ]phenanthrene
*1-methylpyrene	1-methylpyrene	3-methylpyrene

contact may also play an essential role. The good skin penetration of individual PAH has been demonstrated in humans and animal studies. There is no reason to assume that there is much difference in the penetration behaviour of those PAH that have not been investigated.

## 2 Mechanism of Action

PAH are relatively inert hydrophobic compounds that can be converted to highly reactive (+)-*anti*-, (-)-*anti*-, (+)-*syn*- and (-)-*syn*-dihydrodiol epoxides during metabolism in mammals. These diol epoxides react with double strand and single strand DNA, preferably with the  $N^6$  position of guanine and the  $N^2$  position of adenine, with *cis* and *trans* adducts being formed for every *syn* and *anti* enantiomer (Jerina et al. 1991; Scicchitano 2005). Depending on their reactivity and structure, activated PAH can form further adducts. The example of DNA adducts of *anti*-dihydrodiol epoxides was used to demonstrate that certain changes in the conformation of the DNA are associated with the covalent binding. Thus, the aromatic ligand may become positioned in the minor DNA groove without inducing any major changes in conformation, it may intercalate between base pairs in such a way that the double helix is only stretched or it may push between base pairs in such a way that their hydrogen bonds break and individual DNA bases are dis-



placed from the double-helix structure into the outer section of the helix (Geacintov et al. 1997).

Numerous PAH were investigated for their tumourigenicity. Observations were made that compounds that have a bay region, an indentation caused by an angular benzene ring, are severe carcinogens; however, compounds with a fjord region are even more carcinogenic. The structural characteristics of PAH understandably influence both their metabolic activation and the stereochemistry after DNA binding.

There are no conclusive structure-response regularities from which a carcinogenic potency could be derived. The binding frequency (modification density) alone is only a rough standard for the tumourigenic effect. However, the type of DNA binding is of greater influence, because it decides whether programmed cell death or DNA repair occurs; whether the latter proceeds rapidly enough relative to the cell cycle; whether the DNA barrier can be overcome during DNA replication and if so, at the cost of which copy errors (Chakravarti et al. 2000; Conney et al. 2001) and other possible reactions. Mutations in the oncogenes *H-ras*, *K-ras* and *p53* are a frequent finding (DeMarini et al. 2001; Hecht et al. 1998; Noda et al. 2004; Ross and Nesnow 1999). It is difficult to foresee the biological consequences of the numerous types of DNA modifications (Baird et al. 2005).

PAH also lead to gene activations (Ma 2001). The induction of xenobiotic-degrading enzymes of phase I (e.g. cytochrome P4501A1 and -1A2) and phase II (e.g. glutathione *S*-transferase and UDP-glucuronosyl transferase) (Kondraganti et al. 2005) has been well studied; they lead to an increased (and activating) metabolism of PAH and also catalyze the unphysiological degradation of endogenous metabolites. Overexpression, e.g. of the proto-oncogene *c-myc* (Fields et al. 2004), of the transferrin receptor gene (Kemp et al. 2006) and of further genes, has been described.

### 3 Toxicokinetics and Metabolism

#### 3.1 Absorption, distribution and elimination

PAH are absorbed by inhalation, via the skin and via the gastrointestinal tract. The distribution of PAH in the body after administration was investigated in rodents. The levels that were determined in different tissues depend on the specific PAH, the type of administration, the vehicle, the time of determination in the specific tissue after administration and the presence or absence of inducers or inhibitors of hydrocarbon metabolism in the organism. The studies have shown that, after exposure, PAH are detected in every soft tissue, adipose tissues may be a depot from which the PAH are released again and the gastrointestinal tract contains particularly high levels of hydrocarbons and metabolites even if a route of administration other than the oral one was selected (WHO 1998).

Animal studies have demonstrated that PAH are rapidly transported from the site of administration (gastrointestinal tract, lungs or skin) to other tissues via the blood and lymph (Mitchell 1982). This was also evident from the fact that high concentrations of DNA adducts were found in the lungs after application of PAH to the rodent skin (Carmichael et al. 1990; Randerath et al. 1988; Schoket et al. 1988). Absorption of PAH through the skin of humans has been well documented. Very high dermal exposure of workers exposed to creosote was concluded from a field study since the daily excretion of 1-hydroxypyrene exceeded the daily inhalation by a factor of 50 (Elovaara et al. 1995). By means of 1-hydroxypyrene it was demonstrated in 12 coke oven workers that an average of 75% of the total absorbed amount of pyrene enters the body through the skin (Van Rooij et al. 1993 a). Wearing protective clothing reduced skin contamination among workers exposed to creosote by 35% and thus the urinary excretion of 1-hydroxypyrene as a measure of internal exposure by 50% (Van Rooij et al. 1993 b). When a coal-tar ointment was applied epicutaneously to volunteers, the urinary excretion of 1-hydroxypyrene was increased. It was estimated that about 2 µg pyrene/cm<sup>2</sup> was systemically available (Van Rooij et al. 1993 c). After application of a coal-tar ointment to eczema patients, aromatic DNA adduct levels were increased about 2 to 4 times in monocytes, lymphocytes and granulocytes and about 20 times in skin. The urinary excretion of 3-hydroxybenzo[a]pyrene correlated with the adduct levels in the skin (Godschalk et al. 1998). After topical application of a 2% solution of crude coal tar in petrolatum, phenanthrene, anthracene, pyrene and fluoranthene were detected in the peripheral blood (Storer et al. 1984). Volunteers who had been treated with creosote (100 µl) or pyrene (500 µg, applied in a toluene solution) and psoriasis patients who used a shampoo containing coal tar excreted 1-hydroxypyrene as a conjugate in the urine. In both cases, maximum amounts were excreted 10 to 15 hours after treatment (Viau et al. 1995 a).

An *in vitro* study using full-thickness monkey skin showed that the penetration of acenaphthene, anthracene, phenanthrene, fluoranthene, naphthalene, pyrene and fluorene from a lubricating oil is slower than from acetone and artificial sweat. For benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene, it was possible to demonstrate absorption through the skin only when the compounds were dissolved in acetone/artificial sweat (Sartorelli et al. 1999). The penetration of radioactively labelled benzo[a]pyrene was detected in flow-through cells with human skin after up to 24-hour exposure. The study does not contain any quantitative data on the flux level. Treatment of the skin with two skin barrier creams did not reduce absorption; even increased absorption rates were observed in some cases (Van der Bijl et al. 2002).

Excretion of orally or intraperitoneally administered PAH is completed after about 3 days (Aitio 1974; Grimmer et al. 1988 a, 1991 b; Jacob et al. 1989). Excretion of 57% of the administered amount of pyrene in the urine and of 18% in the faeces of rats was observed within 24 hours (Viau et al. 1999), whereas half-lives of 5 to 7 hours were determined for this species (Bouchard et al. 1998).

The phenols and dihydrodiols formed in the metabolism of PAH are excreted in urine in the form of the more readily water-soluble sulfates and glucuronides after conjugation with sulfuric acid catalyzed by sulfotransferases and conjugation with glucuronic acid catalyzed by UDP-glucuronosyl transferases, respectively. It was demonstrated, at least for rodents, that arene oxides are also metabolized to glutathione conjugates by the catalytic effect of glutathione *S*-transferase; these are finally excreted after cleavage of two amino acid residues (glycine and glutamic acid) and subsequent acetylation as mercapturic acids (MCS). It is not known whether this process, which is one of the main routes of elimination in rodents, is also of relevance for humans. In principle, this route of elimination cannot be excluded since water-soluble metabolites with a molecular weight of <475 Dalton (molecular weights for MCS of phenanthrene, pyrene and benzo[*a*]pyrene are 357, 381 and 431 Dalton, respectively) are excreted mainly in the urine whereas those with higher molecular weights are excreted in the faeces (Nau 1994). Moreover, the excretion of other mercapto derivatives of PAH metabolites such as mercaptoacetic acid, mercaptolactic acid and mercaptopyruvic acid were described in the urine of rodents (Horning et al. 1987). It is unclear whether these are also formed in humans.

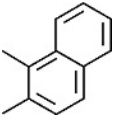
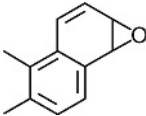
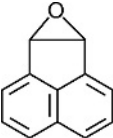
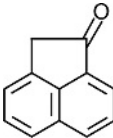
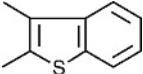
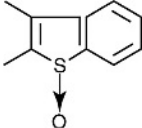
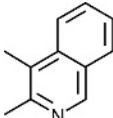
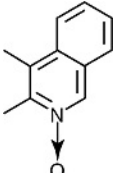
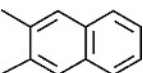
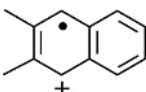
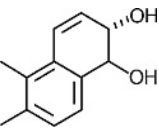
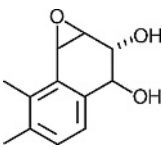
Cigarette smokers excrete the inhaled PAH within 24 hours (Jacob 2007 a). This has also been substantiated by a study of Brzezniński et al. (1997) for a group of volunteers exposed to PAH (half-life of 9.8 hours) at an aluminium plant. After exposure of volunteers to pyrene by ingestion and dermal application, a half-life of about 12 hours was determined (Viau et al. 1995 a), whereas a half-life of 4.4 hours was found in a different study after administration of pyrene with the diet (Buckley and Lioy 1992).

## 3.2 Metabolism

### 3.2.1 Enzymes and biotransformation reactions in the metabolism of PAH

**Cytochromes P450** (CYP) mainly function as monooxygenases that can introduce an epoxide group (Table 3, A), or – less frequently – a phenolic hydroxyl group directly into PAH (Jerina 1983). Many PAH epoxides spontaneously react with nucleophilic compounds such as DNA, water or glutathione, forming DNA adducts, *trans*-dihydrodiols or glutathione conjugates. Alternatively, they can spontaneously isomerize to yield phenols or – less frequently – tautomeric carbonyl compounds (Table 3, B). Depending on the structure of the epoxide, only some of these reaction pathways are often found. The ratios may be substantially modified in the presence of enzymes like epoxide hydrolases (EH) and glutathione transferases (GST). Moreover, CYP frequently form considerable amounts of benzylic alcohols (Engst et al. 1999) and *S*-oxides (Jacob et al. 1991) from PAH with partial aliphatic structures or thiaarenes. A review of the toxicology of thiaarenes may be found in

**Table 3** Oxidative metabolism of aromatics

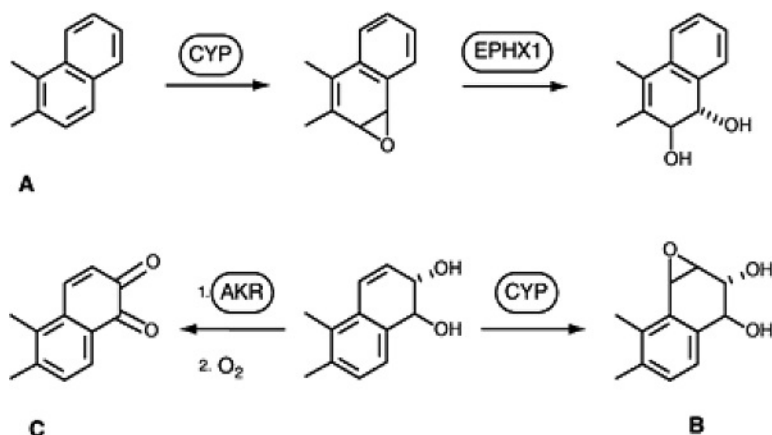
Substrate	Enzyme	Product
A 	cytochrome P450	
B 	spontaneous	
C 	cytochrome P450	
D 	cytochrome P450	
E 	cytochrome P450	
F 	cytochrome P450; myeloperoxidase; lipoxygenase; prostaglandin H synthase	

Jacob (1990). A positive effect was observed for two isomers of benzo[*b*]naphthothio-*phene* in *Salmonella typhimurium* (Pool et al. 1989). By contrast, *N*-oxidation seems hardly to play a role in the biotransformation of azaarenes (Schneider et al. 1994; Ye et al. 1995). Benzylic alcohols, *S*-oxides (Table 3, C) and *N*-oxides (Table 3, D) are of low reactivity under physiological conditions. Benzylic alcohols can be metabolized further to both reactive metabolites (sulfuric acid esters) and excretable metabolites (e.g. carboxylic acids and glucuronides) (Glatt et al. 2003; Ma et al. 2002; Watabe et al. 1982). There is only little information about the importance of *S*- and *N*-oxidation for the further metabolism of PAH.

Cytochromes often catalyze the first biotransformation step of PAH. However, it is quite common that further monooxygenase reactions take place at the same molecule. The formation of dihydrodiol epoxides at terminal benzo rings is particularly relevant toxicologically (Jerina 1983; Thakker et al. 1985). In principle, numerous CYP forms are able to metabolize PAH (Jacob et al. 1996 a). However, members of the CYP1 family (CYP1B1, CYP1A1 and less frequently CYP1A2) are far more active than other forms; this particularly applies to the first biotransformation step of a PAH. It is remarkable that most of the individual CYP forms can lead to the formation of several different products from one PAH substrate although the product profiles of the diverse CYP forms may vary considerably (Engst et al. 1999; Jacob et al. 1996 a).

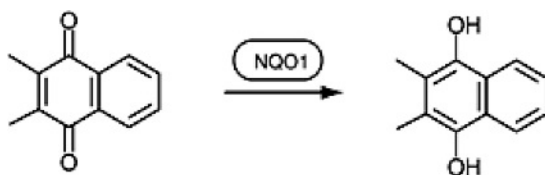
CYP can act on PAH not only as monooxygenase but also as one-electron oxidase, with a short-lived radical cation forming (Cavalieri and Rogan 1995) (Table 3, E). Similar reactions can be mediated by numerous **peroxidases** such as lipooxygenases (Kulkarni 2002) and prostaglandin H synthases (Degen et al. 2002). When radical cations react with DNA, the N<sup>7</sup> position is strongly preferred. The resulting adducts are unstable and leave apurinic sites in the DNA after disintegration. Alternatively, stable metabolites such as phenols and quinones can be formed via radical cations. Dihydrodiols seem to be converted to dihydrodiol epoxides not only via monooxygenase reactions, but also peroxidatively (as can be derived from the resulting DNA adducts and tetraoles as successive metabolites), for example by prostaglandin H synthases (Marnett et al. 1979), lipooxygenases (Hughes et al. 1989), myeloperoxidase (Trush et al. 1991) and a peroxidase from the placenta (Madhavan and Naidu 2000) (Table 3, F).

Epoxides can be hydrolyzed by **epoxide hydrolases** to form trans-dihydrodiols (Figure 1, A) (Arand and Oesch 2002; Glatt et al. 1982; Jerina 1983). For PAH epoxides only one form seems to be of importance, i.e. the membrane-bound form mEH (microsomal epoxide hydrolase). The hydrolysis of epoxides is primarily regarded as a detoxification since dihydrodiols are far less reactive than epoxides. However, highly problematical products such as dihydrodiol epoxides (Figure 1, B) and ortho-quinones (Figure 1, C) may form from dihydrodiols in secondary reactions. Elimination of the *Ephx1* gene sequence (knockout) thus led to the total resistance of mice to the carcinogenic effect of 7,12-dimethylbenzo[*a*]anthracene after systemic administration and to high resistance in the initiation-promotion skin assay (Miyata et al. 1999).



**Fig. 1** Formation of dihydrodiols from PAH via CYP oxidation to yield arene oxides and subsequent hydrolysis by microsomal epoxide hydrolase; EPHX1 (**A**). Formation of quinones (**C**) from dihydrodiols by AKR (aldo-keto reductases) and further CYP oxidation to yield dihydrodiol epoxides (**B**)

After the introduction of oxygen-containing functional groups, various oxido-reductases can intervene in the biotransformation of PAH. These particularly include **dihydrodiol dehydrogenases** (which belong to the superfamily of **aldo-keto reductases**) (Glatt et al. 1979, 1982; Jez and Penning 2001; Oppermann and Maser 2000; Penning 2004), **carbonyl reductase** (and other members of the short-chain dehydrogenases/reductases; SHR) (Wermuth et al. 1986), **quinone reductase** (DT-diaphorase; NQO1) (Chesis et al. 1984; Lind et al. 1982), **CYP reductase** (Chesis et al. 1984; Hermersdörfer et al. 1997), and – in the case of PAH with side chains – **alcohol dehydrogenases** (ADH) and **aldehyde dehydrogenases** (ALDH) (Ma et al. 2002). Dihydrodiol dehydrogenases can convert *trans*-dihydrodiols to catechols, which readily autoxidize to *ortho*-quinones (Penning 2004) (Figure 1, C). Quinone reductase reduces *ortho*- and *para*-quinones and multinucleate quinones by two-electron transfer to the relevant hydroquinones (Figure 2), which are generally good substrates for sulfotransferases (Figure 6) and UDP-glucuronosyl transferases (Figure 5). The regular function of CYP reductase consists of the acquisi-



**Fig. 2** Two-electron reduction of quinones to hydroquinones by NQO1 (quinone reductase; DT-diaphorase)

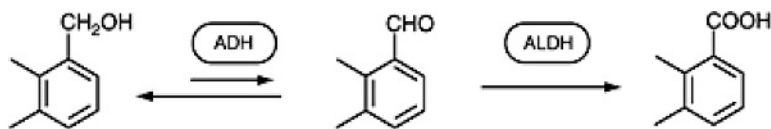


Fig. 3 Alcohol/aldehyde balance between benzylic alcohol and aryl-aldehyde and its further oxidation to aryl carboxylic acid by ALDH (ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase)

tion of two electrons from NADPH and their transmission as individual electrons on CYP. However, low-molecular substances like quinones may also function as electron acceptors; these are then converted to semiquinones. They can again autoxidize readily to quinone with air/oxygen, forming superoxide anions. Alcohol dehydrogenases can prevent the bioactivation of benzylic alcohols to reactive sulfuric acid esters by converting alcohols to aldehydes (Ma et al. 2002). However, the alcohol/aldehyde balance is generally on the side of alcohol. Therefore, this inactivation only functions if aldehyde is rapidly metabolized further, aldehyde dehydrogenases being suitable for this purpose (Figure 3).

Three classes of conjugating enzymes have a prominent position in the biotransformation of PAH, *i.e.* **glutathione transferases (GST)**, **UDP-glucuronosyl transferases (UGT)** and **sulfotransferases (SULT)**. GST metabolize electrophilic substrates, whereas UGT and SULT attack nucleophilic structures (mainly hydroxyl groups).

PAH epoxides can be catalyzed by numerous GST forms (Dostal et al. 1988; Glatt et al. 1983; Seidel et al. 1998 b; Sundberg et al. 1997, 1998 a, 1998 b) (Figure 4, A). GST P1 seems to have particularly favourable kinetic properties for some dihydrodiol epoxides (Seidel et al. 1998 b; Sundberg et al. 1998 a) (Figure 4, B). GST can also catalyze quinones, whereby hydroquinones with one or several C-terminal glutathionyl residues form as Michael addition products (Monks and Lau 1998) (Figure 4, C). Some of these substituted hydroquinones are more sensitive to oxidation than the corresponding unsubstituted hydroquinones (Monks and Lau 1998). No data are known about which human GST forms catalyze PAH quinones particularly well. Both electrophilic phase I metabolites and electrophilic phase II metabolites may function as substrates for GST. Thus, some polycyclic benzylic sulfuric acid esters in rats were inactivated particularly well by GST T2 (Hessel 2006; Hiratsuka et al. 1994) (Figure 4, D); there are no relevant data for human GST. Before elimination, glutathione conjugates are generally processed further, particularly frequently to mercapturic acids. The occurrence of mercapturic acids in the urine or faeces is a biomarker for the formation of an electrophilic metabolite and its detoxification. Mercapturic acids that were formed from dihydrodiol epoxides, K-region epoxides and benzylic sulfates were detected using sophisticated analytical methods (Boyland and Sims 1962 a; Ma 2002; Upadhyaya et al. 2006; Yang et al. 1999).

Phenols, hydroquinones, dihydrodiols, benzylic alcohols and carboxylic acids are among the PAH metabolites that can be glucuronidated (Lind 1985; Ma et al. 2002;

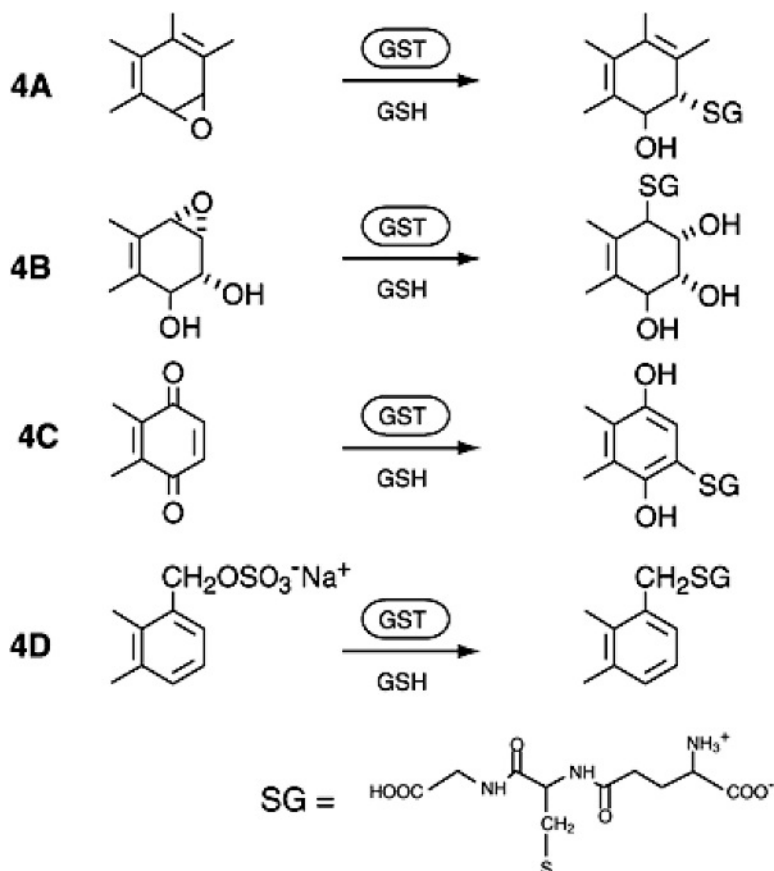
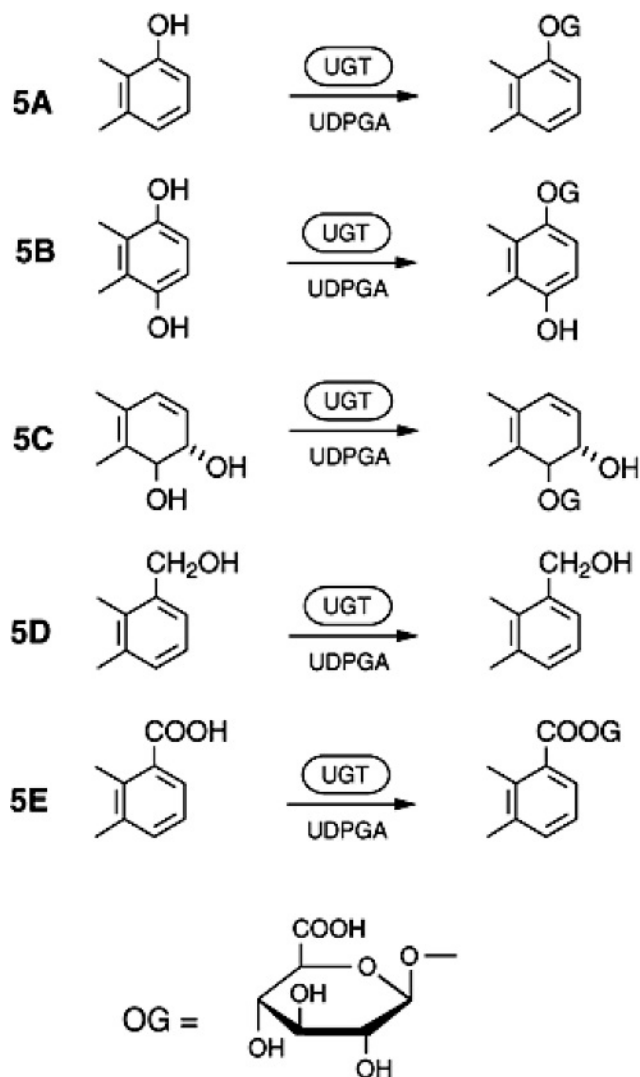


Fig. 4 Conjugation of electrophilic metabolites such as arene oxides (4A), dihydrodiol epoxides (4B), quinones (4C), and benzylic sulfate esters (4D) with glutathione (GSH), catalyzed by glutathione S-transferases (GST)

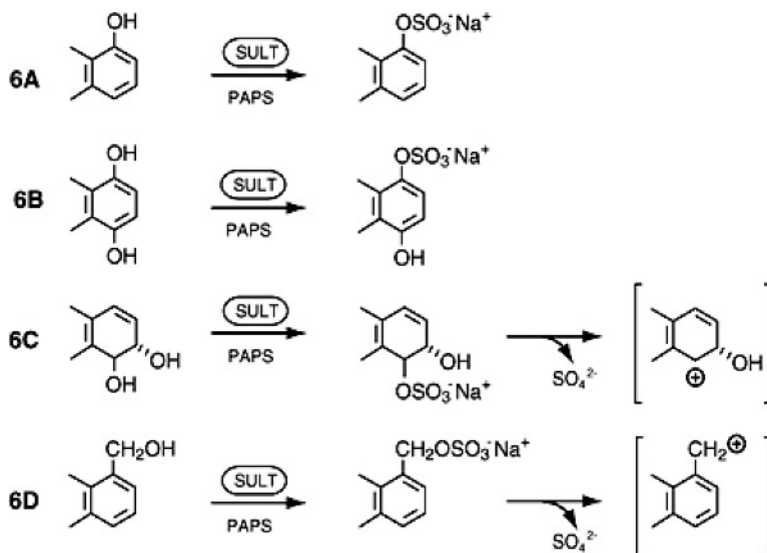
Nemoto and Gelboin 1976) (Figure 5, A–E). They do not include any reactive metabolites, but their precursors, which can thus be sequestered by UGT. The resulting glucuronides – as far as they have been investigated – show low reactivity and are highly excretable. Thus glucuronidation in PAH metabolism generally means inactivation if the possibility of a cleavage of the conjugates by bacterial  $\beta$ -glucuronidases in the intestinal tract is not considered; in this case, glucuronides might function as forms of transport of proximal carcinogens/mutagens.

Sulfate conjugation must be assessed in a more differentiated way. Sulfation of phenolic hydroxyl groups (also in hydroquinones) leads to the formation of stable, excretable sulfates (Nemoto et al. 1978) (Figure 6, A–C). However, on account of





**Fig. 5** Conjugation of nucleophilic PAH phenols (**5A**), hydroquinones (**5B**), dihydrodiols (**5C**), benzylic alcohols (**5D**) and aryl carboxylic acids (**5E**) with glucuronic acid catalyzed by UDP-glucuronosyl transferases (UGT)



**Fig. 6** Sulfate conjugation of phenols (**6A**), hydroquinones (**6B**), dihydrodiols (**6C**) and benzylic alcohols (**6D**) catalyzed by sulfotransferases (SULT)

the resonance stabilization of the resulting cation, sulfate is a good leaving group in a benzylic position (Figure 6, D). Sulfoxymethyl metabolites are actually ultimate mutagens/carcinogens of some methylated PAH, for example of 1-methylpyrene (Glatt 2000; Rogan et al. 1986; Surh et al. 1990 a; Watabe et al. 1982). Dihydrodiols and tetrols also contain benzylic (and possibly) allylic hydroxyl groups. A leaving group – similar to oxirane oxygen – can be generated by sulfoconjugation; in this way, the same cation as is formed from the specific epoxides (Figure 6) can possibly be transmitted to acceptor molecules. For some dihydrodiols (Surh et al. 1993) and tetrols (Glatt et al. 1998), *in vitro* studies showed that this reactivation actually takes place, but its practical relevance *in vivo* is not known.

### 3.2.2 Reactive PAH metabolites and their role in carcinogenicity

**PAH epoxides** are important intermediates in the biotransformation of PAH, but their chemical and biochemical properties may considerably differ from each other. Arene oxides at central aromatic positions (e.g. benzo[a]pyrene-1,2-oxide and benzo[a]pyrene-2,3-oxide) isomerize immediately after their formation to phenols (Yang et al. 1977). Unlike direct hydroxylation, a transient occurrence of the epoxide is of interest for the mechanism of the enzyme reaction, but of no relevance for toxicology. Epoxidation at terminal benzo rings, for example at the 7,8- or 9,10-positions of benzo[a]pyrene, has a less drastic effect on the aromatic system, the specific arene oxides thus having longer half-lives. They can react with DNA

and were shown to be mutagenic, although only to a slight extent. Compared with isomerization, their spontaneous hydrolysis is negligible. However, in the presence of microsomal epoxide hydrolase, hydrolysis can become the dominant pathway (Shou et al. 1994). In a third group, epoxidation takes place at an isolated double bond, which is conjugated with the aromatic system, but has a largely olefinic character. Epoxides at ethylene bridges (e.g. cyclopenta[*cd*]pyrene-3,4-oxide) (Gold and Eisenstadt 1980; Wood et al. 1980) and at terminal benzo rings after saturation of the vicinal double bond (e.g. *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-oxide) (Sims et al. 1974) are examples of this. The formation of epoxides at K regions of PAH (e.g. benzo[*a*]pyrene-4,5-oxide) is also an example of epoxidation at electron-rich positions with a partial double-bond character (Oesch and Glatt 1976). Reactivity with nucleophilic compounds is dominant among these epoxides; no or hardly any isomerization reactions occur. These epoxides are generally potent mutagens unless they are metabolically inactivated. However, they may vary considerably from each other as regards their metabolic inactivation. Thus, benzo[*a*]pyrene-4,5-oxide is a potent mutagen in *Salmonella* in the absence of mammalian enzymes, but it can be detoxified very effectively by microsomal epoxide hydrolase and many different GST forms. Almost the same applies to benzo[*a*]anthracene-5,6-oxide (Glatt et al. 1982, 1983). Its direct application to rodents may elicit tumourigenicity, but only after local treatment with a relatively high dose at which an overload of the detoxification systems occurs (Flesher et al. 1976). Benzo[*a*]pyrene-4,5-oxide is mainly responsible for DNA adduct formation in mussels exposed to benzo[*a*]pyrene; they presumably have no efficient detoxification systems. *Anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-oxide and cyclopenta[*cd*]pyrene-3,4-oxide are considered to be important ultimate carcinogens of the relevant PAH. These epoxides are able to induce tumours in test animals much more readily than benzo[*a*]pyrene-4,5-oxide, which is substantially due to their lower detoxification. Depending on their stereochemistry, vicinal dihydrodiol epoxides are no or only poor substrates for microsomal epoxide hydrolase, the hydrophilic hydroxyl groups close to the oxirane ring obviously impairing the interaction with the enzyme (Glatt et al. 1982). The metabolism of dihydrodiol epoxides by GST is several orders of magnitude slower than that of simple arene oxides (Glatt et al. 1983).

**Benzylic sulfates** occur as reactive intermediates, particularly in the biotransformation of alkylated PAH. Like epoxides, they can also be chemically synthesized and directly examined for toxicological effects. Benzylic sulfates proved to be carcinogenic in animal studies (Cavalieri et al. 1978; Flesher et al. 1998 a, b; Horn et al. 1996; Surh et al. 1990 a). They react with DNA in a similar way as epoxides. The exocyclic amino groups of the purine bases are the preferred target structures, with stable adducts forming. After administration of alkylated pyrenes, which had induced tumours in animal studies (Rice et al. 1987), the same DNA adducts were detected as are also formed by the specific alkyl sulfates (Glatt 2007 b). However, comparable data are available for only few alkylated PAH. In the case of 7,12-dimethylbenzo[*a*]anthracene, its carcinogenicity is apparently determined by dihydrodiol epoxides rather than sulfate esters (Surh et al. 1991).

Unlike epoxides, sulfates exist as anions under physiological conditions; this restricts their distribution in the organism. Sulfooxymethylpyrenes are actively taken up into cells by the organic anion transporters OAT1 and OAT3 (Bakhiya et al. 2006). These transporters are expressed primarily in renal tubular cells, which is presumably the reason for the particularly strong renal DNA adduct formation by 1-sulfooxymethylpyrene (Glatt et al. 2003). It is also remarkable that usually only small amounts of 1-hydroxymethylpyrene in rats are activated *in vivo* to form the specific sulfuric acid ester. The main amount is oxidized by alcohol dehydrogenases and aldehyde dehydrogenases to form carboxylic acid. If this pathway is blocked, for example by the administration of ethanol, *i.e.* a competing substrate of alcohol dehydrogenases and aldehyde dehydrogenases, adduct formation rises dramatically (in some cases more than 100-fold) (Ma et al. 2002).

**PAH radical cations** can be formed directly from hydrocarbons in one-electron oxidation (Cavalieri and Rogan 1995). This may lead to the formation of unstable N<sup>7</sup> PAH purine adducts both *in vitro* and *in vivo*. Various reactions in cell-free systems were designed in such a way that adduct formation had to have taken place in the DNA (since there were no other substantial purine resources). The glycosidal binding becomes labile due to binding at the N<sup>7</sup> position; this leads to the release of the modified base and the formation of an apurinic site in the DNA. These base adducts are also formed *in vitro* and *in vivo*, but the extent to which they form at DNA, RNA or free nucleotides is unclear. It is expected that the radicals form mainly in the cytoplasm and in the outer nuclear membrane and react with the nearest target structures. It must also be considered that a large number of apurinic sites in the DNA arise without any impact of xenobiotics, for example by spontaneous deamination of cytosine residues to uracil residues (about 200 per day and cell), misincorporation of deoxyuridine and subsequent base excision repair by uracil glycosidase (Atamna et al. 2000) or by spontaneous depurination (about 9000 AP sites that are formed per day and cell as a result of the spontaneous deamination of thymidine residues in the DNA) with subsequent repair by AP endonuclease 1 (Ape1) (Nakamura and Swenberg 1999). Under these circumstances, the possible role of PAH radical cations in PAH carcinogenicity remains unclear. 7,12-Dimethylbenzo[*a*]anthracene belongs to those PAH that form a particularly large number of N<sup>7</sup> base adducts (Devanesan et al. 1993). A knockout of *Ephx1* should not impair this formation of radicals, but leads to total resistance to the carcinogenic effect of 7,12-dimethylbenzo[*a*]anthracene in mice (Miyata et al. 1999). It can be concluded from this that PAH radical cations alone are not sufficient to induce tumours.

The biological activity of **PAH quinones** greatly depends on the model used. They show no or only weak mutagenic or cytotoxic activity in bacteria (Chesis et al. 1984; Flowers-Geary et al. 1996; Wislocki et al. 1976; Wood et al. 1977 b). This is presumably due to the highly reductive milieu in bacterial cells. However, several PAH quinones are highly toxic, and some of them are also genotoxic in mammalian cells *in vitro*, with mainly chromosomal lesions or also gene mutations being induced depending on the substance (Flowers-Geary et al. 1996; Ludewig et al. 1991). The mechanisms involved are heterogeneous. Some quinones react with

nucleophilic compounds as Michael acceptors. In other cases, binding to macromolecules occurs after a one-electron reduction to semiquinone. A redox cycle with the formation of reactive oxygen is also frequently involved. Almost all of these findings were derived from cell culture models. However, only few results are available with PAH quinones in test animals; they are mainly based on benzo[*a*]-pyrene-quinone (Joseph et al. 2000; Slaga et al. 1978 b). Marginal initiation of papillomas was observed after local application to the mouse skin. *In vivo*, these quinones are presumably reduced rapidly, mainly to hydroquinones, which can readily be conjugated (Lilienblum et al. 1985) and then excreted (Ruzgys et al. 2005). In particular, *ortho*-quinones are postulated to play a role in PAH carcinogenicity (Palackal et al. 2002; Penning et al. 1999); however, there are no supporting *in vivo* findings for this. Naphthalene, which is classified in Carcinogen Category 2, is a special case (2001 Supplement “Naphthalene”, this volume). Although the mechanism of its carcinogenicity has not been clarified, *in vivo* protein adducts of 1,2- and 1,4-naphthoquinone (besides adducts of naphthalene-1,2-oxide) were detected in test animals (Troester et al. 2002; Waidyanatha et al. 2002). Naphthalene is relatively rapidly metabolized as compared with other PAH.

PAH can moreover be photoactivated. Epidioxides were isolated as **photoactivation products**, for example (Tu et al. 1979). This mechanism is probably of no appreciable significance for human exposure to PAH.

Altogether it may be stated that a large variety of reactive products can be formed from PAH. The evidence of their actual role in carcinogenic and other adverse effects of PAH differs in quality. The data available for epoxides are convincing. Particularly dihydrodiol epoxides are decisive ultimate carcinogens among purely aromatic PAH with a bay or fjord region (e.g. benzo[*a*]pyrene) and individual methylated congeners (e.g. 7,12-dimethylbenzo[*a*]anthracene). In a few cases, simple epoxides like cyclopenta[*cd*]pyrene-3,4-oxide may also be ultimate carcinogens – although the possibility of further activation by microsomal epoxide hydrolase and SULT cannot be completely ruled out; such a reactivation has at least been well substantiated *in vitro* (Glatt et al. 1994 b; Hsu et al. 1999; Surh et al. 1993). A SULT-related activation can be regarded as reliable for some carcinogenic alkyl pyrenes; the DNA adducts are formed by reactive sulfates, and the sulfates were found to be carcinogenic. It is expected that this pathway is important for further alkylated PAH, but there are no data (for occurrence of alkylated PAH see Section 6.2). Further reactive metabolites such as free radicals, quinones and photoactivation products are possibly involved in the carcinogenicity of PAH; however, this assumption is based exclusively on *in vitro* findings or not very conclusive end points (*N*<sup>7</sup> PAH purine adducts of unknown origin). There are no reliable *in vivo* findings with the exception of protein quinone adducts of naphthalene, which is classified in Carcinogen Category 2 (2001 Supplement “Naphthalene”, this volume). Other *in vivo* findings (loss of 7,12-dimethylbenzo[*a*]anthracene carcinogenicity after *Ephx1* knockout; carcinogenicity studies with multinucleate PAH quinones) rather militate against a role of this activation pathway in the models used; however, the data are not yet sufficient to derive a conclusive assessment.

## 4 Effects in Humans

### 4.1 Single exposures

There is evidence of percutaneous PAH absorption in humans. After topical application of a 2% solution of crude coal tar in petrolatum, phenanthrene, anthracene, pyrene and fluoranthene were detected in the peripheral blood (Storer et al. 1984). Volunteers who had been treated with creosote (100 µl) or pyrene (500 µg; applied in a toluene solution) and psoriasis patients who used a shampoo containing coal tar excreted 1-hydroxypyrene as conjugates in the urine. In both cases, maximum amounts were excreted 10 to 15 hours after treatment (Viau et al. 1995 a). Naphthalene, which is classified in Carcinogen Category 2 (2001 Supplement “Naphthalene”, this volume), is often used as an agent to control insects in homes. Acute haemolytic anaemia is a typical systemic effect among humans after oral, dermal or inhalation exposure. The lethal oral doses in cases of poisonings with naphthalene were specified to be 5–15 g for adults and 2 g for a 6-year-old child (1995 MAK documentation “Naphthalene”, Volume 11, present series). Between 1949 and 1959, 10 cases of poisonings with naphthalene caused by mothballs (sucking or ingestion) were described in the United States. Some of the children were found to have haemolytic anaemia (Anziulewicz et al. 1959; Mackell et al. 1951; Zuelzer and Apt 1949).

The signs of naphthalene ingestion became manifest after one or several days in the form of nausea, vomiting and convulsions, often followed by diarrhoea. Other symptoms included disturbances of consciousness, lethargy, incoordination, coma and hemiplegia. Haemolytic anaemia with haemoglobin concentrations of up to 40% was often followed by haemoglobinuria. More or less pronounced jaundice was also observed, and there was liver necrosis in one lethal case (Konar et al. 1939).

### 4.2 Repeated exposures

Haemolytic anaemia after inhalation of naphthalene was observed in newborn who were wrapped in blankets that had previously been treated with mothballs and who inhaled the evaporating naphthalene (Valaes et al. 1963). Lens opacities were observed in 5 of 9 female workers under 40 years who had been exposed to high naphthalene concentrations in the inhaled air for 5 years (no other details). Various authors also reported corneal ulcerations and cataracts after occupational exposure to naphthalene as a vapour or dust. Around 1900, naphthalene was administered for intestinal diseases at doses of 3 to 5 g per day. Strangury with severe burning of the urethra and, in some patients, swelling and reddening of the external urethral orifice were observed after administration for several days. Transplacental naphthalene poisoning was also described in two cases. Diffuse lens opacities after dermal absorption were found in 8 of 20 female workers who produced a dye inter-

mediate. Cataract formation in both eyes and unilateral chorioretinitis were reported in 2 workers who had direct contact with naphthalene and naphthalene powder, respectively (no other details). It was not possible to rule out inhalation exposure (1995 MAK documentation "Naphthalene", volume 11, present series).

Table 4 lists some of the many studies on occupational exposure to PAH that only consider benzo[*a*]pyrene. The table illustrates that the concentrations for comparable and even for identical workplaces are very variable; however, it also shows that great progress was made in improving the workplace situation for PAH emissions through the modernization of industrial plants. This is especially true for coking plants; in Germany, maximum exposure limits of 5 µg benzo[*a*]pyrene/m<sup>3</sup> for the topside area of the oven and 2 µg benzo[*a*]pyrene/m<sup>3</sup> for other workplaces are prescribed by law (BMA 1989); in France, 0.15 µg benzo[*a*]pyrene/m<sup>3</sup> are stipulated for plants manufacturing graphite electrodes (Lafontaine et al. 1990). For human biomonitoring, however, it must be pointed out that the PAH profiles vary not only from one emission source to the other but also as a function of time for certain workplaces; therefore, an extrapolation of the concentrations for phenanthrene and pyrene is generally not possible on the basis of the measured benzo[*a*]pyrene concentration. The PAH profiles measured at diverse workplaces (manufacture of graphite electrodes; manufacture and processing of fireproof materials) show considerably different relative benzo[*a*]pyrene levels with variations by a factor of 5 (Preuss et al. 2006). By means of 1-hydroxypyrene it was possible to show in 12 coke oven workers that an average of 75% of the total absorbed amount of pyrene entered the body through the skin (Van Rooij et al. 1993 a).

#### 4.2.1 Human biomonitoring of PAH metabolites

Occupational medicine has shown in practice that persons who work in a single trade and whose external exposure (PAH in the air) is almost identical may nevertheless show considerable interindividual differences in their systemic PAH exposure (Grimmer et al. 1997; Seidel et al. 2002). However, besides the very unlikely oral exposure (hand-mouth contact) and possible pharmacokinetic factors, dermal absorption of PAH is regarded as the main cause of these interindividual differences; it accounts for a substantial fraction of the total PAH exposure that cannot be neglected (Gündel et al. 2000; McClean et al. 2004; Vanaanan et al. 2005; Van Rooij et al. 1992, 1994). At the same time, meaningful associations with moderate correlation coefficients between PAH concentrations measured in the air and metabolite concentrations measured in the urine are obtained only if persons with dermal exposure are excluded from the statistical analysis (Unwin et al. 2006). Since PAH are also known to be absorbed through the skin, pyrolysis products and other mixtures containing PAH are handled like substances designated with an "H" (DFG 2007). Altogether, human biomonitoring by determination of PAH-specific metabolites in the urine is much more suitable for assessing workplace exposure than airborne PAH measurements at the workplace on account of dermal PAH absorption.

**Table 4** Benzo[a]pyrene concentrations at different workplaces

Workplace	Country	Year	Benzo[a]pyrene ( $\mu\text{g}/\text{m}^3$ )	References
<b>Coking plant</b>				
oven topside area	Poland	1978	20–383	Braszczyńska et al. 1978
modernized			0–6.8	Braszczyńska et al. 1978
oven topside area	Sweden	1982	9.4–13.5	Lindstedt and Sollenberg 1982
charging-car driver			4.7–17	Lindstedt and Sollenberg 1982
topside worker	Germany	1981	22.3–33.0	Blome 1981
oven topside; machine operator	Germany	2002	7.44 (mean)	Strunk et al. 2002
bench side; machine operator	Germany	2002	1.26 (mean)	Strunk et al. 2002
oven area	Germany	2002	0.94 (mean)	Strunk et al. 2002
charging-car driver			4.5	Blome 1981
ramp worker			1.33	Blome 1981
driver			0.16–0.93	Blome 1981
battery topside	Germany	1992	10.6–15.8	Grimmer et al. 1993 b
charging-car driver			5.8–10.1	Grimmer et al. 1993 b
employee farther from the centre of emission			0.9–4.9	Grimmer et al. 1993 b
battery side; oven topside; repair work at combustion chamber wall	Germany	3-year period	0.62 (median) 5.05 (90th percentile)	Preuss et al. 2003 c
<b>Various workplaces</b>				
aluminium plant (Soederberg technology)	Norway	1978	11.3–854	Bjørseth et al. 1978 a, 1978 b
copper, brass and zinc foundries	Germany	1983	<0.05–0.1	Blome 1983
steel foundry			0.38–57.5*	
	Canada	1982		Coenen 1988
	Germany	1982		Verma et al. 1982
	Finland	1983		Blome 1983
		1986		Knecht et al. 1986
		1981		Schimberg 1981



Table 4 (Continued)

Workplace	Country	Year	Benzo[a]pyrene ( $\mu\text{g}/\text{m}^3$ )	References
light-metal foundry	Germany	1983	0.05–0.26	Blome 1983
road construction				
using coal-tar bitumen	Germany	1989	0.7–22.0	Knecht and
using petro bitumen	Germany	1990	0.02 (median)	Woitowitz 1989
	Denmark	1989	4.0 (median)	Tobias et al. 1990
				Hansen 1989
roofing companies	Germany	1983	14.0 (maximum level)	Blome 1983
	United States	1982	0.4–11.0	Malaiyandi et al. 1982
optical industry	Germany	1983	<0.05–19.7	Blome 1983
tar refinery	Germany	1979	3.6	Schunk 1979
tar distillation	Germany	3-year period	0.14 (median) 4.74 (90th percentile)	Preuss et al. 2003 c
bitumen production	Germany	1983	<0.5	Blome 1983
bitumen processing	Germany	2003	<0.06	Preuss et al. 2003 b
brickworks	France	1987	3.4	Lesage et al. 1987
rubber industry	United States	1980	0–32.3	Williams et al. 1980
	Finland	1982	<0.02–0.25	Enwald 1982
	Germany	1983	<0.05	Blome 1983
production of graphite electrodes	Germany	3-year period	0.49 (median) 4.08 (90th percentile)	Preuss et al. 2003 c
	Germany	1996	0.003–3.39	Angerer et al. 1997 a
production of fireproof materials	Germany	3-year period	0.17 (median) 3.44 (90th percentile)	Preuss et al. 2003 c
production of fireproof materials	Germany	5-year period	0.10 (median) 1.72 (90th percentile) 38.15 (maximum level)	Preuss et al. 2006
production of fireproof materials	Germany	3-year period	0.63 (median) 23.25 (90th percentile)	Preuss et al. 2003 c

\* benzo[a]pyrene concentration range in the 5 cited steel foundry studies

Not only the good dermal absorption of PAH but also its systemic toxicity are regarded as relevant for a carcinogenic hazard to humans. The total amount of PAH absorbed systemically, *i.e.* by inhalation, through the skin and also orally, at the workplace or from the environment is of decisive importance here. It can best be recorded by human biomonitoring. Since the excretion of PAH-specific metabolites in the general population is influenced mainly by smoking and eating habits and by the environmental exposure of the examined persons, human biomonitoring of PAH-specific metabolites has also proved useful in environmental medicine – in addition to occupational medicine – and is here, too, regarded as being superior to air monitoring or recording exposure by questionnaires on account of the main routes of absorption and the mode of the systemic toxicity of PAH (Gunier et al. 2006; Hu et al. 2006).

1-Hydroxypyrene was used as a parameter for measuring systemic PAH exposure in most studies (Levin 1995 b). It represents the main metabolite of pyrene in mammals (Boyland and Sims 1964; Grimmer et al. 1994; Harper 1958; Harper and Legator 1987; Jacob et al. 1982; Keimig et al. 1983) and has become accepted as a sensitive and specific parameter of PAH exposure; a review is described by Jongeneelen (1997). Apart from 1-hydroxypyrene, further metabolites of pyrene, such as *trans*-4,5-dihydroxy-4,5-dihydropyrene, 1,6- and 1,8-dihydroxypyrene, 1,6- and 1,8-pyrenequinone and dihydrodiol phenols, were detected in the urine (Boyland and Sims 1964; Harper 1957, 1958; Harper and Legator 1987; Jacob et al. 1982; Keimig et al. 1983). Selected data on the excretion of 1-hydroxypyrene in the urine of non-exposed persons and PAH-exposed workers at different workplaces are shown in Tables 5a and 5b. All information is specified in µg/g creatinine or µg/l urine; for individual studies, only data in µg/24-hour urine were available. These data were converted assuming a daily excretion of 1.2 g creatinine for women and 1.8 g for men so that they could be compared more readily. Data in concentrations of µmol/mol creatinine were converted to µg/g creatinine.

The interindividual variations that were observed for the excretion of 1-hydroxypyrene were initially due to the different levels of exposure in diverse trades and diverse working areas within one trade. When 1-hydroxypyrene was measured, exposure in the production of fireproof materials and the production of graphite electrodes and special carbon products was thus higher than for example in coking plants or the further processing of fireproof materials (BGFA 2005; Rossbach and Angerer 2002). These results were confirmed by the measurement of poly-hydroxylated PAH metabolites (Jacob and Seidel 2002).

Interindividual differences in the excretion of 1-hydroxypyrene may also vary considerably within one trade (factors between 10 and 100; Petry et al. 1996); these variations may be explained by at times substantial dermal PAH absorption at individual workplaces or by different hygiene measures of the workers in the trade examined (McClean et al. 2004). Interindividual differences in the excretion of 1-hydroxypyrene can however hardly be explained by genetic polymorphisms in PAH-metabolizing genes. The analysis of 11 polymorphisms in a total of eight enzymes involved in the xenobiotic metabolism of PAH (CYP1A1, CYP1A2, CYP1B1,

CYP3A4, microsomal epoxide hydrolase, GSTM1, GSTT1 and GSTP) in a group of 170 workers exposed to PAH yielded an impact on the urinary excretion of 1-hydroxypyrene for only three polymorphisms; it was moreover negligibly small with factors between 1.4 and 1.6 (Rihs et al. 2005).

1-Hydroxypyrene is not the end product of a metabolic pathway of pyrene, but is further oxidized enzymatically to 1,6-dihydroxypyrene and 1,8-dihydroxypyrene. These dihydroxypyrenes are endogenously in a redox balance with pyrene quinones and were only recently detected and determined in the human urine (Ruzgyte et al. 2005; Seidel et al. 2005). Ruzgyte et al. (2005) used the method of high-performance liquid chromatography with fluorescence detection to determine 1,8-dihydroxypyrenes after their conversion to diacetyl derivatives. In contrast, Seidel et al. (2005) used the determination established for phenols, GC/MS after derivatization to methyl ethers. In studies with a total of more than 300 volunteers (non-smokers) from Germany and three other European countries, excretion of these 1,8-dihydroxypyrenes at the same or even higher concentrations than those of 1-hydroxypyrene was found in the general population (1-hydroxypyrene/1,8-dihydroxypyrene ratio: 1.0 to 0.5; (Seidel and Jacob 2005; Seidel et al. 2005). These excretion ratios of 1-hydroxypyrene to 1,8-dihydroxypyrene drastically change among workers with occupational exposure. Thus, 1-hydroxypyrene/1,8-dihydroxypyrene ratios of about 19:1 were found among workers involved in graphite electrode production, about 10:1 in coke oven workers, about 4:1 in workers in converter lining and about 3:1 in workers involved in the production of fireproof materials (Seidel and Jacob 2005). The results indicate that higher PAH exposure leads to a shift in pyrene metabolism in favour of an excretion of 1-hydroxypyrene. However, 1-hydroxypyrene accounts for only about 50% of the phenolic pyrene metabolites determined in the urine at low exposure. An overview is provided in Tables 5a and 5b.

Recent studies have not only determined 1-hydroxypyrene in the urine but have also examined the excretion of further PAH metabolites – particularly of phenanthrene phenols (1-, 2-, 3-, 4- and 9-hydroxyphenanthrene). In the evaluation of the data, these are specified as sum parameters of all five isomers ( $\Sigma$ hydroxyphenanthrenes) in most cases and have the advantage that  $\Sigma$ hydroxyphenanthrenes are influenced by individual smoking or eating habits to a much lesser extent than 1-hydroxypyrene in the urine. Determination of  $\Sigma$ hydroxyphenanthrenes is generally a more sensitive and more specific method of recording occupationally induced PAH exposure than the determination of 1-hydroxypyrene in the urine. The lesser effect of smoking on the excretion of  $\Sigma$ hydroxyphenanthrenes in the urine as compared with 1-hydroxypyrene had already been observed in earlier studies. Only a slight increase in  $\Sigma$ hydroxyphenanthrenes was observed among smokers as compared with non-smokers, whereas no differences whatsoever were detected for passive smokers as compared with non-smokers (Hoepfner et al. 1987; Martin et al. 1989). Different eating habits also have a minor influence on the urinary excretion of  $\Sigma$ hydroxyphenanthrenes. An increase in the concentration of phenanthrene in the diet by about a factor of 10 (from 0.5  $\mu\text{g/kg}$  to 4.4  $\mu\text{g/kg}$ ) led to only a slight

**Table 5a** Urinary excretion of 1-hydroxypyrene (median or range) in control volunteers

Persons exposed	Number	Country	1-Hydroxypyrene		References
			(µg/g creatinine)	(µg/l urine)	
<b>general population</b>	2747	United States	0.044	0.047	CDC 2005
women	1399		0.042	0.043	2001–2002
men	1348		0.046	0.055	2001–2002
<b>general population</b>	2312	United States	0.073	0.078	CDC 2003
women	1206		0.077	0.071	1999–2000
men	1106		0.070	0.085	1999–2000
<b>general population</b>					
non-smokers	389	Germany	0.08	0.10	UBA 1998
– West Germany	182			0.09	
– East Germany	45			0.11	
smokers	184		0.19	0.25	
<b>general population</b>	140	Canada	0.17		Viau et al. 1995 b
non-smokers	95		0.14		
smokers	45		0.23		
<b>general population</b>					UBA 1990
non-smokers		Germany			
– West Germany	75			0.12	
– East Germany	75			0.30	
<b>general population</b>	139	Germany	<0.04–0.13		Göen et al. 1995
non-smokers	80		<0.04–0.054		
smokers	59		<0.04–0.13		
<b>general population</b>	70	People's Republic of China	0.8–4.6		Zhao et al. 1990
<b>general population</b>		France			Lafontaine et al. 2006
non-smokers	27		0.06 (0.02–0.29)		
smokers	27		0.22 (0.08–1.02)		
<b>general population</b>	8	Germany		0.3	Grimmer et al. 1991 b
non-smokers	10	Germany	0.54±0.34 <sup>a</sup>		Jacob et al. 1999 a
smokers	9		0.41±0.18 <sup>a</sup>		
non-smokers	11	Germany	<0.1–0.3	<0.1–0.3	Angerer et al. 1992
smokers	11		<0.1–0.8	<0.1–0.8	
non-smokers	97	Germany	0.06–1.56		Gündel et al. 1996
smokers	27			0.18–1.50	

Table 5a (Continued)

Persons exposed	Number	Country	1-Hydroxypyrene		References
			( $\mu\text{g/g}$ creatinine)	( $\mu\text{g/l}$ urine)	
<b>general population</b>		Italy			Granella and Clonfero 1993
non-smokers	19		0.04		
smokers	20		0.08		
patients treated with tar ointment	8	Germany	0.94–5.81		Angerer et al. 1992
	25	Italy		7.60	Clonfero et al. 1989

<sup>a</sup> mean

increase in the excretion of  $\Sigma$ hydroxyphenanthrenes, *i.e.* by a factor of 2 (Martin et al. 1989). This was substantiated by findings from another study, in which persons who had consumed charcoal grilled meat with an about 250-fold higher benzo[a]pyrene concentration as compared with that consumed by the corresponding control persons revealed a level of 1-hydroxypyrene in the urine that was increased by a factor of 4 to 12 (Buckley and Lioy 1992). It is unclear, however, whether the pyrene concentration of the grilled goods was increased to the same extent as benzo[a]pyrene. Selected data on the excretion of  $\Sigma$ hydroxyphenanthrenes in the urine of non-exposed persons and PAH-exposed workers at different workplaces are shown in Tables 6a and 6b. All data are specified in  $\mu\text{g/g}$  creatinine or  $\mu\text{g/l}$  urine.

The varying PAH profile in the diverse trades and therefore the different results in the urinary excretion of 1-hydroxypyrene and  $\Sigma$ hydroxyphenanthrenes among the trades lead to a change in the “hierarchy” of the exposure levels for  $\Sigma$ hydroxyphenanthrenes as compared with 1-hydroxypyrene in the individual trades. As in the case of 1-hydroxypyrene, the highest concentrations of  $\Sigma$ hydroxyphenanthrenes were obtained in the production of fireproof materials and in the production of graphite electrodes and special carbon products. However, in contrast to the results for 1-hydroxypyrene, high exposures in the form of  $\Sigma$ hydroxyphenanthrenes were established both in coking plants and in the further processing of fireproof materials and tar distillation (BGFA 2005; Jacob and Seidel 2002; Rossbach and Angerer 2002).

Altogether, it may be stated that the results of the measurements of 1-hydroxypyrene and  $\Sigma$ hydroxyphenanthrenes in the urine of PAH-exposed workers demonstrate higher exposures to PAH mainly in the production and further processing of fireproof materials and graphite electrodes as well as in coking plants and tar distillation, whereas fire damage restoration and hydraulic engineering are rather of subordinate relevance for exposure to PAH. Only low level exposures were demonstrated in the latter branches of industry by determinations of both 1-hydroxypyrene and  $\Sigma$ hydroxyphenanthrenes.

**Table 5b** Urinary excretion of 1-hydroxypyrene (median or range) in occupationally exposed persons

Persons exposed	Number	Country	1-Hydroxypyrene		References
			(µg/g creatinine)	(µg/l urine)	
<b>different industries (total)</b>	237	Germany	5.38 (0.12–279.63)		BGFA 2005; Jacob and Seidel 2002; Rossbach and Angerer 2002
production of graphite electrodes and special carbon products	71		8.65 (0.36–90.84)		
production of fireproof materials	68		11.27 (0.31–279.63)		
coking plant	47		4.30 (0.51–34.82)		
production of fireproof materials	24		4.98 (0.28–27.14)		
tar distillation	18		1.51 (0.31–5.08)		
fire damage restoration	5		0.46 (0.13–2.09)		
hydraulic engineering and corrosion protection	4		1.98 (0.83–7.88)		
<b>production of graphite electrodes</b>	34	Norway	19.7		Bentsen-Farmen et al. 1999
	67	Germany	8.7		Angerer et al. 1997 b
	67	Germany	0.2–326		Mannschreck et al. 1996
	23	Germany	7.1–125.0		Göen et al. 1995
	6	Germany	7.1–82.0	9.7–73.5	Angerer et al. 1992
	4		18.1–129.6	20.1–50.4	
	14		2.2–125.0	3.2–160.8	
	13		0.58–16.8		
<b>coal-tar impregnation plants</b>	3	Netherlands	1.3–38.5		Jongeneelen 1992
	1		80.9–158		
	3		8.2–134.9		
	6		4.8–146.5		
	9		1.2–55.9		
	3	Germany	7.1–41.2	12.7–77.1	Angerer et al. 1992

Table 5b (Continued)

Persons exposed	Number	Country	1-Hydroxypyrene		References
			(µg/g creatinine)	(µg/l urine)	
creosote workers	3	Netherlands	1.0–9.1		Jongeneelen et al. 1986
		Canada	3.14 (0.35–20.19)		Viau et al. 1995 b
coal tar distillation	4	Netherlands	2.3–25.1		Jongeneelen et al. 1986
coking plant	15	People's Republic of China	36.3		Zhao et al. 1990
oven topside area	31	Sweden	15.9		Levin et al. 1995 a
	10		1.9–34.7		
	24	Germany	3.3–79.4		Strunk et al. 2002
	8	Germany		14.1–118.5	Grimmer et al. 1993 b
charging-car driver	4	Germany		8.2–21.0	Grimmer et al.
machine operator	4			2.0–6.9	1993 b
aluminium smelter	5	Netherlands	2.3–17		Vu-Duc and Lafontaine 1996
steelworks	25	Germany	0.1–126.2		Göen et al. 1995
	37	Germany	0.7–126.2	0.2–99.2	Angerer et al. 1992
	12	People's Republic of China		3.5	Zhao et al. 1990
	10	Germany	0.85 (0.1–3.8)	<0.1–4.2	Göen et al. 1995; Angerer et al. 1992
road construction					
road construction (in general)	49	Germany	0.22	0.18	Marczynski et al. 2006
hot bitumen processing	4	Germany		2.6	Grimmer 1993 c
	31	Netherlands	0.8–16.4		Jongeneelen et al. 1988
	66	Germany	0.44	0.30	Marczynski et al. 2006
petro bitumen processing	20	Germany		0.4–2.4	Knecht and Woitowitz 1989
coal-tar bitumen processing	12			5.3–92.1	
waste incineration					
municipal plant	53	Germany	<0.1–0.8	<0.1–1.3	Göen et al. 1995; Angerer et al. 1992

**Table 5b** (Continued)

Persons exposed	Number	Country	1-Hydroxypyrene		References
			(µg/g creatinine)	(µg/l urine)	
industrial plant	43	Germany	<0.1–0.8		Göen et al. 1995
meat smokehouse	13		<0.1–1.1	<0.1–1.1	Göen et al. 1995; Angerer et al. 1992
railway sleeper plants	14	Netherlands	0.4–8.7		Jongeneelen 1992
fire fighting (fire brigade)	43	Canada	<0.1–6.9		Caux et al. 2002
car repair shops		Italy			
non-smokers	40		0.07		Granello and
smokers	25		0.13		Clonfero 1993

**Table 6a** Urinary excretion of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene (Σhydroxyphenanthrenes) (median or range) in control volunteers

Persons exposed	Number	Country	Σhydroxyphenanthrenes		References
			(µg/g creatinine)	(µg/l urine)	
general population	≥2741	United States	0.36	0.41	CDC 2005 2001–2002
women	1397		0.37	0.38	
men	≥1344		0.35	0.45	
general population	≥2179	United States	0.35	0.41	CDC 2003 1999–2000 1-, 2- and 3-hydroxyphenanthrene
women	≥1131		0.38	0.41	
men	≥1048		0.33	0.41	
general population		Germany			UBA 1998
non-smokers	389		0.67	0.84	
smokers	184		1.00	1.38	
general population	8	Germany		3.5	Grimmer et al. 1991 a
non-smokers	10	Germany	1.3		Jacob et al. 1999 a
smokers	6		2.4		
smokers with lung cancer	10		1.6		



**Table 6b** Urinary excretion of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene ( $\Sigma$ hydroxyphenanthrenes) (median or range) in occupationally exposed persons

Persons exposed	Number	$\Sigma$ hydroxyphenanthrenes		References
		( $\mu\text{g/g}$ creatinine)	( $\mu\text{g/l}$ urine)	
<b>different industries (total)</b>	237	8.48 (0.19–122.18)		BGFA 2005; Jacob and Seidel 2002; Rossbach and Angerer 2002
production of graphite electrodes and special carbon products	71	6.87 (0.54–122.18)		
production of fireproof materials	68	9.81 (0.67–116.84)		
coking plant	47	10.29 (3.30–79.36)		
production of fireproof materials	24	13.96 (0.19–84.22)		
tar distillation	18	7.05 (2.11–29.53)		
fire damage restoration	5	2.17 (0.85–6.53)		
hydraulic engineering and corrosion protection	4	5.61 (3.18–17.07)		
<b>coking plant</b>	8	12.0		Strunk et al. 2002
oven topside area	24		70.0	
	4		103–686 <sup>a</sup>	
charging-car driver	4		45.0–88.0 <sup>a</sup>	
machine operator	4		7.0–22.0 <sup>a</sup>	Grimmer et al. 1991 a Grimmer et al. 1993 b
<b>road construction</b>				
road construction (in general)	49	1.15	0.95	
	4		35.0	
	6	1.50		Martin et al. 1989
hot bitumen processing	66	2.37	1.56	
bitumen processing	7	4.9 to 322 <sup>b</sup>		Marczynski et al. 2006
treatment with creosote (wood preservation)	1	542 <sup>b</sup>		

<sup>a</sup> 24-hour urine;<sup>b</sup> taken from a figure

Analogous to 1-hydroxypyrene, the interindividual differences observed for the excretion of  $\Sigma$ hydroxyphenanthrenes can be explained by the different levels of exposure found in the various trades. The excretion of  $\Sigma$ hydroxyphenanthrenes is also subject to interindividual variations within a trade, as in the case of 1-hydroxypyrene. On the basis of the high factors between 10 and 100, these variations can be explained only by the sometimes considerable dermal absorption of PAH. By contrast, as in the case of 1-hydroxypyrene, interindividual differences associated with genetic polymorphisms in PAH-metabolizing genes are also only of subordinate relevance for  $\Sigma$ hydroxyphenanthrenes (Rihs et al. 2005). For  $\Sigma$ hydroxyphenanthrenes, the analysis of 11 polymorphisms in a total of eight enzymes involved in the xenobiotic metabolism of PAH (CYP1A1, CYP1A2, CYP1B1, CYP3A4, microsomal epoxide hydrolase (EPHX1), GSTM1, GSTT1 and GSTP) shows that only four polymorphisms had a potential influence on the urinary excretion of  $\Sigma$ hydroxyphenanthrenes. The factors with values between 1.5 and 2.0 are negligibly small as compared with the wide ranges of variation with factors of 10 to 100, which may only be due to different dermal absorption at otherwise identical workplaces. Most carcinogenic PAH including benzo[a]pyrene, which has been investigated best, reveal a structural parameter, *i.e.* a bay region that is critical for the biological effect. Bay-region *anti*-benzo[a]pyrene-7,8-dihydrodiol-9,10-oxide, which results from further enzymatic oxidation of the intermediate benzo[a]pyrene-*trans*-7,8-dihydrodiol, is formed as the ultimate biologically active main metabolite of benzo[a]pyrene. By structural analogy to benzo[a]pyrene, phenanthrene as the simplest representative of PAH with a bay region is metabolized to bay-region *anti*-phenanthrene-1,2-dihydrodiol-3,4-oxide via phenanthrene-*trans*-1,2-dihydrodiol (Jacob et al. 1996 b; Nordqvist et al. 1981). Both the intermediate phenanthrene-1,2-dihydrodiol (Grimmer et al. 1993 a, b; Hecht 2002; Jacob et al. 1999 b) and phenanthrenetetrol, which is excreted as the end product of this metabolic pathway (Hecht et al. 2003) and is formed by hydrolysis of bay-region *anti*-phenanthrene-1,2-dihydrodiol-3,4-oxide, are determined in human urine. Whereas the excretion of the monohydroxylated metabolites (phenols) of the non-carcinogenic phenanthrene corresponds to a detoxification pathway, the proposal is made that phenanthrenetetrol should be measured as a qualitative surrogate marker for the metabolic activation of PAH (Hecht et al. 2003) since the specific enzymatic steps proceed analogously to those in carcinogenic PAH like benzo[a]pyrene.

Phenanthrene-1,2-dihydrodiol was determined by means of a GC/MS method after derivatization to methyl ethers (Jacob et al. 1999 a) among workers with occupational exposure (Seidel and Jacob 2005). A median of 0.7  $\mu\text{g/g}$  creatinine was found in workers involved in graphite electrode production, 3.6  $\mu\text{g/g}$  creatinine among coke oven workers, 51.7  $\mu\text{g/g}$  creatinine in workers in converter lining and 21.2 or 127.7  $\mu\text{g/g}$  creatinine in workers involved in the production of fireproof materials depending on the material used. Both these increasing values and the levels for  $\Sigma$ hydroxyphenanthrenes (BGFA 2005) can be explained by an increasing exposure to phenanthrene at the workplaces. However, it is unusual that the ratio of excreted phenanthrene 1,2-dihydrodiol to  $\Sigma$ hydroxyphenanthrenes considerably

increases with an increasing exposure level; this indicates an induction of this metabolic pathway among the persons exposed.

In the United States, the excretion of phenanthrenetetrol was examined among non-smokers, smokers, coke oven workers and psoriasis patients treated with a tar ointment by use of a GC/NICI MS method after derivatization to silyl ethers (Hecht et al. 2003). The results demonstrated that phenanthrenetetrol is a sensitive biomarker for PAH exposure that effectively reflects changes in the exposure level. Accordingly, significant differences in the excretion of phenanthrenetetrol were found in the 4 listed exposure groups with increasing exposure (non-smokers:  $n = 30$ , mean = 1.51 pmol/mg creatinine; smokers:  $n = 31$ , mean = 4.58 pmol/mg creatinine; coke oven workers:  $n = 32$ , mean = 25.7 pmol/mg creatinine; psoriasis patients:  $n = 20$ , mean = 791 pmol/mg creatinine). Both  $\Sigma$ hydroxyphenanthrenes and phenanthrenetetrol excretion were also measured in 300 volunteers of the European general population (non-smokers) from Italy, Poland and Serbia (Seidel et al. 2005). About the same or even higher amounts were excreted in these cohorts with slight exposure as compared with  $\Sigma$ hydroxyphenanthrenes (Italy: medians of 0.37  $\mu\text{g/g}$  and 0.53  $\mu\text{g/g}$  creatinine for  $\Sigma$ hydroxyphenanthrenes and phenanthrenetetrol, respectively; Poland: medians of 2.01  $\mu\text{g/g}$  and 1.77  $\mu\text{g/g}$  creatinine for  $\Sigma$ hydroxyphenanthrenes and phenanthrenetetrol, respectively; Serbia: medians of 1.05  $\mu\text{g/g}$  and 1.10  $\mu\text{g/g}$  creatinine for  $\Sigma$ hydroxyphenanthrenes and phenanthrenetetrol). A 6-week longitudinal study among smokers and non-smokers showed that the excreted concentrations of  $\Sigma$ hydroxyphenanthrenes, phenanthrenetetrol and the ratio of  $\Sigma$ hydroxyphenanthrene/phenanthrenetetrol varied in the individual volunteers (mean coefficients of variation ranging from 29% to 46%). The increased ratio of phenanthrenetetrol/ $\Sigma$ hydroxyphenanthrenes in smokers versus non-smokers (1.39 versus 0.78) was interpreted as an induction of the metabolic activation pathway in smokers (Hecht et al. 2005). The available studies substantiate that phenanthrenetetrol can be used as a good biomarker of systemic PAH exposure.

The human biomonitoring described so far on the basis of the excretion of phenanthrene and pyrene metabolites in the urine does not reflect the systemic load posed by carcinogenic PAH in pyrolysis products or other mixtures containing PAH. Accordingly, efforts are being made to record metabolites of carcinogenic PAH like naphthalene, chrysene and benzo[*a*]pyrene analytically.

Since naphthalene has been re-evaluated as a carcinogenic hazardous substance (2001 Supplement "Naphthalene", this volume) based on an NTP study (NTP 2000), the question of human biomonitoring also arises for this substance. A substantial fraction of the low-boiling naphthalene is absorbed via the lungs. Its metabolites, for example 1- and 2-naphthol, are excreted via the urine. These metabolites are thus easily available for human biomonitoring in the urine. In the meantime, 1- and 2-naphthol have been measured in a number of studies, the results being specified both separately and as a sum ( $\Sigma$ naphthols). The most comprehensive studies in the general population for these two metabolites – and also for all other PAH metabolites – are available from the Center for Disease Control & Prevention in the United States, which tests the body burden of the American general

population for various environmental contaminants (including PAH) in thousands of urine samples on a two-year cycle. The Third National Report on Human Exposure to Environmental Chemicals of the National Health and Nutrition Examination Survey (CDC 2005) specifies an excretion of 4.0 µg/l and 3.5 µg/g creatinine (median) for  $\Sigma$ naphthols measured in 2748 persons. The level for men ( $n = 1349$ ) was 4.5 µg/l and 3.8 µg/g creatinine, whereas the corresponding level for women was 3.6 µg/l and 4.0 µg/g creatinine. Smokers had higher levels for  $\Sigma$ naphthols (Nan et al. 2001; Serdar et al. 2003). Similar values were also found in the urine of pre-school children (Wilson et al. 2003), middle-school students (Kang et al. 2002) and adults (Kim et al. 2003; Kuusimäki et al. 2004; Serdar et al. 2003). The CDC 2005 data (from samples that were collected between 2001 and 2002) were however only at levels of 50% of the values measured by other authors in 983 persons (smokers and non-smokers) in the United States (Hill et al. 1995). The median levels for 1- and 2-naphthol excretion in the urine were 4.4 and 3.4 µg/l, respectively. The corresponding 95th percentiles were 43 and 30 µg/l. Urinary concentrations of 1- and 2-naphthol were also measured in 72 adults and 35 children from Germany to assess the systemic exposure to naphthalene. Smokers had 4 times higher levels of  $\Sigma$ naphthols (related to creatinine) than non-smokers (Preuss et al. 2004 b). Preliminary reference values (95th percentile) of 41.2 µg/g creatinine for adult non-smokers and 23.5 µg/g creatinine for children have been proposed in Germany for  $\Sigma$ naphthols in the urine.

Studies on occupational exposure have also shown that 1- and 2-naphthol are specific and sensitive parameters of an exposure to naphthalene (Nan et al. 2001; Preuss and Angerer 2004 a; Preuss et al. 2003 a, 2004 b, 2005; Rappaport et al. 2004; Serdar et al. 2003).

Higher body burdens of naphthalene of up to about 1300 µg/l were found in occupationally exposed persons as compared with the general population. In coke oven workers, urinary concentrations of 1- and 2-naphthol were between 5.3 and 386.4 µg/l and 4.8 and 174.3 µg/g creatinine, respectively. For workers involved in the production of fireproof materials, the specific concentrations were in a range between 1.5 and 205.8 µg/l (1.5 to 109.5 µg/g creatinine), whereas they were between < detection limit and 216 µg/l (< detection limit and 150.5 µg/g creatinine) for workers in graphite electrode production. The authors found corresponding concentrations for a non-exposed cohort in a range between 1.4 and 83.6 µg/l (1.4 and 84.6 µg/g creatinine) (Preuss et al. 2005).

Unlike naphthalene, higher-boiling PAH (e.g. chrysene and benzo[a]pyrene) are excreted mainly with the faeces (Jacob et al. 1990). The analytical procedures to be developed for this have to be extremely sensitive and specific to be able to detect the small amounts of hydroxylated metabolites present in the urine with sufficient diagnostic validity. The studies initially concentrated on 3-hydroxybenzo[a]pyrene in the urine as the metabolite of the primary substance benzo[a]pyrene.

A method for the determination of 3-hydroxybenzo[a]pyrene after reduction with hydriodic acid was described for the first time in 1983 (Becher and Bjørseth 1983). The concentrations measured with this method were 0.12 µg/l in the urine of

PAH-exposed workers. However, the procedure did not become generally accepted as a routine method. Subsequently, a HPLC method for the determination of 3-hydroxybenzo[a]pyrene was published with recovery rates of 43% and a limit of quantification of 1 ng/24 hour urine (Jongeneelen et al. 1985 a). In a later study, a considerably higher detection limit of 1 µg/l (4 nmol/l) was specified (Jongeneelen et al. 1987). However, since the concentrations of 3-hydroxybenzo[a]pyrene in the urine of workers of a coal-tar distillation plant were below the detection limit, the method did not seem to be very suitable for human biomonitoring. The sensitivity and reproducibility of the HPLC method was improved by the addition of ascorbic acid to the eluent (Bouchard et al. 1994). Later a HPLC method was described with fluorescence detection using a silica precolumn modified with copper phthalocyanine, as originally developed by Lintelmann et al. (1994 a, b) and Boos et al. (1992); detection limits of 6 ng/l and 8 ng/l were found for the simultaneously determined 3-hydroxybenzo[a]pyrene and 3-hydroxybenzo[a]anthracene, respectively (Gündel and Angerer 2000). Concentrations of 3 to 198 ng 3-hydroxybenzo[a]pyrene/g creatinine and 15 to 1871 ng 3-hydroxybenzo[a]anthracene/g creatinine were measured in post shift urine samples from workers (n = 19) engaged in the production of fireproof materials. According to the authors, 3-hydroxybenzo[a]anthracene might be a biomarker that represents the group of carcinogenic PAH (Gündel et al. 2000). The results were in a range that was also found for 3-hydroxybenzo[a]pyrene in another study for both non-exposed persons (6 ng/l; n = 48) and coke oven (370 ng/l; n = 40) and road construction workers (19 ng/l; n = 10) (Grimmer et al. 1990, 1991 a). A correlation was established between the amount of benzo[a]pyrene inhaled within 8 working hours and the excretion of its phenolic metabolites (sum of 3-, 7- and 9-hydroxybenzo[a]pyrene) (Grimmer et al. 1993 a). The inhaled amount of benzo[a]pyrene of 17.71 µg corresponded to an excreted amount of phenol of 0.82 µg. However, the detection limit for 3-hydroxybenzo[a]pyrene using the usual fluorescence detection is too high for a sensitive and specific determination of this analyte in the urine of occupationally non-exposed volunteers (e.g. children) (Hollender et al. 2000). After other, more sensitive methods had initially been established, such as HPLC with laser-induced fluorescence detection (LIF) using an improved sample accumulation or  $\gamma$ -cyclodextrin-modified micellar electrokinetic chromatography with LIF, it was possible to determine lower concentrations in a range of 0.5 to 8 g/l (Ariese et al. 1994; Smith et al. 1998). Although significantly increased 1-hydroxypyrene concentrations were measured in the urine of coke oven workers as compared with non-exposed volunteers, no correspondingly elevated 3-hydroxybenzo[a]pyrene concentrations were observed in most samples (Ariese et al. 1994).

Meanwhile, three sensitive and specific methods have been developed that can routinely be used in studies for the detection of 3-hydroxybenzo[a]pyrene. One method is based on capillary gas chromatography with high-resolution mass spectrometry (GC-HRMS) (Romanoff et al. 2006). After enzymatic hydrolysis, purification by means of solid-phase extraction and silylation of the hydroxylated PAH metabolites, 3-hydroxybenzo[a]pyrene can be detected down to the low ng/l range

using this method. The 95th percentile of 3-hydroxybenzo[a]pyrene determined by the American Environmental Survey with this method in the urine of 2152 persons from the general population was 0.18 µg/l. The median was below the detection limit.

A second method is based on high-pressure liquid chromatography with an APCI tandem mass spectrometer as the detection system (Fan et al. 2006). In the Multiple Reaction Monitoring Mode (MRM), tandem mass spectrometry is another excellent method for the quantitative determination of PAH metabolites in the urine, with only a simple solid phase extraction being required for sample preparation because of the high selectivity after the enzymatic cleavage of the conjugates. The method shows a limit of quantification of 1.03 µg/l for 3-hydroxybenzo[a]pyrene. Besides 3-hydroxybenzo[a]pyrene, 1-hydroxypyrene was also recorded in the urine with this method. In a student cohort of smokers and passive smokers in China, means of  $0.007 \pm 0.011$  and  $0.253 \pm 0.138$  µmol/mol creatinine were found for the excretion of 3-hydroxybenzo[a]pyrene and 1-hydroxypyrene, respectively. In contrast, the means of 3-hydroxybenzo[a]pyrene and 1-hydroxypyrene were  $0.006 \pm 0.012$  and  $0.192 \pm 0.129$  µmol/mol creatinine (corresponding to  $0.011 \pm 0.023$  and  $0.37 \pm 0.249$  µg/g creatinine) for non-smokers (Fan et al. 2006).

A third method is based on high-performance liquid chromatography with four-fold column switching for online purification of the urine samples with subsequent evidence of 3-hydroxybenzo[a]pyrene by means of fluorescence detection (4D HPLC FLD) (Lafontaine et al. 2006; Simon et al. 2000). The median of the concentrations determined by this method was 0.055 ng/g creatinine ( $< 0.024$ – $0.199$  ng/g) in 27 smokers, whereas it was 0.026 ng/g ( $< 0.024$ – $0.107$  ng/g) in 27 non-smokers (Lafontaine et al. 2006). In addition to the regular metabolites 1-hydroxypyrene and  $\Sigma$ hydroxyphenanthrenes, 3-hydroxybenzo[a]pyrene was analyzed in urine samples of a total of 225 PAH-exposed workers from different industrial trades after workshift using the described method (Förster et al. 2007). The median of 3-hydroxybenzo[a]pyrene was 0.8 ng/g creatinine ( $<$  limit of detection to 19.5 ng/g; 95th percentile: 6.7 ng/g) and thus about 40 times higher than the value for non-smokers in the study by Lafontaine et al. (2006). Only 1% of the analyzed samples yielded values below the detection limit. The 3-hydroxybenzo[a]pyrene values revealed a strongly positive association with the specific values of 1-hydroxypyrene and  $\Sigma$ hydroxyphenanthrenes in the urine; no associations between benzo[a]pyrene levels in the air and 3-hydroxybenzo[a]pyrene in the urine were found because of both dermal absorption and inadequate detection limits of benzo[a]pyrene in the air.

The methods of Romanoff et al. (2006) and Lafontaine et al. (2006) are completely automated and require only minor manual sample preparation. In the studies carried out by the American Environmental Survey and by (Lafontaine et al. 2006 and Förster et al. 2007), both methods proved their routine practicability, the 4D HPLC FLD method by Lafontaine *et al.* being more sensitive for the evidence of 3-hydroxybenzo[a]pyrene in the urine. The values of 3-hydroxybenzo[a]pyrene measured in the non-exposed American general population were about 5 to 10 times

higher than those concentrations of the occupationally non-exposed general population in Germany. It has not yet been clarified whether the differences between the two countries were due to the method or to differences in exposure to PAH. The method developed by Romanoff et al. (2006) not only makes it possible to determine 3-hydroxybenzo[*a*]pyrene within one analytical run but also to routinely measure further PAH metabolites with detection limits in the low ng/l range, for example 1-, 3- and 9-benzo[*a*]anthracene ( $\Sigma$ hydroxybenzo[*a*]anthracenes), 1-, 2-, and 3-hydroxybenzo[*c*]phenanthrene ( $\Sigma$ hydroxybenzo[*c*]phenanthrenes), 1-, 2-, 3-, 4- and 6-hydroxychrysene ( $\Sigma$ hydroxychrysenes), 3-hydroxyfluoranthene and 2-, 3- and 9-hydroxyfluorene ( $\Sigma$ hydroxyfluorenes). In this way, a median of 17.5  $\mu\text{g/l}$  urine and 14.7  $\mu\text{g/g}$  creatinine was determined for 3-hydroxyfluoranthene in samples of 2152 persons of the American general population (CDC 2005), whereas the median levels for  $\Sigma$ hydroxyfluorenes were 0.67  $\mu\text{g/l}$  urine and 0.54  $\mu\text{g/g}$  creatinine. The median values were below the detection limit for all other metabolites. The 95th percentile was 0.04  $\mu\text{g/l}$  urine for  $\Sigma$ hydroxybenzo[*a*]anthracenes, 0.06  $\mu\text{g/l}$  urine for  $\Sigma$ hydroxybenzo[*c*]phenanthrenes, 0.26  $\mu\text{g/l}$  urine for  $\Sigma$ hydroxychrysenes, 0.1  $\mu\text{g/l}$  urine for 3-hydroxyfluoranthene and 0.55  $\mu\text{g/l}$  urine for  $\Sigma$ hydroxyfluorenes. At the CDC, this method has recently also been extended to the measurement of 7-hydroxybenzo[*a*]pyrene (Li et al. 2006).

On account of their nucleophilic character, the excreted monohydroxylated PAH (phenols) are biomarkers that represent detoxified metabolites. Ideally, a metabolite of a carcinogenic PAH, such as of benzo[*a*]pyrene, which represents a bioactivation for example to the electrophilically reactive dihydrodiol epoxides, would be a desirable biomarker. Benzo[*a*]pyrenetetrol, which is formed by hydrolysis of *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-oxide (see Section 3.2.2), would be such a biomarker. However, measurement of benzo[*a*]pyrenetetrol in the urine (Bentsen-Farmen et al. 1999; Bowman et al. 1997; Simpson et al. 2000; Wu et al. 2002) has up to now not become established in occupational and environmental medicine since the excreted amounts are very small and the detection limits are not sufficient.

Human PAH exposure at different workplaces in various trades is always characterized by substantial differences in PAH compositions, the spectrum ranging from low-molecular representatives, such as the still volatile naphthalene, to higher-molecular representatives, such as benzo[*a*]pyrene and dibenzopyrenes. The latter are almost completely bound to particles. Therefore, metabolites covering a broad spectrum of exposure are particularly suitable as biomarkers, the amounts excreted in the urine generally decreasing with an increasing molecular weight. Taking into account urine excretion and the well-established methods of determination, phenols are an important class of metabolites for the measurement of PAH exposure. In particular, the phenols of naphthalene, pyrene and phenanthrene are well established biomarkers that can routinely be determined in the urine using various methods. Recent analytical developments also allow a specific and sensitive determination of the relatively small amounts of 3-hydroxybenzo[*a*]pyrene occurring in the urine as exposure markers for the carcinogenic benzo[*a*]pyrene. Since phenols fundamentally represent a detoxification of PAH, it is important to determine further

classes of metabolites that allow in particular an assessment of the hazard potential of PAH. Current analytical developments concentrate on the determination of tetrols that are formed as hydrolysis products from the bay-region dihydrodiol epoxides that are important for the biological activity and on dihydrodiols from which the specific bay-region dihydrodiol epoxides are formed. Non-carcinogenic phenanthrene currently allows such a determination of several classes of metabolites as biomarkers, a metabolite profile being able to be established from several phenols, dihydrodiols and a tetrol formed from a bay-region dihydrodiol epoxide. The latter reflects the most important metabolic activation pathway for PAH because of the structural analogy mentioned before. Further investigations are necessary to establish whether the excretion of phenanthrenetetrol is associated with the formation of dihydrodiol epoxides of carcinogenic PAH such as benzo[*a*]pyrene or with the formation of DNA adducts as their effect markers.

#### 4.2.2 Susceptibility

Interindividual differences in the excretion of PAH metabolites are attributed to sequence variants in genes that are involved in PAH metabolism. Modified genes may affect the catalytic properties (if there is a change in the amino acid sequence) or the amount of the coded enzyme (Alexandrie et al. 2000; Glatt 2004; Kim et al. 2003; Kuljucka-Rabb et al. 2002; Nerurkar et al. 2000; Rihs et al. 2005; Wu et al. 1998, 2002).

**Susceptibility associated with the excretion of 1-hydroxypyrene** The interindividual differences observed in the excretion of 1-hydroxypyrene can generally be caused by genetic polymorphisms of the enzymes involved in the metabolism of pyrene, such as genetic variants of CYP enzymes (phase 1) and various glutathione *S*-transferases (GST) (phase 2) (Autrup 2000; Bartsch et al. 2000). The study of a possible influence of the *CYP1A1 MspI* genotype on the excretion of 1-hydroxypyrene in coke oven workers in Taiwan showed that the 1-hydroxypyrene level among individuals with the homozygous variant (*aa*) of this genotype was twice as high as that of the group with the wild type (*AA*) and the heterozygous variant (*Aa*) ( $p = 0.04$ ) (Wu et al. 1998). In addition, a positive trend was observed for 1-hydroxypyrene excretion in decreasing order. It was more pronounced in the topside oven workers with the homozygous variant trait than in those with the heterozygous variant trait. This trend was less pronounced in side oven workers, but it was also more prevailing in those with the homozygous variant trait ( $p < 0.001$ ). Similar results were obtained in a study conducted among traffic policemen with PAH exposure via contaminated ambient air in municipal areas. Higher concentrations of 1-hydroxypyrene in the urine were found in policemen who smoked fewer than 15 cigarettes/day and belonged to the heterozygous variant of the *CYP1A1 MspI* genotype than in policemen who were homozygous for the reference type. In contrast, no effect of the genotype on 1-hydroxypyrene excretion was found if the policemen were either non-smokers or had smoked more than 15 cigarettes/day



(Merlo et al. 1998). A study of non-occupationally exposed volunteers consisting of Japanese, Hawaiians and Caucasians showed that the urinary excretion of 1-hydroxypyrene in smokers with the heterozygous *CYP1A1 MspI* genotype was twice as high as in smokers with the reference type after adjusting for age, ethnicity and number of cigarettes smoked ( $p = 0.02$ ). Similar results were obtained in this study for the *CYP1A1 Ile462Val* genotype (gene: *CYP1A1\*2B*) (Nerurkar et al. 2000). An elevated urinary 1-hydroxypyrene level in carriers of the homozygous *CYP1A1 462Val* variant was also described in a study carried out among 162 Chinese coke oven workers (Zhang et al. 2001). However, no impact of the *CYP1A1 Ile462Val* genotype on 1-hydroxypyrene excretion was found in a study with coke oven workers in China (Pan et al. 1998). A study among aluminium smelter workers showed a non-significant increase in the 1-hydroxypyrene excretion for individuals with the heterozygous *CYP1A1 Ile462Val* genotype (Alexandrie et al. 2000). Here, the highest urinary 1-hydroxypyrene concentrations were observed among volunteers who additionally had no *GSTM1* expression (null genotype). Unlike these findings, no impact of the *CYP1A1 Ile462Val* genotype was reported among aluminium production workers (Schoket et al. 2001). Further *CYP* genotypes examined such as *CYP1A1 MspI*, *CYP1B1 Leu432Val*, *CYP2C9 Arg144Cys* and *CYP2C9 Ile359Leu* did not affect the 1-hydroxypyrene excretion of the volunteers either (Schoket et al. 2001). In one publication it is pointed out that not only the extrahepatic *CYP1A1* in the lungs but also other forms of *CYP*, particularly forms that occur in the liver and catalyze pyrene metabolism, contribute to 1-hydroxypyrene excretion (Alexandrie et al. 2000). This is substantiated by a study in coke oven workers in Korea. In this study, the *CYP1A1 Ile462Val* polymorphism had no impact; the authors associated this with a clearly lower expression of *CYP1A1* in Asian populations as compared with the Caucasian population. It is interesting that a higher excretion of 1-hydroxypyrene was found among individuals with the *c1/c2* or *c2/c2* genotype of the *CYP2E1* (Nan et al. 2001). The available data suggest that individuals with certain genetic variants of the *CYP1A1* and *CYP2E1* gene have an increased capacity for metabolism of pyrene to 1-hydroxypyrene.

The relationship between *GST* genotypes and the urinary excretion of 1-hydroxypyrene is only of a descriptive nature since the 1-hydroxypyrene concentration is determined by the excreted glucuronide and sulfate conjugates in the urine. No metabolic basis is obvious since phenols are not substrates for *GST*; nor are there any indications that the pyrene-1,2-oxide that is formed intermediately is conjugated with glutathione. The impact of *GSTM1* and *GSTT1* polymorphisms on the urinary excretion of 1-hydroxypyrene as a glucuronide was initially examined in smokers. Whereas 1-hydroxypyrene excretion was higher in smokers with a *GSTM1* deficiency (null genotype) than in individuals with a positive *GSTM1* genotype, it was increased in *GSTT1* positive smokers as compared with those with *GSTT1* deficiency (Hong et al. 1999). An elevated 1-hydroxypyrene excretion linked with the *GSTM1* null genotype was found among coke oven workers in Italy (Brescia et al. 1999). This observation is also supported by the study in aluminium production workers mentioned before (Alexandrie et al. 2000). However, among aluminium

smelter workers, a non-significant decrease in 1-hydroxypyrene excretion was found in individuals who showed a *GSTM1* null genotype. These authors also found an interaction between *GSTM1* and *GSTP1*. Volunteers with a *GSTM1* deficiency showed a significantly lower 1-hydroxypyrene excretion as compared with the *GSTM1*-positive genotype if the *GSTP1* genotype Ile105/Ile105 was present at the same time (Schoket et al. 2001). Other studies among traffic policemen in Italy (Merlo et al. 1998) and coke oven workers in Sweden (vrebø et al. 1998) and in China (Zhang et al. 2001) have led to the result that a GST polymorphism in the enzymes *GSTM1*, *GSTP1* or *GSTT1* has no or only a slight marginal impact on 1-hydroxypyrene excretion. The study among coke oven workers in Korea revealed no impact of *GSTM1* on 1-hydroxypyrene excretion, whereas the concentration of 1-hydroxypyrene in the urine of *GSTT1*-positive volunteers was higher, although not statistically significantly, than that of *GSTT1* null genotypes (Nan et al. 2001). This is consistent with the results from a study among United States Army soldiers serving in Kuwait (Poirier et al. 1998). A recent study carried out among coke oven workers in the Netherlands demonstrated that neither *GSTM1* nor *GSTT1* affect the urinary excretion of 1-hydroxypyrene after occupational exposure to PAH (Van Delft et al. 2001).

It is interesting that the evaluation of a recent Korean study carried out among 661 volunteers without occupational PAH-exposure revealed a 1-hydroxypyrene level in the urine that, after adjustment for smoking and lifestyle, was 1.5-fold higher in *GSTT1* carriers ( $p < 0.05$ ) than in volunteers with the *GSTT1* null genotype. This trend intensified for *GSTT1* carriers if the *GSTM1* gene (*GSTM1*\*0) was not present, i.e. the 1-hydroxypyrene level was increased by a factor of 2. However, other polymorphisms investigated individually in the genes *CYP1A1*, *CYP1B1* and *GSTM1* did not affect 1-hydroxypyrene excretion (Yang et al. 2003).

**Susceptibility associated with the excretion of hydroxyphenanthrenes** Phenanthrene metabolites were used for the human biomonitoring of PAH in numerous studies (the terms hydroxyphenanthrenes and phenanthrols are used synonymously in the literature). Phenanthrene can be metabolized to five different phenols (1-, 2-, 3-, 4- and 9-hydroxyphenanthrene), three *trans*-dihydrodiols (1,2-, 3,4- and 9,10-dihydroxydihydrophenanthrene) and phenanthrenetetrol as the hydrolysis product of *anti*-phenanthrene-1,2-dihydrodiol-3,4-oxide; they are preferably excreted in the form of their sulfates and glucuronides (Boyland and Sims 1962 a, b, c; Boyland and Wolf 1950; Grimmer et al. 1991 b; Hecht et al. 2003; Lertratanangkoon et al. 1982; Sims 1962; Struble et al. 1986). A significant association was found between the excretion of phenanthrene metabolites and the urinary level of 1-hydroxypyrene (Mannschreck et al. 1996).

Since phenanthrene can be oxidized at three different molecule regions and higher amounts of it than of pyrene are metabolized to dihydrodiols by microsomal epoxide hydrolases, its urinary metabolite profile also provides information about the balance of the enzymes involved in these processes. Different CYP forms show different region specificities for the oxidation of phenanthrene (Jacob et al. 1996 a, 1996 b). Accordingly, the induction of individual CYP forms can be identi-

fied from the profile of the phenanthrene metabolites excreted in the urine; for example, an induction of CYP1A2 was found for cigarette smokers (Jacob et al. 1999 a) on account of the modified ratio of the oxidation of phenanthrene at the 3,4-position to the 1,2-position. Furthermore, phenanthrenetetrols, which are formed as a result of the further metabolization of 1,2-dihydrodiol, are described as a valuable biomarker that does not only indicate the biological metabolic pathway of PAH but is also able to detect fine differences in the exposure to phenanthrene in a specified PAH mixture (Hecht et al. 2003).

The influence of genetic polymorphisms on the metabolization of phenanthrene has not been sufficiently investigated so far. A study that included 170 PAH-exposed workers demonstrated that carriers of the *CYP1A1* variant 3801TC, which is identical with the heterozygous variant *CYP1A1* *MspI* mentioned before (Merlo et al. 1998; Wu et al. 1998), had a 1.6-fold higher, significant hydroxyphenanthrene concentration as compared with the homozygous reference (3801TT;  $p = 0.03$ ) (Rihs et al. 2005). The heterozygous *GSTP1* variant 114AV led to a 2-fold increased excretion ( $p = 0.001$ ) of hydroxyphenanthrene and a 1.5-fold higher excretion ( $p = 0.03$ ) of hydroxypyrene versus the reference *GSTP1* 114AA. The same study also revealed an effect on excretion for the two homozygous variants of the microsomal epoxide hydrolase EPHX1 113His/113His and 139Arg/Arg. The variant 113His/113His and the variant 139Arg/139Arg were associated with increased ( $p = 0.05$ ) and reduced ( $p = 0.02$ ) hydroxyphenanthrene excretion, respectively, as compared with the reference genotypes 113Tyr/113Tyr and 139His/139His.

**Susceptibility associated with phenanthrenetetrol excretion** Whereas phenanthrols are biomarkers of detoxification, phenanthrenetetrols are biomarkers of metabolic activation, whose application has recently been described (Hecht et al. 2003, 2005). In a study with 346 smokers, 11 different polymorphisms of various PAH-metabolizing enzymes were also investigated for their possible impact on the ratio of the r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene/3-hydroxyphenanthrene metabolites determined in the urine (Hecht et al. 2006). The heterozygous and homozygous *CYP1A1* Ile462Val polymorphism showed a significant association ( $p = 0.02$ ) with an increased phenanthrenetetrol/3-hydroxyphenanthrene ratio. In contrast, two heterozygous polymorphisms in the *CYP1B1* gene (Arg48Gly and Ala119Ser) were associated with a significantly reduced phenanthrenetetrol/3-hydroxyphenanthrene ratio ( $p < 0.02$ ).

#### 4.3 Local effects on skin and mucous membranes

Skin contact with small amounts of naphthalene (wearing clothes that had been treated with mothballs) induced irritation up to severe dermatitis (Gerarde 1960 a). Contact with clothes that were exposed to mothballs may cause severe erythema. Workers had inflammation of the skin on the hands, arms, legs and lower abdomen after contact with mineral oil containing 1% to 1.5% naphthalene or devel-

oped dermatitis after skin contact with naphthalene. Eye irritation was found at airborne naphthalene concentrations of 15 ml/m<sup>3</sup> and above (1995 MAK documentation “Naphthalene”, Volume 11, present series).

Healing occurred in a man with exfoliative dermatitis after all contact with naphthalene had been eliminated (Fanburg 1940). Cases of haemolytic anaemia were observed in newborn whose nappies had previously been stored together with mothballs (Anziulewicz et al. 1959). Repeated exposure to naphthalene vapours or naphthalene dust led to ulceration of the cornea, lenticular opacity and cataracts (Sandmeyer 1981).

#### 4.4 Allergenic effect

Anthracene increases the sensitivity of the skin to sunlight (Gerarde 1960 a). A positive reaction to naphthalene was observed in the patch test carried out in a 43-year-old patient who suffered from acute recurrent dermatitis. One of 598 patients who were examined because of dermatoses reacted to naphthalene in the patch test. A frequency of 0.13% is specified for allergy to naphthalene (1995 MAK documentation “Naphthalene”, Volume 11, present series).

#### 4.5 Reproductive and developmental toxicity

Two reports are available on transplacental naphthalene poisoning (Anziulewicz et al. 1959; Zinkham and Childs 1958). In both cases, expectant mothers had sucked or chewed naphthalene-containing mothballs for a prolonged period in the last trimester of pregnancy. Haemolytic anaemia with jaundice was observed in the newborn 7 hours and 3 days after birth, respectively.

#### 4.6 Genotoxicity

Genotoxic effects induced by PAH were found in human cells *in vitro* and *in vivo* (Glatt 2005).

#### 4.7 Carcinogenicity

PAH are complex mixtures of substances with several hundreds of different individual compounds, which were assessed anew by the International Agency for Research on Cancer (IARC) in 2005 (Straif et al. 2005; IARC 2010). Benzo[a]pyrene is the only individual substance that has been classified as a confirmed human carci-

nogen to date (IARC Group 1). Furthermore, cyclopenta[*cd*]pyrene, dibenzo[*a,h*]anthracene and dibenzo[*a,l*]pyrene are considered to be probably carcinogenic to humans (IARC Group 2A). Tar oils (oily liquids that form in the fractionated distillation of coal tar; also called creosote) contain PAH mixtures and are also classified as probably carcinogenic to humans (IARC Group 2A).

In 2005, the IARC classified occupational exposures during coal gasification, coke production, coal tar distillation, chimney sweeping, paving and roofing when handling coal tar and aluminium production as carcinogenic to humans (IARC Group 1) (Straif et al. 2005; IARC 2010).

#### 4.7.1 Reference to the workplace

Occupational exposure to PAH generally consists of combustion gas from organic material absorbed via the respiratory tract and of coal tar products absorbed through the skin. In numerous studies, a significant increase of various types of cancer mainly in the lungs, urinary bladder and skin were detected in industries with high PAH exposure. At certain workplaces today, occupational PAH exposure is still far higher than the impact by tobacco smoking. Boffetta et al. (1997) specified the relative cancer risk posed by PAH exposure for various industries. Bosetti et al. (2006) based their review on that by Boffetta *et al.* and assessed the studies published up to 2005 for cancer of the respiratory tract and urinary tract. Armstrong et al. (2004) quantified the dose-response relationships for lung cancer in the various industries in their review. An adjustment made in the studies for smoking has been taken into account.

In Germany, more than 200.000 workplaces must be assumed to have occupational PAH exposure higher than the background contamination in the environment (CAREX 2007). High PAH exposures may occur at workplaces in coking plants and aluminium, iron and steel works, in industrial plants producing and processing coal tar, pitch, creosote, mineral oil, soot and carbon black as well as in production plants of fireproof materials and graphite electrodes. Wide ranges of possible PAH concentrations may be found here. In a multicentre study recently carried out in Germany, PAH exposures were measured several times at workplaces in four different industries (production of fireproof materials and graphite electrodes, coking plants and charging of converters) in the period from 1999 to 2003. Mean exposures of 13.5 µg/m<sup>3</sup> in the production of graphite electrodes, 22.5 µg/m<sup>3</sup> in coke production, 30.2 µg/m<sup>3</sup> in the production of fireproof materials and 576 µg/m<sup>3</sup> in the charging of converters resulted for total PAH (sum of the concentrations of 16 U.S. EPA particle-bound and gaseous PAH measured in the breathing zone) (Rihs et al. 2005).

Particularly in the past, road construction and roofing workers had high PAH exposures caused by the use of coal tar. Tar was prohibited for road construction in Germany in 1984. The general use of coal tar was considerably restricted by the German Tar-Oil Ordinance (Teerölverordnung) of May 27, 1991 and the German

Banned Chemicals Ordinance (Chemikalien-Verbotsverordnung), which became valid in 2002. In road construction, bitumen replaced tar as a binder, which clearly reduced occupational PAH exposure. The composition of vapours and aerosols also changed with the removal of tar. However, there may occasionally be exposure to tar in road construction, for example in the reconstruction of tar-containing roads. Such an exposure to tar can be clearly detected by biomonitoring as high levels of PAH metabolites (Schott et al. 2003).

Sir Percival Pott (1775) observed an unusually high incidence of scrotal carcinomas in chimney sweeps (chimney sweep's cancer) even more than 200 years ago. In later studies, an increased incidence of neoplastic changes of the respiratory tract and other organs was reported in this occupational group (Cadez 1983; Gustavsson et al. 1987, 1988; Hogstedt et al. 1982). Markedly elevated skin cancer incidences (scrotum) and neoplastic changes in other organs were later observed in further occupational groups exposed to coal tar, coal tar pitch or creosote in wood impregnating plants (Karlehagen et al. 1992). Also of historical importance are the studies published in 1936 and 1947 on mortality from lung cancer and laryngeal carcinomas based on occupations specified on death certificates (Kennaway and Kennaway 1936, 1947).

Besides the significantly increased cancer incidences for the lungs, bladder and skin in typical industries with high PAH exposure, evidence of cancer in further organs such as the kidneys, larynx, oesophagus and stomach is listed in the literature. The findings are less consistent and cannot be evaluated conclusively under the aspect of possible mixed exposures and a lack of adequate data overall. However, there are data indicating that there might also be an association with laryngeal carcinoma.

There may be co-exposure under various exposure conditions, particularly by exposure to heterocyclic amines, aromatic amines and benzene. In a study among 6423 men who were employed at an aluminium smelter between 1954 and 1997, the cumulative exposure variables for benzo[*a*]pyrene and benzene-soluble substances were highly correlated ( $r = 0.94$ ) (Friesen et al. 2007).

#### 4.7.2 Lung cancer

The lung is considered to be the main target organ for airborne occupational PAH exposure. As early as in their studies of 1936 and 1947, Kennaway and Kennaway reported an increase in lung cancer mortality caused by PAH for coke oven workers, gas workers and road construction and asphalt workers. Numerous studies were subsequently carried out to examine the relative lung cancer risk posed by PAH; the major ones (with at least 100 lung cancer cases) were those by Andjelkovich et al. (1990, 1992, 1994), Armstrong et al. (1994), Boffetta et al. (2003), Constantino et al. (1995), Doli et al. (1972), Gibbs (1985), Hammond et al. (1976), Hurley et al. (1983), Rockette and Arena (1983), Romundstad et al. (2000), Sorahan et al. (1994), Swaen et al. (1991) and Xu et al. 1996.

In Germany, lung cancer induced by coke oven emissions was included in the List of Occupational Diseases (BK 4110) in 1989 and a technical exposure limit (Technische Richtkonzentration; TRK value) was introduced for benzo[*a*]pyrene (up to 2005). In 1998, the Medical Experts Forum "Occupational Diseases" at the German Federal Ministry of Labour and Social Affairs recommended that the following new occupational disease should be included in the Annex of the German Ordinance on Occupational Diseases: "Lung cancer caused by polycyclic aromatic hydrocarbons if there is evidence of an impact of a cumulative dose of at least 100 benzo[*a*]pyrene years [ $(\mu\text{g}/\text{m}^3) \times \text{years}$ ]" (BMA 1998).

An increase of the relative lung cancer risk caused by occupational PAH exposure has also been substantiated in meta-analyses (Armstrong et al. 2004; Bosetti et al. 2006). However, the estimated risk increase depends on the industry and is moderate in most branches.

A total of 39 cohort studies carried out between 1972 and 2001 were analyzed in a review (Armstrong et al. 2004). The studies covered nine different industries: coking plants (10 cohort studies), gas production from coal (4), aluminium smelters (8), carbon anode production (4), asphalt use (3), tar distillation (3), chimney sweeps (2), thermoelectric power plants (3) and carbon black production (2). The total number of lung cancer cases analyzed was more than 2800. Unlike other meta-analyses, this review also considered quantitative dose-response relationships. The risk estimates were specified as URRs (unit relative risks), *i.e.* as RRs (relative risks) related to 100  $\mu\text{g}/\text{m}^3$  benzo[*a*]pyrene years.

The relative lung cancer risk was significantly increased (1.2 at 100  $\mu\text{g}/\text{m}^3$  benzo[*a*]pyrene years; 95% CI: 1.11–1.29). This means that the lung cancer risk increases by 20% at a cumulative exposure of 100  $\mu\text{g}/\text{m}^3$  benzo[*a*]pyrene years. However, the authors point out that the estimate is to be interpreted cautiously because of heterogeneity, for example, differences in exposure among the branches; risk assessments should therefore be considered specific for the industry. According to the authors, the heterogeneity may be explained by the diverse compositions and therefore different carcinogenicity of PAH mixtures occurring in the various industries (Armstrong et al. 2004). A relatively consistent increase of the relative lung cancer risks was observed for coke, gas and aluminium production. An increased relative lung cancer risk of 1.17 at 100  $\mu\text{g}/\text{m}^3$  benzo[*a*]pyrene years (95% CI: 1.12–1.22) was obtained for the three industries taken together. Except for the carbon black branch (URR = 0.00; 95% CI: 0 to more than 1000), the relative lung cancer risk was considerably higher for the other six branches, but clearly less reliable because of a smaller number of cases and lower exposure levels (URR = 4.30 for carbon anode production; 95% CI: 0.81–22.79; up to URR = more than 1000 for thermoelectric power plants; 95% CI: 0 to more than 1000). There was a significant relative risk increase for asphalt workers and chimney sweeps, but not for workers in carbon anode production, tar distillation, power plants and carbon black production. Exposures in the analyzed studies showed a wide range and were between 0.75 to 805  $\mu\text{g}/\text{m}^3$  benzo[*a*]pyrene years (this corresponds to an airborne concentration of about 0.04–40  $\mu\text{g}/\text{m}^3$ ). It was concluded from the results that occupa-

tional exposure to PAH by inhalation is associated with a significant impact on the lung cancer risk. The average unit relative risk for 100 µg/m<sup>3</sup> benzo[a]pyrene years (URR) was 1.2 (95% CI 1.11–1.29) (Armstrong *et al.* 2004).

Cohort studies from 1997 to 2005 were analyzed in a meta-analysis (Bosetti *et al.* 2006). Studies that had already been assessed in the review by Boffetta *et al.* (1997) were also included in the risk estimates. The results of a total of 57 cohort studies from ten different branches were summarized. Unlike Armstrong *et al.*, however, Bosetti *et al.* calculated the pooled risk estimates as a weighted mean of the SMR (standardized mortality ratios) rather than estimating dose-response relationships for PAH exposures and the relative lung cancer risk. The analyzed studies showed clear heterogeneities for the measured relative risks among the analyzed studies and also within the branches. A significantly increased relative lung cancer risk was found for asphalt workers (RR = 1.14; 95% CI: 1.07–1.22), carbon black production (RR = 1.30; 95% CI: 1.06–1.59), iron and steel foundries (RR = 1.40; 95% CI: 1.32–1.49), roofing workers (RR = 1.51; 95% CI: 1.28–1.78), coking plants (RR = 1.58; 95% CI: 1.98–2.64) and coal gasification (RR = 2.29; 95% CI: 1.98–2.64). The results for coking plants and gas production substantiate the results obtained by Armstrong *et al.* (2004). Unlike Armstrong *et al.* (2004), Bosetti *et al.* (2006) found no significant risk increase for aluminium smelters (RR = 1.03; 95% CI: 0.95–1.11). According to Bosetti *et al.* (2006), the results for carbon black production must be interpreted with restrictions since the considered data are not very reliable. The role of other contaminants that might be involved in the development of lung cancer has been discussed for coal gasification and iron and steel foundries. These are for example heavy metals and quartz in gas production and heavy metals, quartz and asbestos in iron and steel foundries. No significant increase of the relative lung cancer risk was observed for coal anode production, tar distillation or creosotes.

Fayerweather (2007) updated a meta-analysis for paving and roofing workers by Partanen and Boffetta (1994). A significant risk increase was found for roofing workers (RR = 1.67; 95% CI: 1.39–2.20); after adjustment for coal tar, it was no longer significant (RR = 1.10; 95% CI: 0.91–1.33). The increased relative lung cancer risk of roofing and paving workers was attributed to PAH exposures in coal tar.

In summary, an association between occupational PAH exposure and lung cancer can be derived from the available epidemiological studies on the basis of a relative risk. The meta-analysis by Armstrong *et al.* (2004) revealed a statistically significant dose-response relationship between cumulative PAH exposure (in benzo[a]pyrene years) and lung cancer, which was generally even more pronounced in the subgroup of studies adjusted for smoking as compared with the average for all studies.

#### 4.7.3 Bladder cancer

Evidence was already available in the 1990s that an association between occupational PAH exposure and bladder cancer can be assumed on the basis of epidemiological findings obtained in individual studies (e.g. Pesch *et al.* 2000 a; Steineck



et al. 1990). This has been substantiated by reviews (e.g. Boffetta et al. 1997), meta-analyses (e.g. Armstrong et al. 2003; Bosetti et al. 2006; Gaertner and Theriault 2002) and pooled analyses (e.g. Kogevinas et al. 2003), although the association is less pronounced than for lung cancer. There may be different reasons for this. In particular, it must be considered that the lower incidence of bladder cancer in cohort studies as compared with lung cancer leads to a smaller number of cases, which result in a higher uncertainty in risk assessment.

Boffetta et al. (1997) related the risk increases for bladder cancer mainly to exposures to coal tar and coal pitch. In this case, however, there may also be concomitant exposure to aromatic amines, which are not relevant confounders for lung cancer. In a meta-analysis (Bosetti et al. 2006), evidence of increased risks was provided for coal gasification (pooled RR = 2.39; 95% CI: 1.36–4.21), but lower risks were established for aluminium production (pooled RR = 1.29; 95% CI: 1.12–1.49) and steel production (pooled RR = 1.29; 95% CI: 1.06–1.57). Another meta-analysis of 40 cohort studies among foundry workers yielded an only weakly significantly increased relative risk of 1.11 (95% CI: 1.05–1.17). It was only slightly higher if the analysis was restricted to studies for which more adequate exposure estimates were available (Gaertner and Theriault 2002).

In a meta-analysis of 27 cohort studies, an association between occupational exposure and bladder cancer was concluded and a significantly increased relative risk of 1.33 (95% CI: 1.16–1.52) was estimated (Armstrong et al. 2003). The model assumptions used as a basis had a high impact on the risk estimators.

Cohort studies carried out at workplaces with moderate PAH exposure provided less clear evidence of a PAH-associated risk of developing bladder cancer. The relative risk was determined for 48 cases of bladder cancer in a cohort of 7928 asphalt paving workers (Burstyn et al. 2007). Since the number of cases was small, potential confounders could not adequately be controlled. Cumulative PAH exposure was not associated with an increased incidence of bladder cancer, but a doubling of the relative risk was observed at higher concentrations.

Population-based cohorts support the assumption of an association between occupational PAH exposure and bladder carcinomas only to a limited extent. Thus, an expert assessment of the individual occupational histories in a cohort in the Netherlands revealed a relative risk (incidence rate ratio) of 1.24 (95% CI: 0.68–2.27) for high PAH exposure (Zeegers et al. 2001). Smoking behaviour was answered in the negative in all 19 cases. The relative risk of bladder cancer was somewhat, but not significantly higher among active smokers (RR = 1.61; 95% CI: 0.62–4.17). If an adjustment was made after exposure for example to aromatic amines, the relative risk was lower (RR = 1.18; 95% CI: 0.62–2.24).

Various case-control studies also provide evidence of an increased relative risk of bladder cancer caused by occupational PAH exposure. PAH exposure in these studies is generally assessed by a job exposure matrix (JEM). Thus, one of the largest case-control studies that has been carried out to date, the German multicentre urothelial and renal cancer study (MURC) demonstrated that high PAH exposure (determined by means of a British JEM by Pannett et al. 1985) was associated with

a significant relative urothelial carcinoma risk (OR = 1.6; 95% CI: 1.1–2.3) (Pesch et al. 2000 a). A pooled analysis of European case-control studies (Kogevinas et al. 2003), which also included the German data, also established a significant relative risk of 1.23 (95% CI: 1.07–1.40) for high PAH exposure using the Finnish JEM. The authors pointed out that the applied JEM did not take aromatic amines into account as an exposure category. Another major case-control study conducted in British Columbia with 1129 bladder cancer cases and 2998 cancer controls (except for lung cancer) yielded increased relative risks in the smelting of non-ferrous ores including aluminium production, but did not assess PAH exposure quantitatively (Band et al. 2005).

The relatively consistent findings support an association between PAH exposure and cancer of the urinary tract although coal tar products contain aromatic amines such as 2-naphthylamine (Grimmer et al. 1987; Hofmann and Boente 1933).

#### 4.7.4 Renal cell cancer

The kidney is currently not a confirmed target organ for occupational PAH exposure. The IARC classified employment in coke production as “carcinogenic to humans” (Group 1), with the kidney being included as a possible target organ (IARC 1987).

In the past, no clear evidence of a PAH-associated relative kidney cancer risk was obtained in cohort studies for workers in industries with high PAH exposure. Indications of an increased risk were found in various case-control studies after occupational contact with tar, pitch and coal tar products (Mandel et al. 1995; Partanen et al. 1991; Sharpe et al. 1989; Siemiatycki 1991; Siemiatycki et al. 1987, 1988). However, this was not always significant (see McLaughlin et al. 1984, 1985). In a small case-control study, a weak association for an increased relative risk of kidney cancer in men was found for workers in oil refineries who had come into contact with hydrocarbons (no other details) (Kadamani et al. 1989). Increased relative risks were reported, for example in oil refineries, in a Danish case-control study (Mellempgaard et al. 1994). In a relatively large German case-control study, marginally increased relative risks were found for PAH exposure after exposure assessment by means of a British JEM (job exposure matrix) (Pesch et al. 2000 b).

In numerous studies, exposure to benzene and other solvents could not be ruled out, and had even been reported. Therefore, Boffetta et al. (1997) considered the available epidemiological evidence to be inconclusive on its own. The inadequate data from cohort studies and a significantly increased risk for lung and bladder cancer after adjustment for smoking (Pesch et al. 2000 b; Van Dijk et al. 2006) demonstrate that the evidence of renal cell cancer has still not been clarified sufficiently.

#### 4.7.5 Skin cancer

The skin is an important target organ for dermal exposure to PAH, particularly in the form of tar and products obtained from coal tar. Malignant skin tumours generally form via pre-cancerous stages, referred to as tar keratoses, which may already be occupational diseases as such.

Sir Percival Pott (1775) observed an unusually high incidence of scrotal carcinoma in chimney sweeps more than 200 years ago. After these initial observations by Pott, a high incidence of scrotal cancer was observed also after occupational exposure to PAH-containing derivatives from coal tar, among cotton mule spinners after contact with spindle oils (Henry 1946), in car repair shops (Waterhouse 1971) and in the metal industry after exposure to non-water miscible mineral oils such as drilling, cutting, cooling and cylinder oils (Järvholm and Lavenius 1987; Roush et al. 1964; Waldron et al. 1984). An increased risk of squamous cell carcinomas of the skin was found among persons who had been exposed to PAH via mineral oil (OR = 1.46; 95% CI: 1.06–2.05) (Kubasiewicz et al. 1991). A doubling of the relative risk of melanomas established in a study (Bell et al. 1987) was however not detected in different studies and may be due to an observer bias (more intensive and frequent examination of the skin among PAH-exposed persons because of the known risk of the development of squamous cell carcinomas and their preliminary stages) (Letzel and Drexler 1998).

Reliable recent cohort studies on the risk of skin cancer caused by PAH or coal tar and its products are not known. Reviews of epidemiological findings on skin cancer after exposure to PAH-containing materials, in particular to tar, pitch, creosote and other products obtained from coal tar, were published by Bolm-Audorff (1998), by the CBEAP (1972) and by the IARC (1973, 1983, 1984 a, b, 1987).

Cohort studies are not very reliable for predicting the relative skin cancer risk of non-melanoma skin cancer (NMSC) because, except for melanomas, all forms of skin cancer, that is squamous cell carcinomas and their preliminary stages and basalomas, are often not fatal and have therefore generally not been documented in cancer registries. Exposure to UV light and skin type are important co-factors for NMSC and melanomas (Grodstein et al. 1995; Ramos et al. 2004; Veierod et al. 2003). The role of skin hygiene is less well known. It is also difficult to quantify dermal exposure. Initial methods were developed to assess dermal exposure with a semiquantitative contamination index (van Wendel de Joode et al. 2005 a, 2005 b) and with pads (Tsai et al. 2001).

#### 4.7.6 Cancer of the larynx, pharynx and oral cavity

The importance of PAH for the development of malignant tumours of the upper respiratory tract is currently being discussed. Cancer of the larynx, pharynx and oral cavity belong to the potential mode of action of PAH on the basis of mechanistic considerations (Norpoth 1990). The data of a causal relationship resulting from toxicological mechanistic approaches have not been adequately substantiated

by specific epidemiological evidence. The impact of the various risk factors depends on the specific sublocalization. A particular consideration for head-neck tumours are the technical difficulties in diagnosing the sublocalizations and assessing them separately.

With regard to the distinction that must be made between PAH exposure and smoking, this localization poses the added problem that the severe effects of alcohol consumption on the mucous membranes in combination with smoking must also be taken into account. It is currently estimated that 75% of these tumours are due to the combined effect of smoking and alcohol consumption (Hashibe et al. 2007). However, specific data to control these strong confounders are frequently missing in cohort studies. In certain industries, further occupational exposures, in particular exposure to asbestos or welding fumes, are also confounders (Gustavsson et al. 1998).

A high incidence of cancer of the larynx after occupational exposure to PAH has been reported in various historical observations (e.g. Kennaway and Kennaway 1936). However, up to now few cohort studies have specifically determined relative cancer risks for sublocalizations in the larynx and the oral and pharyngeal cavities caused by PAH exposure, particularly not in areas with high exposure to PAH.

Relatively comprehensive case-control studies were carried out for head-neck tumours. Thus, for laryngeal carcinomas increased relative risks have been reported for persons who had ever been exposed as road construction workers (OR = 6.4; 95% CI: 2.4–17.3) (Becher et al. 2005). In 1010 cases with tumours in men and 2176 controls, only indications of an increased relative risk posed by solvents were found for tumours of the larynx and hypopharynx (Berrino et al. 2003).

On the basis of the available data, the special problems of the method and the strong confounders, an impact of occupational PAH exposure on these localizations is assumed for reasons of plausibility although there are no reliable epidemiological data.

#### 4.7.7 Other cancer localizations

PAH compounds are generally able to induce other epithelial tumours as well. However, no confirmed epidemiological data for cancer of other localizations caused by occupational PAH exposure are available. Boffetta et al. (1997) consider epidemiological evidence to be provided for the target organs lungs, skin and bladder after high occupational exposure to PAH. For renal cell cancer and head-neck tumours, there are indications of possible, but not sufficiently confirmed relationships with occupational PAH exposure. There is currently no reliable evidence of a causal relationship between further cancer localizations and occupational PAH exposure.

Stomach and intestinal tumours have been discussed repeatedly because ingestion of PAH, for example through insufficient hygiene or by breathing through the mouth, cannot be ruled out particularly among persons exposed to tar. For instance, an increased incidence of stomach carcinoma was observed among roofing workers in the United States (Hammond et al. 1976). Observations by other

authors (Manz 1984 and Silverstein et al. 1985) reveal an increased number of gastrointestinal tumours after exposure to tar and pitch or in coking plants, but there are also contradictory findings (e.g. Swaen et al. 1991). Other substances with possibly syncarcinogenic effects may also have been involved in causing the cases of observed stomach cancer.

The available documentation (Boffetta et al. 1997; IARC 1973, 1983, 1984 a, b, 1985, 1986 a, 1987) lists leukaemias as other cancer localizations. It must be pointed out that in some areas such as coking plants (Friesen et al. 2007), high exposures may occur through benzenes.

## 5 Animal Experiments and *in vitro* Studies

### 5.1 Acute toxicity

Only few studies are available on the acute toxicity of PAH, with the exception of studies on naphthalene (2001 Supplement "Naphthalene", this volume). The values specified by WHO (1998) show that the acute toxicity is low (Table 16 in the Annex). A single intraperitoneal dose of 10 mg benzo[*a*]pyrene led to an inhibition of growth in young rats (Haddow et al. 1937). Reduced size of the spleen, cellular depletion, haemosiderosis and follicles with large lymphocytes were observed in mice after a single intraperitoneal injection of benzo[*a*]pyrene (dose not specified) (Shubik and Della Porta 1957; WHO 1998). The mitosis rate in the epidermal cells of hairless mice (hr/hr strain) was reduced after a single application of 0.05 ml of a 15% solution of benzo[*a*]pyrene in acetone to the intrascapular region (Elgio 1968). A single intraperitoneal injection of 30 mg chrysene per animal did not affect the growth in rats. One or 2 intraperitoneal injections of 3 to 90 mg dibenzo[*a,h*]anthracene into young rats led to an inhibition of growth within 2 days, and this persisted up to 15 weeks (Haddow et al. 1937).

WHO (1998) tabulated the toxic effects of individual doses of naphthalene (Table A1 in the Annex). In dogs, oral doses of 400 mg/kg body weight led to anaemia and doses of 4000 mg/kg body weight induced heavy diarrhoea. Doses of 1000 to 3000 mg/kg body weight elicited corneal opacity in rabbits; 3000 mg/kg body weight were lethal in rabbits and cats. Bronchial necrosis was observed in mice after inhalation exposure (0.1 mg/l corresponding to 100 mg/m<sup>3</sup>; 4 hours). Effects on the respiratory system were found after intraperitoneal injection into mice, rats and hamsters. More recent studies on the acute toxicity of naphthalene are included in the 2001 Supplement "Naphthalene". In mice, 4-hour inhalation of 2 ml naphthalene/m<sup>3</sup> (10.6 mg/m<sup>3</sup>) led to Clara cell necroses in the epithelium of the proximal bronchiolar respiratory tract of some animals; more severe lesions occurred with increasing concentrations. However, no changes in the cells of the various bronchiolar sections of the trachea were observed in male Sprague-Dawley rats that had been exposed to concentrations of up to 110 ml naphthalene/m<sup>3</sup>.

(586 mg/m<sup>3</sup>) for 4 hours. Lesions of Clara cells were also found in mice after ingestion of 200 mg naphthalene/kg body weight and after intraperitoneal injection at 300 mg/kg body weight and above; the terminal bronchiole epithelium was affected at 200 mg/kg body weight and above.

Repeated inhalation exposure of mice to naphthalene rendered Clara cells resistant to further lesions through a glutathione-dependent mechanism (West et al. 2003).

## 5.2 Subacute, subchronic and chronic toxicity

### 5.2.1 Inhalation

Male Syrian golden hamsters were exposed 4.5 hours daily on 5 days per week for 16 weeks to 9.8 or 44.8 mg/m<sup>3</sup> benzo[a]pyrene by inhalation. No neoplasms were observed in the respiratory tract (Thyssen et al. 1980). Fischer 344/Crl rats were exposed 2 hours per day on 5 days per week for 4 weeks to 7.7 mg benzo[a]pyrene dust/m<sup>3</sup>. Lung lavage, clearance of radioactively labelled particles and histopathological examinations revealed no lesions of the respiratory tract (Wolff et al. 1989).

### 5.2.2 Ingestion

DBA/2N mice having a low affinity Ah receptor (aryl hydrocarbon receptor) were given oral doses of 120 mg benzo[a]pyrene daily for 1 to 4 weeks. The animals died from the toxic effect on the bone marrow. No toxic effect on the bone marrow was observed in C57B1/6N mice with a high affinity Ah receptor; the animals survived 6 months under these conditions (Legraverend et al. 1983). Reduced carboxylase activity was found in the intestinal mucosa of rats that were given 50 or 150 mg benzo[a]pyrene orally for 4 days. The NOAEL (no observed adverse effect level) for the effect on the stomach, liver or kidneys was 150 mg benzo[a]pyrene/kg body weight per day (Nousiainen et al. 1984). The growth of rats that were administered 1.1 g benzo[a]pyrene/kg diet for 100 days was inhibited (White and White 1939; WHO 1998). Ingestion of 100 mg phenanthrene/kg body weight per day for 4 days led to a 30% increase of the carboxylase activity in the intestinal mucosa (Nousiainen et al. 1984). The daily ingestion of benzo[a]anthracene by rats for 4 days resulted in a NOAEL of 150 mg/kg body weight for the effect on the stomach, liver or kidneys. The carboxylase activity in the intestinal mucosa was reduced (Nousiainen et al. 1984).

WHO (1998) listed studies of naphthalene in a table (Table A2). In rats, 150 to 220 mg/kg body weight administered for 14 weeks led to enlarged liver with cellular oedema, damage to the liver parenchyma and signs of an inflammation of the kidneys. The administration of 1000 mg/kg in the diet for 46 to 60 days led to the formation of cataracts; 2000 mg/kg in the diet inhibited growth and induced enlar-

gement and fatty degeneration of the liver. Mice were treated with 27, 53 and 267 mg/kg body weight on 7 days/week for 14 days. The findings observed in the animals of the highest dose group included weight reduction of the thymus and spleen. In a second test (5.3, 53 and 133 mg/kg body weight on 7 days per week for 90 weeks), the relative spleen weights were reduced. In dogs, diarrhoea and anaemia occurred for 7 days after administration of 220 mg/kg body weight (see also 1995 MAK documentation "Naphthalene", Volume 11, present series).

Groups of 20 male or 20 female CD-1 mice were given pyrene doses of 75, 125 or 250 mg/kg body weight by gavage for 13 weeks. Nephropathy was detected in all 4 male control animals, 1 male of the low dose group, 1 male of the middle dose group and 9 males of the high dose group and, correspondingly, in 2, 3, 7 and 10 females. The relative and absolute kidney weights were reduced in the animals of the middle and highest dose groups. A NOAEL of 75 mg/kg body weight per day and a LOAEL (lowest observed adverse effect level) of 125 mg/kg body weight per day were determined on the basis of nephropathy and reduced kidney weights (USEPA 1989; WHO 1998).

### 5.2.3 Dermal absorption

An *in vitro* study of the dermal penetration of benzo[a]pyrene or pyrene in guinea pigs showed that pyrene mainly diffuses passively, whereas benzo[a]pyrene undergoes biotransformation during absorption and 7,8,9,10-tetrol is found as a metabolite in the receptor fluid of the diffusion cells (Ng et al. 1992). Both the parent substance and a wide spectrum of metabolites were found in the receptor fluid in *in vitro* studies with skin samples from mice, rats, rabbits, guinea pigs, marmosets and humans that had been treated with benzo[a]pyrene (Kao et al. 1985). Doses of 1.25 to 125 µg/cm<sup>2</sup> of <sup>14</sup>C-labelled benzo[a]pyrene had been applied to the necks of mice, and the animals were sacrificed 7 days later. Proportions of 6% and 40% of the amount applied were no longer detected within 1 hour and 24 hours, respectively. After 7 days, 7% was still detected at the application site. The major fraction of benzo[a]pyrene was excreted via the hepatobiliary system and in the faeces, *i.e.* 35% after 24 hours, 58% after 48 hours and 80% after 7 days. Only 10% of the radioactivity was found in the urine. The absorption showed a saturation effect at >15 µg/cm<sup>2</sup>; this was regarded as evidence of an increased risk of tumour induction in the epithelium of the skin (Sanders et al. 1986). The binding of benzo[a]pyrene to DNA and proteins in the mouse skin was 15 to 20 times higher if acetone was used as a vehicle instead of a low-viscosity oil (Ingram and Phillips 1993). While <sup>14</sup>C-benzo[a]pyrene dissolved in acetone easily penetrated the skin removed from human cadavers (diffusion cells; 24-hour period; receptor fluid from human plasma), a substantially lower proportion of benzo[a]pyrene from soil penetrated into human skin. A transition of benzo[a]pyrene from such human skin into the receptor fluid was not observed. In an *in vivo* study with rhesus monkeys and benzo[a]pyrene contained in soil, absorption was significantly less as compared with

human skin (Wester et al. 1990). There is evidence of an absorption through the skin (WHO 1998).

### 5.2.4 Subcutaneous and intraperitoneal injection

The weekly subcutaneous injection of 0.25 mg benzo[*a*]anthracene into mice for 40 weeks resulted in an accumulation of iron in lymph nodes and a decrease in the number of lymphatic cells (Hoch-Ligeti 1941). In mice, weekly subcutaneous injections of 0.25 mg dibenzo[*a,h*]anthracene per animal led to soft, enlarged livers with signs of degeneration, an accumulation of iron in the lymph nodes and a decrease in the number of lymphatic cells (Hoch-Ligeti 1941). Haemolympathic changes including extravascular erythrocytes in the region of the lymph nodes and large, pigmented cells were observed in male rats after subcutaneous injections of 0.28 mg dibenzo[*a,h*]anthracene had been given per animal on 5 days/week for 4 weeks (Lasnitzki and Woodhouse 1944).

Mice that had received intraperitoneal pyrene injections of 500 mg/kg body weight per day for 7 days survived (Gerarde 1960 b). WHO (1998) listed studies of naphthalene in a table (Table A2 in the Annex). The intraperitoneal injection of 50 to 200 mg/kg body weight for 7 days showed no morphological effects in mice. The dose of 300 mg/kg body weight injected on the 8th day was also tolerated. Effects in rabbits after subcutaneous injection of 0.1 to 1 ml/kg body weight for 123 days were associated with hypoxaemia induced by haemolytic anaemia (see also 1995 MAK documentation “Naphthalene”, Volume 11, present series).

## 5.3 Local effects on skin and mucous membranes

### 5.3.1 Skin

In 1912, Yamagiwa and Ichikawa (1915) demonstrated for the first time the carcinogenic effect of coal tar on the rabbit skin. A large number of studies followed that showed this effect for numerous coal tar products on the mouse skin (IARC 1985). It was when Cook et al. (1932) applied benzo[*a*]pyrene to mouse skin that numerous PAH started to be investigated in this test system with the aim of both providing evidence of their carcinogenicity and studying dose-response and structure-effect relationships. Reviews of the generation of skin cancer in test animals were compiled by Arcos et al. (1968), Boutwell (1964), Eckardt (1959), Hueper (1964), IARC (1983, 1985), Weisburger and Weisburger (1967), Wynder and Hoffmann (1970), and Pott and Heinrich (1990) (see Section 5.7).

The adverse dermatological effects that were observed in animals after acute and mid-term dermal exposure to PAH included destruction of the sebaceous glands, dermal ulcers, hyperplasia, hyperkeratosis and alterations of epidermal cell growth. Perylene, benzo[*e*]pyrene, phenanthrene, pyrene, anthracene, naphthalene,



acenaphthene, fluorene and fluoranthene generated no sebaceous gland suppression; benzo[*a*]anthracene, dibenzo[*a,h*]anthracene and benzo[*a*]pyrene reduced the sebaceous gland index to  $> 1$  (Bock and Mund 1958). The sebaceous gland index is used to compare the number of active sebaceous glands in the skin of animals treated with a carcinogenic substance with the number of active sebaceous glands in the skin of animals treated with a non-carcinogenic substance. Index 3 means complete destruction of the sebaceous glands after application of the carcinogenic substance; index 2 refers to degeneration of more than half and index 1 to less than half of the sebaceous glands. Index 0 describes the intact skin (Smith 1956; Suntzeff et al. 1955, 1957). In Swiss mice that were treated daily with benzo[*a*]pyrene solutions for 3 days, a concentration of 0.1% destroyed less than half and 0.2% more than 50% of the sebaceous glands (Suntzeff et al. 1955). Single doses of 6.25 to 200 nmol dibenzo[*a,l*]pyrene or its metabolites were applied once to the skin of female SENCAR mice. Dibenzo[*a,l*]pyrene and dibenzo[*a,l*]pyrene-11,12-dihydrodiol, a metabolic precursor of the highly active diol epoxide, caused erythema 5 to 6 days after treatment (Casale et al. 1997).

**Benzo[*a*]pyrene** An  $ID_{50}$  value (irritant dose 50) of  $5.6 \times 10^{-5}$  mmol benzo[*a*]pyrene was determined for dermal irritation to the mouse ear 24 hours after application (Brune et al. 1978).

**Naphthalene** The application of 495 mg to the rabbit skin without occlusion led to slight irritation (Sax and Lewis 1984).

### 5.3.2 Eyes

A single dose of 100 mg naphthalene had a slightly irritant effect on the rabbit eye (Sax and Lewis 1984).

## 5.4 Allergenic effect

**Benzo[*a*]pyrene** A total dose of 250  $\mu$ g benzo[*a*]pyrene in complete Freund's adjuvant was injected into 4 adult female guinea pigs; 2 or 3 weeks later, they were treated with solutions of 0.001, 0.01, 0.1 or 1% benzo[*a*]pyrene in acetone and olive oil to investigate contact sensitization. After 24 hours, slight to marked hypersensitivity was observed (Old et al. 1963). C3H mice were treated with an epicutaneous application of 100  $\mu$ g benzo[*a*]pyrene in 0.1% acetone solution. Contact hypersensitivity was achieved 5 days later by applying 20  $\mu$ g benzo[*a*]pyrene to the ear dorsum. The response was determined via ear swelling, which reached its maximum 3 to 5 days after application. The LOAEL for contact sensitization was 120  $\mu$ g (Klemme et al. 1987). A positive result was also observed for benzo[*a*]pyrene in the local lymph node assay (LLNA). Various concentrations of the test substance (0.5,

1.0 and 2.5% benzo[a]pyrene in acetone/olive oil) or the vehicle were applied to the dorsum of both ears of mice (no other details) on 3 consecutive days. After 5 days, <sup>3</sup>H-thymidine was injected into the animals and lymphocyte proliferation as compared with the control animals was determined via incorporated <sup>3</sup>H-thymidine. Benzo[a]pyrene was classified as sensitizing (Ashby et al. 1995).

**Anthracene** No contact sensitization induced by anthracene was observed in guinea pigs (Old et al. 1963).

**Phenanthrene** No contact sensitization was observed for phenanthrene (Old et al. 1963; WHO 1998).

**Dibenzo[a,l]pyrene** Single doses of 6.25 to 200 nmol dibenzo[a,l]pyrene or selected metabolites were applied once to the skin of female SENCAR mice. Dibenzo[a,l]pyrene and dibenzo[a,l]pyrene-11,12-dihydrodiol, a precursor of dihydrodiol epoxide, caused erythema 5 to 6 days after treatment. The intensity and duration of erythema were dose-dependent, whereas the delayed appearance of erythema was constant and not related to the dose. This was regarded as evidence of contact sensitization induced by these compounds. Histological findings in the skin were consistent with contact hypersensitivity; epidermal hyperplasia and the presence of mononuclear leukocytes in the dermis were observed in association with erythema. The application of a second dose to mice for antigen exposure after 5 days in a second test in which ear swelling was measured confirmed contact sensitization induced by dibenzo[a,l]pyrene (Casale et al. 1997).

## 5.5 Reproductive and developmental toxicity

The results of studies on the embryotoxicity of benzo[a]anthracene, chrysene and dibenzo[a,h]anthracene in rats and of benzo[a]pyrene and naphthalene in mice and rats (Table A3), on the effect of benzo[a]pyrene on fertility (Table A4) and on the effect of benzo[a]pyrene on the postnatal development of mice (Table A5) are summarized in the Annex. The tables have been taken from WHO (1998). In mice, benzo[a]pyrene had adverse effects on the fertility of females and on the postnatal development of the offspring. In a study in young mice, a NOEL (no observed effect level) of 150 mg/kg body weight per day was established for benzo[a]pyrene on the basis of the effects on fertility (sperm in the testicular lumen; litter size) and of embryotoxicity (malformations) (Rigdon and Neal 1965).

## 5.6 Genotoxicity

A review of the genotoxicity of PAH is included in Table 1. The classification of germ cell mutagenicity is noted on the cover page of this documentation.

### 5.6.1 In vitro

The results of studies in *Salmonella typhimurium* with anthanthrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*b*]naphtho[2,1-*d*]thiophene (not listed in WHO 1998), benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenzo[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene, naphthalene (see also 2001 Supplement "Naphthalene", present volume), phenanthrene and pyrene are listed in Table A6 (Annex) based on WHO (1998). The purity of the test substances and details of the test conditions were not listed because of the great deal of available data. Differences in the S9 fractions with regard to the age, sex and strain of the rats used for this purpose and the use of different enzyme inducers might have had a considerable impact on the established results and might also explain discrepancies. In an evaluation of short-term tests carried out by WHO, benzo[*a*]pyrene and pyrene were used as carcinogenic and non-carcinogenic, structurally related test substances, respectively (IPCS 1988).

Studies of DNA damage caused by PAH in prokaryotes are listed in Table 82 of the WHO monograph (WHO 1998). The data compiled in Table A7 (Annex) on DNA damage caused by PAH in eukaryotes were also taken from the WHO monograph; they refer to benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*b*]naphtho[2,1-*d*]thiophene (not listed in WHO 1998), benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenzo[*a,h*]anthracene, phenanthrene and pyrene. No DNA damage was detected for chrysene, phenanthrene or pyrene (with one exception). Evidence of an induction of DNA damage was provided for all other compounds.

More recent studies on the *in vitro* genotoxicity of naphthalene are included in the 2001 Supplement "Naphthalene" (present volume). In CHO cells, naphthalene induced an increase of sister chromatid exchanges in the presence and absence of a metabolic system as well as an elevated incidence of chromosome aberrations only with S9 mix. A significantly increased number of CREST-negative micronuclei was found in the micronucleus test. Naphthalene caused a significant increase of superoxide anions and hydroxyl radicals in J774A.1 macrophages and an increase of DNA fragmentation (2001 Supplement "Naphthalene", present volume).

DNA adducts of dibenzo[*a,l*]pyrene were detected in C3H10T1/2 cells by means of TLC/HPLC and <sup>32</sup>P-labelling (Nesnow et al. 1997). Evidence of DNA adducts was provided in C3H10T1/2CL8 cells after administration of cyclopenta[*cd*]pyrene by means of <sup>32</sup>P-labelling (Nelson et al. 2002).

DNA adducts were also recorded in a study of the metabolism of dibenzo[*a,h*]anthracene in C3H10T1/2 cells. It was possible to detect DNA adducts of the parent substance and some possible intermediate compounds via <sup>32</sup>P-labelling (Nesnow et al. 1994). Following exposure of C3H10T1/2 cells to <sup>3</sup>H-dihydroxyepoxytetrahydrobenzo[*a*]pyrene and <sup>3</sup>H-benzo[*a*]pyrene, it was demonstrated after processing of the lysate that the major fraction of adducts is contained in mitochondrial DNA rather than in nuclear DNA (Backer and Weinstein 1982).

### 5.6.2 *In vivo*

Studies of benzo[*a*]anthracene, benzo[*a*]pyrene and pyrene in *Drosophila melanogaster* are listed in Table A8 of the Annex (WHO 1998).

Studies of benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*b*]naphtho[2,1-*d*]thiophene (not listed in WHO 1998), benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene, dibenzo[*a,e*]pyrene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene and pyrene for possible chromosomal effects of PAH in mammalian cell systems *in vivo* are summarized in Table A9 of the Annex; DNA binding and adducts as well as sperm abnormalities have also been included. Evidence of DNA adducts or DNA binding was provided for all listed compounds except pyrene (WHO 1998). Benzo[*a*]pyrene and *trans*-benzo[*a*]pyrene-4,5-diol led to damage to the DNA in C3H10T1/2 cells; no stable DNA adducts were demonstrated in the cells treated with *trans*-benzo[*a*]pyrene-4,5-diol (Nesnow et al. 2002). SCE were induced after administration of benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene and phenanthrene, but not after administration of pyrene.

Studies of the *in vivo* genotoxicity of naphthalene are included in the 2001 Supplement “Naphthalene” (present volume). No increase in unscheduled DNA synthesis was found in rat hepatocytes. An increase of lipid peroxidation in mitochondria from the liver and brain and an increase of DNA single strand breaks in the liver were observed in female rats after ingestion of naphthalene.

Although benzo[*a*]anthracene increased the number of chromosome aberrations in the bone marrow of hamsters and the oocytes of mice, it failed to do so in the bone marrow of rats and hamsters in a different study. Both negative and positive findings are available for benzo[*a*]pyrene. Benzo[*b*]fluoranthene, dibenzo[*a,h*]anthracene and phenanthrene induced no increase in chromosome aberrations *in vivo*. No increased number of chromosome aberrations was found in bone marrow cells or spermatogonia of Chinese hamsters, whereas a weak increase of chromosome aberrations was observed in the oocytes of mice (Basler et al. 1977).

An elevated number of micronuclei was detected in rat bone marrow cells and spleen cells after administration of benzo[*a*]anthracene, in mouse bone marrow after administration of benzo[*b*]fluoranthene and benzo[*b*]naphtho[2,1-*d*]thiophene, and in the lungs, blood lymphocytes and bone marrow of rats and in the mouse skin after administration of dibenzo[*a,h*]anthracene. Chrysene induced both a positive (Nishikawa et al. 2005) and a negative finding (He and Baker 1991) in the micronucleus test in keratinocytes. For the induction of micronuclei, numerous positive findings in various tissues of mice, rats and Chinese hamsters and negative findings in mice and hamsters are available for benzo[*a*]pyrene. No increase in the number of micronuclei was caused by naphthalene, phenanthrene or pyrene.

Benzo[*a*]pyrene induced dominant lethal mutations (2001 documentation “Benzo[*a*]pyrene”, present volume).

Evidence of DNA adducts was provided in the lung cells of the mouse strain A/J after administration of cyclopenta[*cd*]pyrene by means of  $^{32}\text{P}$ -labelling (Nelson et al. 2002).

### 5.6.3 Interaction of various PAH with regard to genotoxicity and carcinogenicity

Since workers are mainly exposed to PAH mixtures occupationally, the interaction of PAH among each other with regard to their genotoxicity and carcinogenicity is described below.

#### 5.6.3.1 *In vitro* studies of genotoxicity in the Ames test and SOS chromotest and of DNA adduct formation

In another study with  $^{32}\text{P}$ -postlabelling, DNA adducts were investigated *in vitro* in human lung fibroblasts after treatment with individual carcinogenic PAH in a concentration range of 0.1–10  $\mu\text{M}$  as compared with binary mixtures and complex artificial or natural mixtures. Co-exposure of the cells to 0.1  $\mu\text{M}$  dibenzo[*a,l*]pyrene and 0.1  $\mu\text{M}$  of a second PAH led to a reduction of the adduct rate by 16.6% (chrysene) up to 99.4% (dibenzo[*a,h*]anthracene) as compared with treatment with 0.1  $\mu\text{M}$  dibenzo[*a,l*]pyrene alone. Co-exposure to 1  $\mu\text{M}$  benzo[*a*]pyrene and 1  $\mu\text{M}$  of another PAH led to a reduction of between 27.9% (benzo[*ghi*]perylene) and 99.2% (dibenzo[*a,h*]anthracene) as compared with the adduct rate after exposure to 1.0  $\mu\text{M}$  benzo[*a*]pyrene alone. Cells were treated with 1  $\mu\text{M}$  benzo[*a*]pyrene and different concentrations (0.1  $\mu\text{M}$ , 0.2  $\mu\text{M}$ , 1.0  $\mu\text{M}$ ) of dibenzo[*a,h*]anthracene to investigate an influence of the concentration. A dose-related reduction of the adduct rates of 31.6% (0.1  $\mu\text{M}$  dibenzo[*a,h*]anthracene) up to 98.9% (1.0  $\mu\text{M}$  dibenzo[*a,h*]anthracene) was found as compared with the adduct rate after exposure to benzo[*a*]pyrene alone. Furthermore, after exposure of the cells to 0.1  $\mu\text{M}$ , 0.05  $\mu\text{M}$  or 0.01  $\mu\text{M}$  benzo[*a*]pyrene, the addition of a PAH mixture or an organic extract of inhalable air particles induced an up to 5-fold reduction of the benzo[*a*]pyrene dihydrodiol epoxide DNA adducts in the PAH mixture and a 10-fold reduction in the extract as compared with exposure to benzo[*a*]pyrene alone (Binkova and Sram 2004). The authors assume that these results are due to a competition of the individual compounds for binding sites of the PAH-metabolizing enzymes, their saturation or an inactivation of these enzymes by reactive PAH metabolites.

Various individual PAH compounds (anthracene, phenanthrene, fluoranthene, pyrene, benzo[*c*]phenanthrene, benzo[*a*]anthracene, chrysene, benzo[*b,j,k*]fluoranthenes (equimolar mixture of benzo[*b*]fluoranthene, benzo[*j*]fluoranthene and benzo[*k*]fluoranthene), benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a,h*]anthracene, 3-methylcholanthrene, benzo[*ghi*]perylene) and four different PAH mixtures consisting of the listed individual compounds were investigated in the SOS chro-

motest in *E. coli* for their ability to induce DNA damage. At total PAH mixture concentrations of above 1 µg/ml, the induction of DNA damage was lower than the sum of the lesions induced by the individual compounds. However, at total PAH concentrations below 0.75 µg/ml, the SOS response was only slightly lower than the sum of the DNA lesions caused by the individual compounds. Therefore, additivity of the individual effects is assumed in the low concentration range (White 2002). The authors suggest that the sub-additive effects observed at high concentrations may be due to the relatively poor solubility of PAH and thus to a lower bioavailability.

In the bacterial mutagenicity assay with *Salmonella typhimurium* (TA98; activation with S9 mix or microsomes from rat liver), complex PAH mixtures obtained from coal oils reduced the mutagenic activity of benzo[a]pyrene and 7,12-dimethylbenzo[a]anthracene. The amounts tested were between 0.5 and 10 µg benzo[a]pyrene/plate and 25 µg 7,12-dimethylbenzo[a]anthracene/plate. A severer mutagenic effect was observed only at a benzo[a]pyrene concentration of 0.5 µg/plate when benzo[a]pyrene was tested together with a PAH mixture but not when it was tested on its own (Haugen and Peak 1983). Some non-mutagenic hydrocarbons with 2 or 3 unsubstituted rings such as naphthalene (250 nmol) and anthracene (14.3 nmol) increased the mutagenic effects of benzo[a]pyrene in the bacterial mutagenicity assay with *Salmonella typhimurium*. When administered together, most of the mutagenic PAH examined (333.3 nmol naphthalene, 250 nmol benzo[a]anthracene, 500 nmol fluoranthene, 333.3 nmol triphenylene, 16.7 nmol benzo[e]pyrene, 11.1 nmol dibenzo[a,c]anthracene, 33.3 nmol dibenzo[a,h]anthracene, 33.3 nmol benzo[b]fluoranthene, 10 nmol perylene, 2 nmol dibenzo[a,l]pyrene, 10 nmol dibenzo[a,i]pyrene, 14.3 nmol anthanthrene and 25 nmol coronene) produced a reduction of the mutagenic activity of benzo[a]pyrene at high doses; some of them (5 nmol naphthalene, 10 nmol benzo[a]anthracene, 1 nmol benzo[e]pyrene, 1 nmol benzo[b]fluoranthene and 0.25 nmol anthanthrene) also enhanced the mutagenicity of benzo[a]pyrene at lower doses (Hermann 1981). The author specified increases or decreases in mutagenicity indices (in nmol<sup>-1</sup>) corresponding to the reciprocal value of the concentration at which a PAH led to half the maximum increase or decrease of the mutagenicity of benzo[a]pyrene (1 µg). For better readability, these values were converted in this review and specified as corresponding concentrations.

Subadditive effects were also described in a bacterial mutagenicity assay with *Salmonella typhimurium* TA98 for a mixture of benzo[a]pyrene (1 µg) and dibenzo[a,i]pyrene (2 µg). Compared with benzo[a]pyrene alone, the mutagenic effect of this binary mixture was almost unchanged, whereas a slight increase was found as compared with dibenzo[a,i]pyrene (Salamone et al. 1979 b).

#### 5.6.3.2 *In vivo* studies of carcinogenicity on the skin and in the lungs of mice

Benzo[a]pyrene was investigated in the epicutaneous drip test on the skin of NMRI mice both alone and in mixtures with further carcinogenic PAH, *i.e.* dibenzo[a,h]anthracene, benzo[a]anthracene and benzo[b]fluoranthene (Schmidt et al. 1976).

The dorsal skin of groups of 100 animals was treated with PAH in an acetone solution twice a week. On the basis of the known dose-response relationship in this test (Schmidt et al. 1976), doses of 1.0 µg, 1.7 µg and 3.0 µg benzo[*a*]pyrene were selected in such a way that a tumour yield of between 5% and 50% could be expected and both synergistic and antagonistic effects of the examined PAH mixtures could be recorded. Three different mixtures of benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*a*]anthracene and benzo[*b*]fluoranthene were tested. The three other PAH were added to the selected benzo[*a*]pyrene dose (1.0, 1.7 and 3.0 µg) at ratios corresponding to the relative weight in cigarette smoke condensate (1.0 : 0.7 : 2.8 : 1.8), in automotive exhaust condensate (1.0 : 0.7 : 1.4 : 0.9) and in smokehouse soot and tar (1.0 : 0.8 : 2.6 : 2.5). Mixtures of seven non-carcinogenic PAH were also tested both on their own and in combination with the mixtures of the four carcinogenic PAH. The mixture of the non-carcinogenic PAH included phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*e*]pyrene and benzo[*ghi*]perylene. The three mixtures, which contained all 11 PAH corresponding to the specific matrix cigarette smoke condensate, automotive exhaust condensate and smokehouse soot and tar, were composed according to the principle explained above. In contrast, for the mixtures of the seven non-carcinogenic PAH the doses were equal to and 3, 9 and 27 times higher than the weight percent expressed as benzo[*a*]pyrene. All doses applied in the groups "treatment with benzo[*a*]pyrene", "treatment with carcinogenic PAH", "treatment with non-carcinogenic PAH" and "treatment with all PAH" were tolerated by the mice without any acute toxic damage. The mice were observed for the rest of their lives or sacrificed when a tumour occurred at the application site. The histopathological examination generally demonstrated the formation of squamous cell carcinoma at the application site and sporadically also papillomas, fibrosarcomas and malignant lymphomas.

An increase in effects was observed in all treatment groups with carcinogenic PAH (tumour yield 31–71%) as compared with the group that had been treated with benzo[*a*]pyrene (tumour yield 13–53%) alone. The effects in the group in which all 11 PAH were used seemed to be at least the same or severer, but definitely not weaker than those in the group that had been treated only with carcinogenic PAH. The group of seven PAH regarded as "non-carcinogenic" also induced local tumours but at a relatively low incidence (tumour yield < 5%) with the exception of the highest dose (27-fold higher than the weight percent expressed as benzo[*a*]pyrene; tumour yield 17–33%). The statistical evaluation showed linear dose-response relationships.

This study shows that inhibition of the carcinogenic activity of carcinogenic PAH can not be expected if they are applied together with other non-carcinogenic PAH in mixtures that reflect the volume ratios occurring in the environment. The relatively weaker effects of the mixtures of carcinogenic PAH expressed as that of benzo[*a*]pyrene are due to the large percentage by weight of PAH with weaker carcinogenic activity. An additive effect can be assumed for the activity as a mixture, at least for the four carcinogenic PAH investigated here; this was confirmed by the shift of the mixture's dose-response curve, expected according to the toxic equiva-

lency factors (as in the automotive exhaust condensate; see (Schmähl et al. 1977), compared with that for benzo[a]pyrene. Antagonistic effects for the activity of benzo[a]pyrene or dibenzo[a,h]anthracene together with various non-carcinogenic PAH, as had been described by Falk et al. (1964), were not confirmed.

The interactive carcinogenic activity of binary mixtures of benzo[a]pyrene and dibenzo[a,h]anthracene was investigated in female NMRI mice after subcutaneous injection (Pfeiffer 1977). Benzo[a]pyrene was administered alone at 6 dose levels of 3.12 to 100.0 µg and dibenzo[a,h]anthracene was also administered at 6 dose levels of 2.35 to 75 µg. The binary mixtures corresponding to the two individual dose series were also examined (at a ratio of 1.0 : 0.75, as found in automotive exhaust condensate) (Grimmer et al. 1973). In order to be able to record the effects of an actual PAH mixture even better, the specific binary mixtures were additionally tested in a mixture with ten other non-carcinogenic PAH (benzo[a]pyrene; dibenzo[a,h]anthracene; benzo[e]pyrene (2.15–70.0 µg); benzo[a]anthracene (3.12–100 µg); phenanthrene (125–4000 µg); anthracene (31.25–1000 µg); pyrene (65.1–2100 µg); fluoranthene (28.1–900 µg); chrysene (3.12–100 µg); perylene (0.2–7.0 µg); benzo[ghi]perylene (12.8–410 µg); coronene (3.12–100 µg)) at ratios of 1.0 : 0.75 : 0.7 : 1.0 : 40.0 : 10.0 : 21.0 : 9.0 : 1.0 : 0.07 : 4.1 : 1.0, as in automotive exhaust condensate (Grimmer et al. 1973). The mixture of the ten non-carcinogenic PAH was also examined in a separate test series. The two carcinogenic PAH and the three mixtures mentioned (a total of 5 groups with 6 doses each) were dissolved in tricapriline and administered subcutaneously as a single injection into 100 mice per dose. The tumour incidence was determined after 114 weeks.

An increase in the tumour incidence of 9% to 83% and 37% to 69% was found for the 6 doses in the group with benzo[a]pyrene and dibenzo[a,h]anthracene alone. A tumour incidence of 48% to 79% was determined for the binary mixtures. An increase in the tumour incidence of 41% to 82% was also found for the mixture of all 12 PAH. In contrast, the group with the ten non-carcinogenic PAH was indifferent (tumour incidence varied between 4% and 13%). The statistical evaluation yielded linear dose-response relationships for dibenzo[a,h]anthracene and its binary mixture with benzo[a]pyrene, whereas a relationship significantly deviating from linearity was found for benzo[a]pyrene alone. Low doses of dibenzo[a,h]anthracene have a higher tumour-inducing effect than comparable doses of benzo[a]pyrene, whereas the opposite is true at higher doses. The effect of a mixture of benzo[a]pyrene and dibenzo[a,h]anthracene is greater by a factor of 1.4 (statistically not significant) than that of dibenzo[a,h]anthracene alone. The carcinogenic effects of a mixture of all 12 PAH almost completely depend on the effects of the combination of the two carcinogenic PAH. Thus, the mixture of the ten non-carcinogenic PAH does not influence the effects of the combination of the two carcinogenic PAH.

Further studies on the interaction of binary PAH mixtures confirm effects that are both greater than additive (synergistic) and less than additive (antagonistic) (Arcos et al. 1988). It was thus possible to demonstrate that the carcinogenic activity of benzo[a]pyrene in mice is inhibited in the presence of anthracene, chrysene,



perylene, phenanthrene, pyrene and dibenzo[*a,h*]anthracene (Crabtree 1946; Falk et al. 1964; Finzi et al. 1968). However, a synergistic effect for tumourigenicity was reported in the presence of phenanthrene and pyrene and of 7,12-dimethylbenzo[*a*]anthracene (CAS 57-97-6) and methylcholanthrene (Hoffmann and Wynder 1962; Roe 1962; Rusch et al. 1942). The carcinogenic activity of dibenzo[*a,h*]anthracene is also influenced by other PAH in different ways. Anthracene, benzo[*a*]anthracene and phenanthrene inhibited its tumourigenicity (Falk et al. 1964; Hill et al. 1952; Lacassagne et al. 1945; Steiner and Falk 1951), whereas a synergistic effect was reported together with benzo[*a*]anthracene, benzo[*e*]pyrene, phenanthrene and 3-methylcholanthrene (Falk et al. 1964; Steiner 1955; Steiner and Falk 1951). The synergistic, antagonistic and also neutral effects apparently depend not only on PAH, but also on the relative volume ratio in the tested combination.

The interaction of carcinogenic PAH was also investigated in a lung tumour study in male A/J mice (Nesnow et al. 1998). Adenomas developed in the lungs within 8 months of intraperitoneal injection. It was shown that lung adenocarcinomas developed from these lung adenomas within 18 to 24 months. Mixtures of five carcinogenic PAH were examined. Benzo[*a*]pyrene, benzo[*b*]fluoranthene, dibenzo[*a,h*]anthracene, 5-methylchrysene and cyclopenta[*cd*]pyrene were selected based on their environmental occurrence, structural diversity, metabolic diversity and range of tumourigenic potency (Nesnow et al. 1998). After dose-response studies had been performed with each of the PAH, the doses for the mixtures were selected in such a way that the mortality rate was < 25%, reduction in weight gain was < 10%, a predicted tumour response was between 2 and 100 lung adenomas, and the tumour response observed was potentially 2-fold greater or 4-fold less than the additive tumour response. Based on the dose-response data obtained and the environmental levels, the following doses were selected in mg/kg body weight (high dose/low dose): benzo[*a*]pyrene (75/30), benzo[*b*]fluoranthene (75/30), dibenzo[*a,h*]anthracene (10/2.5), 5-methylchrysene (30/10) and cyclopenta[*cd*]pyrene (100/30). This resulted in a total of 32 test groups representing combinations of quintary PAH at either high or low dose. The influence of non-carcinogenic pyrene on a quintary mixture of the carcinogenic PAH was investigated using the following composition: benzo[*a*]pyrene 30, benzo[*b*]fluoranthene 30, dibenzo[*a,h*]anthracene 2.5, 5-methylchrysene 30 and cyclopenta[*cd*]pyrene 100 mg/kg body weight. It must be pointed out that pyrene was inactive in the tumour model used in a dose range of 10 to 200 mg/kg body weight. The results of these studies yielded significant deviations from additivity that were both less than additive and greater than additive tumour responses. The extent of these deviations ranged from +97.4% to -55% of that expected from additivity. The deviations were dose-related, *i.e.* a greater than additive interaction was found for lower doses and a less than additive interaction was found for higher doses. However, less than additive effects dominated among the quintary PAH mixtures tested. The presence of pyrene caused a 35% reduction in the tumourigenicity of quintary mixtures.

Some of the data generated seem to be contradictory to results that were obtained in studies of the interaction of PAH in binary mixtures. Thus, a greater than additive effect on induction of papillomas on the mouse skin was found for a binary mixture of benzo[*a*]pyrene and cyclopenta[*cd*]pyrene, whereas a less than additive effect was observed in the lung tumour model of A/J mice. Similarly, a greater than additive induction of papillomas was observed for benzo[*a*]pyrene and pyrene in the initiation-promotion test on the mouse skin (Nesnow et al. 1985), whereas pyrene, which caused neither skin tumours (Nesnow et al. 1985) nor lung tumours in A/J mice, led to a significant reduction of lung tumour formation elicited by the mentioned quintary mixture of PAH.

The available studies confirm an interaction of PAH in various mixtures with the carcinogenic activity on the skin and in the lungs, depending on the species, target organ, type of administration and volume ratios administered. Since the tumorigenicity of PAH is a multistage process, it can be assumed that a multiplicative effect for two or more carcinogenic PAH coincides with a parallel effect on different stages, whereas additive effects can be assumed for effects of PAH on the same stage (Gibb and Chen 1986). Synergism was also postulated if the step determining the velocity of tumorigenicity was different for both interacting carcinogens (Reif 1984). The available studies show that although interactions between PAH occur, they are limited in extent (Nesnow et al. 1998). Therefore, it seems to make sense to assume an additive effect as an approximation for an assessment of the effect of a PAH mixture.

#### 5.6.3.3 *In vivo* studies of DNA binding

The binding of benzo[*a*]pyrene or 7,12-dimethylbenzo[*a*]anthracene to DNA under the influence of the presence of various concentrations of benzo[*e*]pyrene was investigated in Sencar mouse epidermis. After exposure to 50 nmol benzo[*a*]pyrene and the simultaneous application of benzo[*e*]pyrene (50, 150 and 500 nmol) for 12 hours, DNA binding was increased by 22% to 40% depending on the benzo[*e*]pyrene dose as compared with treatment with benzo[*a*]pyrene alone. After exposure to 200 nmol benzo[*a*]pyrene and 200, 600 or 2000 nmol benzo[*e*]pyrene for 12 hours, an increase in DNA binding was detected only at the highest benzo[*e*]pyrene dose; however, there was no change in DNA binding after exposure for 24 hours. After co-exposure to 7,12-dimethylbenzo[*a*]anthracene (5 or 20 nmol) and up to 20 times the molar amount of benzo[*e*]pyrene for 3 to 48 hours, DNA binding decreased in relation to the benzo[*e*]pyrene dose (Smolarek et al. 1987). In a comparable study the epidermis of Sencar mice was exposed to 20 nmol benzo[*a*]pyrene; only 69% and 59% of the adducts were observed after co-exposure to 60 nmol and 200 nmol benzo[*e*]pyrene, respectively, as compared with exposure to benzo[*a*]pyrene alone. In contrast, the benzo[*a*]pyrene–DNA adduct rates were increased after exposure for 12, 24 or 48 hours; this increase of 27% and 31% was significant at

60 nmol benzo[*e*]pyrene after 12-hour exposure and at 200 nmol benzo[*e*]pyrene after 24-hour exposure, respectively. Benzo[*e*]pyrene had no influence on the benzo[*a*]pyrene-DNA adduct rates after 48-hour exposure (Lau and Baird 1992). When dibenzo[*a,l*]pyrene, dibenzo[*a,e*]pyrene and benzo[*a*]pyrene were simultaneously applied topically to the mouse skin, a total of 31% fewer DNA adducts was detected than after application of the individual substances; DNA binding was 65% higher after co-treatment with dibenzo[*a,e*]pyrene and benzo[*a*]pyrene as compared with the DNA binding for the sum of the individual substances (Hughes and Phillips 1990). In another study, radioactively labelled benzo[*a*]pyrene (25 µg) was applied dermally to the back of shaved CD-1 mice alone and together with one of five different complex PAH-containing mixtures (5 mg). Benzo[*a*]pyrene bound to DNA was  $6.3 \pm 0.63$  pmol/mg DNA after the application of benzo[*a*]pyrene alone. When benzo[*a*]pyrene was applied together with one of the various mixtures, benzo[*a*]pyrene-DNA binding was reduced to levels between  $2.94 \pm 0.59$  and  $0.9 \pm 0.12$  pmol/mg DNA depending on the complex mixture. This was assumed to be due to the competition of the compounds for binding sites of the CYP involved in metabolism (Springer et al. 1989).

The listed studies demonstrate that simultaneous exposure to several PAH may weaken or intensify the genotoxicity of individual compounds, although no super-additive effects were found. The interaction is influenced by the substances that act together and by their concentration, the exposure period and other factors. Therefore the genotoxic potential of mixtures must be assessed individually.

## 5.7 Carcinogenicity

### 5.7.1 Short-term studies

#### *In vitro*

**Cell transformation studies** Benzo[*a*]pyrene and pyrene were negative in the cell transformation test in macrophages isolated from the rat peritoneum (Andersen et al. 1988). Benzo[*a*]pyrene and *trans*-benzo[*a*]pyrene-4,5-diol induced morphological transformations in C3H10T1/2 cells (Nesnow et al. 2002). In another study with C3H10T1/2 cells, benzo[*a*]pyrene and benzo[*a*]pyrene-7,8-diol induced dose-dependent increases in transformation rates; dibenzo[*a,h*]anthracene induced only a slight increase in transformation rates; phenanthrene caused no transformations (Lubet et al. 1983). When the transformation activities of benzo[*a*]pyrene and dibenzo[*a,l*]pyrene were compared in C3H10T1/2 cells, the effect of dibenzo[*a,l*]pyrene was greater by a factor of 4 to 12. The effect on the transformation rates in C3H10T1/2CL8 cells was less for dibenzo[*a,e*]pyrene and greater for dibenzo[*a,l*]pyrene as compared with benzo[*a*]pyrene (Davis et al. 1999). Cell transformations were also recorded in a study of the metabolism of dibenzo[*a,h*]anthracene in

C3H10T1/2 cells (Nesnow et al. 1994). Benzo[a]pyrene and chrysene were also used in a study of the transformation activities of 6-nitrochrysene and 6-nitrobenzo[a]pyrene in the cell lines BALB/3T3, C3H10T1/2 and Syrian hamster embryo cells. All compounds were active in Syrian hamster embryo cells. Considering concentrations and specific transformation frequencies, benzo[a]pyrene had the highest and chrysene the lowest activity. Only benzo[a]pyrene was clearly active in the two other cell lines (Sala et al. 1987). Cruciani et al. (1996) also observed an increased incidence of transformations induced by benzo[a]pyrene in Syrian hamster embryo cells. A comparison of the two cell lines BALB/c-3T3 and C3H10T1/2 showed that although both are very sensitive, the C3H10T1/2 cells did not react to other carcinogenic compounds (Lubet et al. 1990). When benzo[a]pyrene and dibenzo[a,h]anthracene were used as a mixture, a lower (antagonistic) transformation effect was observed in CH310T1/2CL8 cells (Nesnow et al. 2000).

### ***In vivo***

The Solt-Farber model is based on the observation that some compounds that are carcinogenic in the liver of rats induce resistance to their cytotoxicity in some hepatocytes. Cancer cells may develop from these “resistant” hepatocytes. In the Solt-Farber model, pre-neoplastic cells are generated by the short-term administration of 2-acetylaminofluorene in the diet and their proliferation is intensified by partial hepatectomy (Solt and Farber 1976; Solt et al. 1977). Tsuda et al. (1980) applied this model in studies on chemically induced carcinogenicity. The expression of  $\gamma$ -GT ( $\gamma$ -glutamyl transpeptidase) on the surface of the hepatocytes was used as a histochemical marker for the resistant hepatocytes. The  $\gamma$ -GT-positive hepatocytes could be generated with benzo[a]pyrene, but not with naphthalene (classified in Carcinogen Category 2; see 2001 Supplement “Naphthalene”, this volume), pyrene or phenanthrene.

### **5.7.2 Long-term studies**

Light-dependent reactions that tend to lead to less active or ineffective artefacts are avoided by intrapulmonary injection into Osborne-Mendel rats; therefore, this type of administration seems to be particularly suitable for assessing the carcinogenic potential of PAH to the lung not only because it is the target organ. This tumour model leads to similar results as those obtained after drip application to the mouse skin and thus to a similar sequence of the carcinogenicity of individual PAH.

The carcinogenic potential of benzo[b]naphtho[2,1-*d*]thiophene was investigated after a single intratracheal instillation (Wenzel-Hartung 1992; Wenzel-Hartung et al. 1990). Groups of 35 female Osborne-Mendel rats were treated with 0, 1, 3 or 6 mg benzo[b]naphtho[2,1-*d*]thiophene (99.6% purity) per animal. One carcinoma occurred at a dose of 1 mg/animal and 11 carcinomas were observed at both the middle and the high concentration. The incidences were 2.9% (1 mg/animal) and 31.4%; the average survival period was 102 to 105 weeks. A factor of 0.02 was spe-

cified for the carcinogenicity of benzo[*b*]naphtho[2,1-*d*]thiophene as compared with benzo[*a*]pyrene (factor 1.00).

Animal studies of the carcinogenicity of the polycyclic aromatic hydrocarbons anthanthrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenzo[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene and pyrene are listed in Table 7 and have been compiled from Table A11 of the Annex; the relevant data were taken from the WHO documentation (WHO 1998). After dermal application, carcinogenicity was observed for all tested substances. Other organs, such as the lungs or stomach after ingestion and the liver after intraperitoneal injection, were also affected depending on the type of administration (see Table 7) and species. Tumours of the nose, larynx, pharynx, oesophagus, forestomach and trachea were generated in an inhalation study with benzo[*a*]pyrene aerosol (Thyssen et al. 1981). Direct submandibular administration of benzo[*a*]pyrene led to local carcinomas and sarcomas in rats (Reichhart and Althoff 1980). Phenanthrene and pyrene were carcinogenic only after dermal application. Alternating subcutaneous treatment of 10 mice (alternately into the right and left loin and into the peritoneal cavity) with dibenzo[*a,h*]anthracene twice per week for 50 weeks produced subcutaneous sarcomas in 3 animals (Boyland and Burrows 1935).

No or ambiguous skin tumours were observed after dermal application of naphthalene (Kennaway 1930; Knake 1956; Schmeltz et al. 1978). However, it must be pointed out that dermal application has only limited suitability for carcinogenicity studies because of the high vapour pressure of naphthalene. An inhalative carcinogenicity study in mice (NTP 1992) showed a significantly increased occurrence of pulmonary, alveolar and bronchial adenomas in females. Naphthalene was classified in Carcinogen Category 2 of the *List of MAK and BAT Values* (see 2001 Supplement "Naphthalene", this volume) on the basis of an NTP inhalation study that was carried out in 2000 and that had revealed tumours in the respiratory and olfactory epithelium in male and female rats.

## 5.8 Other effects

Model studies with CYP forms (CYP1A1, CYP1B1 and CYP1A2) of various species (human, rat and mouse) singularly expressed in V79 Chinese hamster cells resulted in different metabolite profiles and concentrations of proximal and ultimate carcinogens for these species (Jacob et al. 1996 a, b, 1999 a; Seidel et al. 1998 a).

Immunotoxic effects were found in male Wistar rats after ingestion of benzo[*a*]pyrene for 35 days at dose levels of 3, 10, 30 or 90 mg/kg body weight. A dose-related decrease in thymus weights was observed in the animals receiving the 3 higher doses, and lymph node weights were reduced in the high dose group. Most toxic effects were observed in this dose group. The relative number of B cells in

Table 7 Target organs of polycyclic aromatic hydrocarbons (data from WHO 1998)

	Administration									
	inhalation	ingestion	i.v.	i.p.	i.pul.	dermal	s.c.	i.m.	i.mam.	i.col.
anthanthrene					lung	skin	no		no	
benzo[a]anthracene		lung	no	no			abnormal finding		abnormal finding	
benzo[b]fluoranthene			abnormal finding	abnormal finding		skin	skin	no	no	
benzo[j]fluoranthene				lung	lung			abnormal finding	abnormal finding	
benzo[k]fluoranthene				liver; lung	lung	skin				
benzo[b]naphtho-[2,1-d]-thiophene					lung	skin	skin			
benzo[a]pyrene	nose; pharynx; larynx; trachea; stomach	stomach; mammary glands	lung	liver; lung	respiratory tract	skin	skin	no	mammary glands	lung; stomach; skin; mammary glands
chrysene		skin		lung; liver	no	skin				
cyclopenta[cd]pyrene				lung	abnormal finding	skin			no	abnormal finding

Table 7 (Continued)

	Administration									
	inhalation	ingestion	i.v.	i.p.	i.pul.	dermal	s.c.	i.m.	i.mam.	i.col.
dibenzo[ <i>a,h</i> ]anthracene		lung	lung		lung		skin; lung	no abnormal finding		
dibenzo[ <i>a,e</i> ]pyrene										
dibenzo[ <i>a,h</i> ]pyrene				liver; lung		skin	skin		mammary glands	
dibenzo[ <i>a,l</i> ]pyrene*						skin			mammary glands	
indeno[1,2,3- <i>cd</i> ]pyrene				liver	lung	skin	skin			
naphthalene	respiratory tract	no abnormal finding		no abnormal finding						
phenanthrene		no abnormal finding		no abnormal finding	no abnormal finding	skin	no abnormal finding			
pyrene				no abnormal finding	no abnormal finding	skin	no abnormal finding			

\* According to Luch and Jacob (in DFG 2004: "Dibenzo[*a,l*]pyren, ein polycyclischer aromatischer Kohlenwasserstoff mit außergewöhnlichen biologischen Wirkungen") all data published until 1966 are questionable and without scientific relevance because of the synthesis route used earlier.  
 Key: i.col.: administration into the colon; i.m.: intramuscular injection; i.mam.: administration into the mammary gland; i.pul.: intrapulmonary injection; i.p.: intraperitoneal injection; i.v.: intravenous injection; s.c.: subcutaneous injection

the spleen was reduced at a dose of only 10 mg/kg body weight (De Jong et al. 1999). The study of an immunotoxic effect of naphthalene in spleen cultures was negative. The authors assume that this is due to the inability of microsomal splenocytes to metabolize naphthalene (Kawabata and White 1990).

The effects of PAH exposure on T lymphocytes and natural killer cells were examined among 10 asphalt workers and 13 coke oven workers. Urinary excretion of 1-hydroxypyrene was significantly increased among the coke oven workers as compared with the asphalt workers. An inhibition of T-lymphocyte proliferation was observed in the workers exposed to PAH as compared with the volunteers of the control group. An increase of natural killer cells only occurred among the asphalt workers (Karakaya et al. 2004).

## 6 Methylpyrene as representative of alkylated PAH

### 6.1 General information

It has long been known that not only unsubstituted aromatic PAH but also many alkylated PAH occur in the environment, particularly those that contain one or several methyl groups or that are bridged with a methylene group in the bay region. Unsubstituted hydrocarbons have been investigated much more thoroughly for their effect and occurrence than these alkylated PAH. One of the reasons for this is that it is difficult to separate and elucidate the structure of many position isomeric compounds, particularly as reference substances are available only to a limited extent.

### 6.2 Occurrence of alkylated PAH

A study by Broman et al. (1987) in which sediments from the Baltic Sea near Stockholm were investigated for PAH by means of GC/MS can be taken as an example of the subject matter under discussion. In addition to phenanthrene (562 ng/g), 4 peaks of monomethyl phenanthrenes (together 528 ng/g), 13 peaks of dimethyl phenanthrenes (767 ng/g) and 17 peaks of trimethyl phenanthrenes (698 ng/g) were found. Since no standards were available for dimethyl and trimethyl phenanthrenes, their exact structure could not be elucidated. For quantification, it was assumed that the yield in processing and the response factor in mass spectrometry were the same as those of the analogous pure PAH. All three possible isomeric methylpyrenes (1-, 2- and 4-methylpyrene) were also detected in the sediment in this study.

Methylpyrene was also detected in cigarette smoke condensates (Grimmer 1988 b), automotive exhaust emissions and cellulose pyrolysis products (Okamoto and



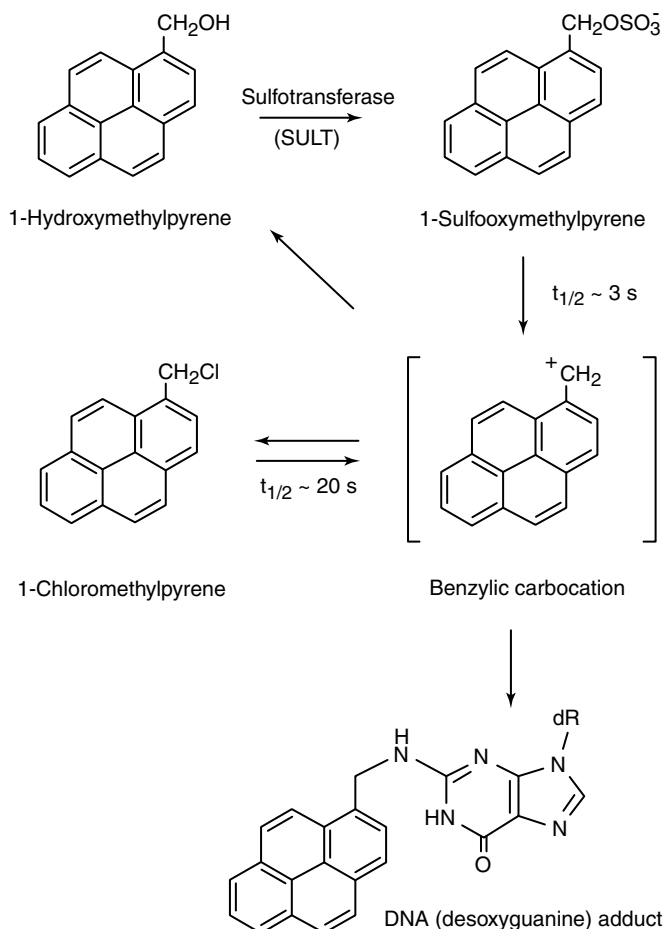
Yoshida 1981), smoked cheese (Guillén and Sopelana 2004), olive oil (Guillén et al. 2004), and in oysters, shellfish and fish (Pancirov and Brown 1977). For deuterated standards of various alkylated PAH – including 1-methylpyrene, 2-methylpyrene, 4-methylpyrene, 1,6-dimethylpyrene and 1,8-dimethylpyrene – it was demonstrated that about the same ratio of all three monomethylpyrenes occurred in all tested samples (cigarette smoke condensates, used oils from various engines, soil and sediment samples, coal tar and the particle fraction from diesel engine emissions); the level of methylpyrenes far exceeded that of benzo[*a*]pyrene (in cigarette smoke condensates by a factor of 8) or was clearly below the benzo[*a*]pyrene level (by a factor of 7 in coal tar). 1,6-Dimethylpyrene and 1,8-dimethylpyrene were also detected in all these samples either individually or as the sum except in coal tar.

### 6.3 Metabolism

On the one hand, alkylated PAH can be activated to reactive metabolites, e.g. bay-region dihydrodiol epoxides, via the same metabolic pathways as their unsubstituted congeners. On the other hand, alkyl substituents provide additional activation pathways, *i.e.* the formation of reactive benzylic sulfuric acid esters. Pyrene and 1-methylpyrene exemplify the relevance of side chain activation. Since neither compound has an angular terminal benzo ring, they cannot be activated to vicinal dihydrodiol epoxides.

Both 1-hydroxymethylpyrene, a benzylic alcohol, and ring-oxidized metabolites were found after incubation of 1-methylpyrene with liver microsomes and NADPH, the co-factor for monooxygenases (Engst et al. 1999). Side-chain oxidation was strongly dominant when microsomes from humans and untreated rats were used. After enzyme induction with 3-methylcholanthrene, the metabolic pathways with rat liver microsomes drastically shifted in favour of ring oxidation. This pathway also dominated in V79 cell lines in which rat CYP1A1 was expressed, *i.e.* in the form that is induced by 3-methylcholanthrene in the liver to a very high extent. In contrast, all tested human CYP forms expressed in V79 cells preferably oxidized at the methyl group. This also applied to CYP1A1, CYP1A2 and CYP1B1, which are well induced by PAH depending on the tissue, and to CYP3A4, the quantitatively most important form in the liver. It is therefore expected that humans metabolize a substantial part of 1-methylpyrene to 1-hydroxymethylpyrene – both with and without enzyme induction by PAH. 1-Hydroxymethylpyrene is an excellent substrate for numerous sulfotransferases of humans and rats (Glatt et al. 2003). The further metabolization of 1-hydroxymethylpyrene is shown in Figure 7. No induction of sulfotransferases by PAH was observed (Glatt 2007 a).

The sulfate group is a good leaving group in some chemical compounds. Its spontaneous cleavage leads to highly reactive carbonium ions (or to nitrenium ions in aromatic amines) that can covalently bind to cellular macromolecules (Glatt et al. 2003). The resulting sulfoconjugate, 1-sulfooxymethylpyrene, has a short



**Fig. 7** Metabolic pathway of 1-hydroxymethylpyrene, formed from 1-methylpyrene via CYP enzymes, to DNA adduct (Glatt et al. 2003)

half-life in water (about 3 min), is highly reactive and forms mutagenic DNA adducts (see also Figure 7). It is remarkable that the expression of sulfotransferase forms that activate 1-hydroxymethylpyrene is greatly concentrated on the liver in rats, whereas in humans extrahepatic sulfotransferase forms also activate 1-hydroxymethylpyrene very efficiently (Glatt et al. 2003). 1-Hydroxymethylpyrene is moreover an excellent substrate for alcohol dehydrogenases (Ma et al. 2002), in humans mainly for form 2 (Kollock et al. 2008). After administration of 1-hydroxymethylpyrene to rats, oxidation to carboxylic acid actually dominated the biotransformation; this competitive reaction reduces sulfotransferase-dependent toxification.

## 6.4 Genotoxicity

**In vitro** 1-Methylpyrene was positive in the test in *Salmonella typhimurium* TA98 with metabolic activation (S9) (50–200 µg/plate taken from published graph). In another study, the metabolites that formed were extracted and separated by means of HPLC. In addition to 1-hydroxymethylpyrene, the 4,5-dihydrodiol and the 9,10-dihydrodiol of 1-methylpyrene were identified (Rice et al. 1988 a). In CYP-expressing V79 cells, the highest absolute rates of benzylic hydroxylation were found in the cells that expressed the forms CYP1B1, CYP1A1 and CYP3A that occur in humans. The relative rates of benzylic hydroxylation (1-hydroxymethylpyrene and 1-pyrenecarboxylic acid as sum parameters) in the cells were 83%, 81%, 80–85% and 45% in CYC3A4, CYC2E1, CYC1B1 and CYC1A1, 3% in CYP1A1 and 45% in CYP1B2 from rats (Engst et al. 1999). The main metabolite in humans, 1-hydroxymethylpyrene, was highly mutagenic in *Salmonella* strains and V79 cell lines that express certain sulfotransferases of humans or other mammalian species (Glatt et al. 2002, 2003). 1-Methylpyrene was a potent inducer of unscheduled DNA synthesis (UDS) in freshly isolated rat hepatocytes (Rice et al. 1987). At a concentration of 1 µM 1-methylpyrene, this effect was so strong that it could no longer be quantified, whereas unsubstituted pyrene remained inactive in the same system even at the highest concentration tested (0.5 mM). 7,12-Dimethylbenzo[*a*]anthracene, a potent carcinogen that was used as a positive control, showed a clearly weaker UDS induction than 1-methylpyrene. The sulfoconjugate, 1-sulfooxymethylpyrene, formed DNA adducts in cell-free systems (Glatt et al. 2003) as well as in bacterial and mammalian cells in culture and induced mutations if a transmembrane transfer was ensured. This could be achieved by the addition of chloride ions to the medium, which led to the spontaneous formation of the charge-neutral reactive species 1-chloromethylpyrene (Glatt et al. 1990).

**In vivo** In rats, intraperitoneal injection of 1-hydroxymethylpyrene induced the formation of DNA adducts *in vivo* in various tissues (Surh et al. 1990 b; Glatt et al. 2003). Male Sprague-Dawley rats received intraperitoneal injections of 1-hydroxymethylpyrene (0.25 µmol/kg body weight) labelled with tritium. After 6 hours, the benzylic DNA adducts accounted for about 60% to 70% of the radioactivity bound in the liver (Surh et al. 1990 b). Evidence of enhanced formation of DNA adducts in the liver and other individual tissues was also provided with the <sup>32</sup>P-postlabelling method (Glatt et al. 2003). The highest adduct incidences were measured in the kidneys although the sulfotransferase activities were substantially lower in this tissue than in the liver (for metabolism see also Figure 7). This is associated with the distribution of 1-sulfooxymethylpyrene formed in the liver via the blood. After intraperitoneal injection into rats, 1-sulfooxymethylpyrene was still detected in the plasma after 3 hours in spite of its short half-life (water; 37°C). The reversible binding to plasma proteins considerably inhibited the hydrolysis of 1-sulfooxymethylpyrene to 1-hydroxymethylpyrene (Glatt et al. 2003). The formation of these DNA adducts was intensified up to 200 times if the further oxidation of 1-hydroxy-

methylpyrene was blocked by inhibition of alcohol dehydrogenases – by the inhibitor 4-methylpyrazol or the competing substrate ethanol. *In vivo*, up to  $10^5$  DNA adducts were achieved per  $10^8$  nucleotides, a frequency that is unusually high for adducts of this structural type.

The sulfo conjugate 1-sulfooxymethylpyrene formed DNA adducts after parental administration to rats (Glatt et al. 2003) and induced mutations by the expression of organic anion transporters in the target cell (Bakhiya et al. 2006).

After administration of alkyl pyrenes, their benzylic alcohols or sulfuric acid esters, the same DNA adduct pattern was found in animals *in vivo* as after treatment of DNA with sulfate ester *in vitro* (Glatt et al. 2007 a). This implies that the described activation pathway is actually responsible for the effects observed *in vivo*.

## 6.5 Carcinogenicity

Several carcinogenicity studies were carried out with 1-methylpyrene. 1-Methylpyrene induced no skin tumours after topical application, which is not surprising for an activation pathway in which hepatic sulfotransferase forms play an important role. A carcinogenic finding was obtained in newborn mice. In a group of 39 newborn mice, each animal was intraperitoneally treated with 150 µg 1-methylpyrene on day 1, 300 µg 1-methylpyrene on day 10 and 600 µg 1-methylpyrene on day 15 of life. The surviving 19 males and 19 females were sacrificed after 52 weeks and their livers examined histopathologically. 1-Methylpyrene led to a significant increase in liver tumours (8 tumours; 1 tumour in control animals). A not significantly increased incidence of lung adenomas and leukaemias was observed in the animals treated with 1-methylpyrene, but not in the control animals. Benzo[a]pyrene was used as a positive control in this study. Although its dose was lower by a factor of 3.8, benzo[a]pyrene induced liver tumours in about twice the percentage of animals and in a number of animals that was higher by about a factor of 7 (Rice et al. 1987). On the basis of these data, an equivalency factor of 0.1 could be attributed to 1-methylpyrene with the restrictions that 1-methylpyrene and benzo[a]pyrene were tested at only one dose level and that newborn mice express relatively few sulfotransferases. The sulfotransferases that are required for the activation of 1-methylpyrene are detoxifying for benzo[a]pyrene.

1-Hydroxymethylpyrene initiated and promoted enzyme-altered foci in the liver of newborn rats (intraperitoneal injection; 9 treated and surviving animals; 12 control animals; sacrifice after 155 days). Induction of such enzyme-altered foci is a good indicator of a hepatocarcinogenic effect (Glatt et al. 1994 a). 1-Sulfooxymethylpyrene initiated tumours on the mouse skin after epicutaneous administration. Groups of 30 female CD-1 mice were treated with 10 topical applications of 1-hydroxymethylpyrene and 1-sulfooxymethylpyrene (0.25 and 0.5 µmol in each case) dissolved in acetone/DMSO. One week after the last application, 2.5 µg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was applied twice weekly for 22 weeks.

All animals survived. After treatment with 1-hydroxymethylpyrene, papillomas were observed in 1 of 30 animals at the low dose and 3 of 30 animals at the high dose; 7 and 10 of 30 animals had papillomas after 1-sulfooxymethylpyrene had been applied. Papillomas occurred in 3 of 30 control animals. Therefore, only 1-sulfooxymethylpyrene induced a significant increase in the incidence of skin papillomas in mice (Surh et al. 1990 b). 1-Sulfooxymethylpyrene is a complete carcinogen on rat skin after subcutaneous injection. Groups of 12 rats (30 days old) were treated subcutaneously with 0.2  $\mu\text{mol}$  of either 1-hydroxymethylpyrene or 1-sulfooxymethylpyrene every other weekday for 20 doses. The vehicle sesame oil/DMSO was injected at a ratio of 9:1 into the animals of one control group; the animals of the second control group remained untreated. After 52 weeks, sarcomas were detected in 7 of the animals treated with 1-sulfooxymethylpyrene, but not in the animals treated with 1-hydroxypyrene. Fibroadenomas developed in 2 animals of this group. No tumours were observed in the animals of the control group (Horn et al. 1996).

## 6.6 Assessment of carcinogenicity

Pyrene was not carcinogenic in mice or golden hamsters (Table A12 in the Annex) and led to negative results in most genotoxicity studies; if there was a positive result in genotoxicity studies, its potency was much weaker than that of benzo[a]pyrene. However, clear evidence of carcinogenicity and genotoxicity has been provided for 1-methylpyrene, and its activation pathway has been elucidated.

1-Methylpyrene is widespread in the environment, but the ratio of the 1-methylpyrene and benzo[a]pyrene levels may vary considerably.

1-Methylpyrene may be activated to produce a genotoxic metabolite both *in vitro* and in animal studies. The activation pathway is well described and deviates considerably from that of benzo[a]pyrene. Studies with cDNA-expressed enzymes suggest that 1-methylpyrene is also activated in humans – presumably even to a higher extent than in mice and rats, particularly if extrahepatic tissues are considered.

Only a few animal studies are available on the carcinogenicity of 1-methylpyrene and its metabolites. Although these clearly substantiate a carcinogenic effect, its potency cannot be sufficiently assessed. 1-Methylpyrene was about one order of magnitude less potent than benzo[a]pyrene in newborn mice. Since the two substances are activated via different metabolic pathways, their relative potencies might also depend on the test system. The enhanced formation of DNA adducts after simultaneous administration of ethanol and 1-hydroxymethylpyrene shows that the effect may depend heavily on the actual enzymatic situation. It is rather problematical to establish equivalency factors under these conditions.

Many other alkylated PAH may be activated to become benzylic sulfuric acid esters, including 1-methylpyrene, 2-methylpyrene, 4-methylpyrene, 1,6-dimethyl-

pyrene and 1,8-dimethylpyrene (Glatt 2007 d). However, the benzylic sulfuric acid esters of 2-methylpyrene and 4-methylpyrene are chemically much less reactive and biologically less active than 1-sulfooxymethylpyrene. 1-Sulfooxymethylpyrene and the sulfuric acid ester of 1,6-dimethylpyrene are active to a similar degree, while the sulfuric acid ester of 1,8-dimethylpyrene is clearly more reactive and biologically more potent. Smaller amounts of 1,6-dimethylpyrene and 1,8-dimethylpyrene than of 1-methylpyrene occur in the investigated matrices; it is also more difficult to analyze them since there may be 15 isomeric dimethylpyrenes and three ethylpyrenes with the same mass. Moreover, only few biological data are available for these compounds. For these reasons, 1-methylpyrene is suggested as a representative of the large class of alkylated PAH in routine analysis. This substance should also be tested in different animal models for the potency and organotropy of its carcinogenicity in comparison with an unsubstituted PAH, e.g. benzo[a]pyrene.

## 7 Potency balances for PAH

Significant progress in understanding a causal relationship between PAH exposure and carcinogenicity was made by balancing the potencies. In these balances, mixtures commonly suspected of being carcinogenic, such as automotive exhaust emissions, used engine oil, coal combustion condensate and tobacco smoke condensate, were chemically fractionated, and these fractions were tested in a suitable animal model (epicutaneous application to mice or intrapulmonary administration in rats) for their carcinogenic potential in comparison with the original mixture (Grimmer 1993 c; Jacob 1996). Table 8 shows results of such potency balance analyses for five different mixtures.

Three conclusions can be drawn:

- 1) In all mixtures examined, carcinogenicity is predominantly (70–90% and more) caused by the class of PAH with 4 or more rings although almost all of them account for less than 4% w/w of the mixture (exception: coal combustion condensate with 23% to 29%).
- 2) The contribution of benzo[a]pyrene to the effect varies widely in the mixtures examined here (0.17% for sidestream cigarette smoke condensate and 4% for the organic extract of diesel engine exhaust condensate, *i.e.* by a factor of 24) and does not correlate with the % w/w of this component in the mixture, which is between  $4 \times 10^{-4}$  and 0.1%.
- 3) Similar results were obtained in two animal models that used different species (mouse and rat) and also two different target organs (skin and lungs), thus confirming the exceptional relevance of the PAH fraction for carcinogenicity in the case of automotive exhaust condensate and coal combustion condensate (85% and 81% (automotive exhaust emission) and 90% (coal combustion)). In contrast, the

**Table 8** Percent contribution of PAH and benzo[a]pyrene to the carcinogenic potency of various mixtures (Jacob 1996)

	Contribution to the effect in %			
	mouse (epicutaneously)		rat (intrapulmonary)	
	PAH*	benzo[a]pyrene	PAH*	benzo[a]pyrene
used engine oil % w/w**	70	18		
	1.1	0.02		
automotive exhaust condensate (petrol engine) % w/w	85	6	81	2.4
	3.5	0.04	2.8	0.05
coal combustion condensate % w/w	90	11	> 90	1.4
	22.7	0.1	29	0.11
diesel engine exhaust condensate (organic extract) % w/w			80	4
			0.9	0.01
sidestream cigarette smoke condensate % w/w			75	0.17
			3.5	0.0004

\* PAH with 4 or more rings; \*\* w/w percentage in the mixture

relative contribution by benzo[a]pyrene of 1.4% to 2.4% (rat; intrapulmonary) and 6% to 11% (mouse; epicutaneously) to the effect deviated considerably in the animal models used. In the case of a 24-month topical application of the PAH fractions, the relative effect of benzo[a]pyrene was overemphasized after epicutaneous application to mice since this component is stable to photo-induced decomposition processes as compared with other less stable carcinogenic PAH (such as anthanthrene, cyclopenta[*cd*]pyrene and PAH with anthracenoid partial structures). Of course, these processes are not relevant for intrapulmonary injection.

For technical reasons, only condensates or extracts were examined in the potency balances mentioned here, whereas the gaseous phase, which may also induce carcinogenic effects (formaldehyde, acrolein, crotonaldehyde, butenolides, etc.), was not considered.

The potency balances carried out with automotive exhaust condensate in the 1970s (Grimmer 1977) tried to simulate the carcinogenic effects of the PAH fraction by administration of a synthetic mixture of the biologically active PAH detected in the condensate. They had already provided evidence that, in addition to the known carcinogenic PAH, previously unknown homologues, most of them with high boiling points, contribute significantly to the total carcinogenicity. However, seven carcinogenic PAH (benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, dibenzo[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene, mixed in the ratio of their occurrence in petrol engine emissions), caused only 10% to 15% of the total effects. It was assumed that the remaining

85% were induced by methylene-bridged PAH of the cyclopenta[*cd*]pyrene type. A simple calculation showed that the effects were not induced only by this component. Its concentration in the exhaust emission was about 12 times higher than that of benzo[*a*]pyrene; at a potency equivalent of 0.15, this corresponds maximally to twice the effect of benzo[*a*]pyrene, which in this mixture contributed a calculated 9% of the effects. Together, these eight components contributed a maximum of 33% of the effects, whereas 67% of the effects could not be explained. Similar ratios were obtained for coal combustion condensate. Here, too, the total carcinogenic effects were practically induced by PAH with 4 or more rings. High-vacuum distillation of the PAH fraction into two subfractions showed that the volatile fraction that accounted for 50% w/w and contained all gas chromatographable PAH also contributed to only half the carcinogenicity. The remaining effects remained in the distillation residue (also 50% w/w of the original fraction), in which hardly any gas chromatographable PAH were detected (Grimmer et al. 1985). This finding indicates that the higher-boiling PAH included either numerous moderately active or some highly active components that have not been identified to date and of which only dibenzo[*a,l*]pyrene is currently known. It is therefore of great importance to elucidate their structures.

## 8 Potency equivalents of PAH

Since the PAH profiles are different, a constant contribution of benzo[*a*]pyrene to the total carcinogenicity of the PAH fraction is not expected in different mixtures. At the high concentration of chrysene and benzo[*b*]fluoranthene, for example, these components may also significantly contribute to the carcinogenic potential although they only have potency equivalents of 0.03 and 0.11, respectively. Dibenzo[*a,l*]pyrene may be of special relevance in PAH mixtures since it has recently been reported to have an extremely high carcinogenic potential (between 10 and 100 times more effective than benzo[*a*]pyrene) (Muller et al. 1995, 1997). Recent studies have demonstrated that the concentration of dibenzo[*a,l*]pyrene in various mixtures (automotive exhaust condensate and coal tar pitch) is about 1/10 of that of benzo[*a*]pyrene. This component was also detected in cigarette smoke condensate (Seidel et al. 2004). One hundredth of the benzo[*a*]pyrene concentration of this component would thus cause the same biological effect as the one expected for benzo[*a*]pyrene. For many mixtures, the carcinogenic effect caused by PAH is therefore expected to be essentially due to dibenzo[*a,l*]pyrene. This also shows that this component must be determined quantitatively in PAH mixtures in the future.

For the assessment of the carcinogenic potency of a PAH mixture, a sum parameter should be calculated in the form of a dose-addition model (IARC 1986 b) based on potency equivalents from carcinogenicity studies in rodents to take into account the variable fraction of benzo[*a*]pyrene in a considered matrix. Currently it cannot be decided whether the carcinogenic potencies of individual PAH actually add up to a total potential or whether synergistic effects, e.g. the induction of cer-



tain CYP enzymes by PAH that are not carcinogenic themselves or are only weakly carcinogenic such as benzo[k]fluoranthene (Jacob et al. 1979; Schmoldt et al. 1981), lead to superadditive effects that are caused by the increased formation of proximal and ultimate carcinogens.

## 9 Equivalency factors of PAH

Proportionality of external and internal exposure is an important criterion if equivalency factors are used to assess adverse effects on health. The mechanism of action of the considered substances on which the assessment is based should be comparable (Neumann 1996). PAH undergo metabolic activation to reactive metabolites that are responsible for the genotoxic effect as a result of DNA binding. The potency equivalency factors assumed for the PAH here are based on carcinogenicity studies primarily carried out in the lung implantation model.

The equivalency factors of individual compounds in relation to benzo[a]pyrene (1.0), which have been published in various reviews, are compiled in Table 9. The authors of these publications point out that the data available on the effect of the exposure route on the equivalency factors are not sufficient, although they assume that the relative carcinogenic potentials of the compounds are the same in the calculations of the equivalency factors irrespective of the type of exposure. They assigned a value to PAH on the basis of the IARC and USEPA classifications, since there was no potency equivalent level available from the literature. A value of 0.001 was assigned to non-carcinogenic compounds (Malcolm and Dobsen 1994).

Other authors used skin painting studies as a basis. The model was based on the assumption that the relative carcinogenic effects of individual PAH determined via intestinal exposure are similar to those after dermal exposure. They considered the total of papillomas and carcinomas per compound to be the end point. When these authors calculated the relative tumour dose (RTD), they obtained the values listed in Table 10 (Rugen et al. 1989).

Another publication presented the results of a model calculation. It was assumed that the probability of tumourigenicity after a certain exposure period to a mixture of PAH with a benzo[a]pyrene-equivalent concentration corresponds to the two-stage birth-death-mutation model. Tissue growth and cell dynamics were included in this model (Krewski et al. 1989). There are no further details about the data used.

The derivation of toxic equivalency factors is based on the following assumptions. A well-investigated compound can be used as a surrogate or as a reference substance for all compounds of the family. The toxic effects of all compounds of the family are qualitatively similar to those of the surrogate compound and can quantitatively be characterized via relative potencies or toxic equivalency factors. Since the toxic equivalency factors of different end points are similar, limited information on the relative toxic potency in one or a few test systems can be used to establish toxic equivalency factors for an individual compound or subgroup of compounds for other end points. The toxic effects of the compounds of a mixture

**Table 9** Table of potency equivalents according to Malcolm and Dobsen (1994)

Compound	Potency equivalent Rugen et al. (1989)	Potency equivalent Nisbet and LaGoy (1992)	Potency equivalent Clement (1988)	Potency equivalent Chu and Chen (1984)	Potency equivalent USEPA (1984)	Potency equivalent Krewski et al. (1989)
naphthalene <sup>a</sup>		0.001			0	
fluoranthene		0.001			0	
chrysene		0.01	0.0044	0.001	1	0.004
anthracene		0.01	0.32		0	
dibenzo[ <i>a,h</i> ]anthracene	0.60	5	1.1	0.69	1	1.11
dibenzo[ <i>a,c</i> ]anthracene						
benzo[ <i>a</i> ]anthracene	0.005	0.1	0.145	0.013	1	0.145
phenanthrene		0.001			0	
1-methylphenanthrene						
benzo[ <i>b</i> ]fluoranthene	0.024	0.1	0.140	0.08	1	0.141
benzo[ <i>j</i> ]fluoranthene	0.08					
benzo[ <i>k</i> ]fluoranthene		0.1	0.066	0.004	1	0.061
pyrene		0.001	0.081		0	0.081
benzo[ <i>a</i> ]pyrene		1	1	1	1	
benzo[ <i>e</i> ]pyrene						0.004
cyclopenta[ <i>cd</i> ]pyrene						0.023
indeno[1,2,3- <i>cd</i> ]pyrene	0.006	0.1	0.232	0.017	1	0.232
perylene						
benzo[ <i>ghi</i> ]perylene		0.01	0.022		0	0.022
fluorene		0.001			0	
acenaphthylene		0.001			0	
acenaphthene		0.001			0	
coronene						

<sup>a</sup> Naphthalene was classified in Carcinogen Category 2 in the 2001 Supplement "Naphthalene"; however, no studies to date have been suitable for deriving an equivalency factor.

are additive. In addition to the publications by Chu and Chen (1984) and Clement (1988), a total of 11 publications with different administration routes (intrapulmonary, dermal, subcutaneous) and with the formation of DNA adducts in *in vitro* studies were used as a basis. A comparison carried out by the authors with results of studies by Schmähl et al. (1977) and Pfeiffer (1977) showed agreement in some cases (Nisbet and LaGoy 1992).

**Table 9** Table of potency equivalents according to Malcolm and Dobsen (1994)

Compound	Potency equivalent Deutsch-Wenzel et al. (1983 a)	Potency equivalent Wenzel-Hartung (1990)	Potency equivalent Wynder and Hoffmann (1959a, 1959b)	Potency equivalent Habs (1980)	Potency equivalent LaVoie (1982)	Potency equivalent Pfeiffer et al. (1977)
naphthalene <sup>a</sup>						
fluoranthene			0			
chrysene		0.03	0.01			
anthracene			0			
dibenzo[ <i>a,h</i> ]anthracene		1.908	1.0			1.0
dibenzo[ <i>a,c</i> ]anthracene						
benzo[ <i>a</i> ]anthracene						
phenanthrene		0.001				
1-methylphenanthrene						
benzo[ <i>b</i> ]fluoranthene	0.11		0.1	0.3	0.1	
benzo[ <i>j</i> ]fluoranthene	0.03					
benzo[ <i>k</i> ]fluoranthene	0.03		0.01	0	0.01	
pyrene			0			
benzo[ <i>a</i> ]pyrene	1	1	1	1	1	1
benzo[ <i>e</i> ]pyrene	0.003		0.01			
cyclopenta[ <i>cd</i> ]pyrene				0.1		
indeno[1,2,3- <i>cd</i> ]pyrene	0.08		0.01	0		
perylene						
benzo[ <i>ghi</i> ]perylene	0.01		0			
fluorene						
acenaphthylene						
acenaphthene						
coronene						

<sup>a</sup> Naphthalene was classified in Carcinogen Category 2 in the 2001 Supplement "Naphthalene"; however, no studies to date have been suitable for deriving an equivalency factor.

**Table 9** Table of potency equivalents according to Malcolm and Dobsen (1994)

Compound	Potency equivalent Phillips et al. (1979)	Potency equivalent Hughes and Phillips (1993) <sup>b</sup>	Potency equivalents or potency ranges	Potency equivalents rounded
naphthalene <sup>a</sup>			0.001	0.001
fluoranthene			0.001	0.001
chrysene			0.0044–0.03	0.01
anthracene			0–0.32 <sup>c</sup>	0.01
dibenzo[ <i>a,h</i> ]anthracene	1.0		0.60–5	1.0
dibenzo[ <i>a,c</i> ]anthracene			0.1 <sup>d</sup>	0.1 <sup>d</sup>
benzo[ <i>a</i> ]anthracene	0.1		0.005–0.145	0.1
phenanthrene			0.001	0.001
1-methylphenanthrene			0.001 <sup>d</sup>	0.001 <sup>d</sup>
benzo[ <i>b</i> ]fluoranthene		XXX	0.024–0.3	0.1
benzo[ <i>j</i> ]fluoranthene			0.03–0.08	0.1
benzo[ <i>k</i> ]fluoranthene		XX	0.004–0.1	0.1
pyrene			0.001–0.081 <sup>c</sup>	0.001
benzo[ <i>a</i> ]pyrene	1		1	1
benzo[ <i>e</i> ]pyrene			0.003–0.01	0.01
cyclopenta[ <i>cd</i> ]pyrene			0.023–0.1	0.1
indeno[1,2,3- <i>cd</i> ]pyrene		X	0–0.232	0.1
perylene			0.001 <sup>d</sup>	0.001 <sup>d</sup>
benzo[ <i>ghi</i> ]perylene			0.01–0.022	0.01
fluorene			0.001	0.001
acenaphthylene			0.001	0.001
acenaphthene			0.001	0.001
coronene			0.001 <sup>b</sup>	0.001 <sup>d</sup>

<sup>a</sup> Naphthalene was classified in Carcinogen Category 2 in the 2001 Supplement “Naphthalene”; however, no studies to date have been suitable for deriving an equivalency factor.

<sup>b</sup> Indication of size since the data were not related to benzo[*a*]pyrene.

<sup>c</sup> Potency equivalents that were not used because they did not agree with the data.

<sup>d</sup> Potency equivalents based on IARC and USEPA classifications.

The effect of benzo[*a*]pyrene, anthanthrene, benzo[*b*]fluoranthene, indeno[1,2,3-*cd*]pyrene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene and benzo[*ghi*]perylene was investigated in 3-month-old female Osborne-Mendel rats (27 to 35 animals per dose group) after a single lung implantation (Stanton et al. 1972) of

**Table 10** Relative doses of the carcinogenic effect of PAH (related to benzo[a]pyrene = 1) calculated via the number of papillomas and carcinomas after skin painting (Rugen et al. 1989)

Compound	Observation period (months)	Relative tumour dose (RTD) related to benzo[a]pyrene 1.0	References
benzo[a]anthracene	18	179	Bingham and Falk 1969
benzo[b]fluoranthene	8	42.5	Wynder and Hoffmann 1959 a
benzo[j]fluoranthene	8	13.1	Wynder and Hoffmann 1959 a
dibenzo[a,h]anthracene	7	1.67	Wynder and Hoffmann 1959 b
indeno[1,2,3-cd]pyrene	10	167	Wynder and Hoffmann 1961

3 doses per substance (2 doses of anthanthrene). The animals were observed for the rest of their lives; afterwards, the lungs were examined. The number of tumour-bearing animals was used as a parameter of the carcinogenic effect. A dose-dependent relation was established for benzo[a]pyrene, benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene, benzo[k]fluoranthene, benzo[j]fluoranthene and anthanthrene on the basis of histopathological assessment and mathematical calculations. Benzo[ghi]perylene had only a weak effect. The relative carcinogenic effects are listed in Table 11. The authors point out that the results agreed with those of other studies after dermal or subcutaneous application of the substances (Deutsch-Wenzel et al. 1983 b).

Other authors also used lung implantation in female Osborne-Mendel rats for the administration of phenanthrene, chrysene, dibenzo[a,h]anthracene, benzo[b]-naphtha[2,1-d]thiophene and benzo[a]pyrene as a reference substance. The animals were observed for the rest of their lives; afterwards, the lungs were examined. The number of tumours per animal was used as a parameter of the carcinogenic effect.

**Table 11** Relative carcinogenic effect of some PAH after lung implantation in Osborne-Mendel rats (Deutsch-Wenzel et al. 1983 b)

Compound	Relative carcinogenic effect
anthanthrene	0.19
benzo[b]fluoranthene	0.11
benzo[j]fluoranthene	0.03
benzo[k]fluoranthene	0.03
benzo[ghi]perylene	0.01
benzo[a]pyrene	1.00
benzo[e]pyrene	0.003
indeno[1,2,3-cd]pyrene	0.08

After probit analysis of the results, potency equivalents of 1.91 for dibenzo[*a,h*]anthracene, 0.03 for chrysene, 0.02 for benzo[*b*]naphtho[2,1-*d*]thiophene and 0.001 for phenanthrene were determined in relation to the carcinogenic effects of benzo[*a*]pyrene (Wenzel-Hartung et al. 1990).

Solutions of 0.5% in acetone of 4 compounds isolated and purified from cigarette smoke condensate (benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene and benzo[*mno*]fluoranthene) were applied to the skin of groups of 20 female Swiss mice 3 times a week for a period of 6 to 13 months. Benzo[*b*]fluoranthene and benzo[*j*]fluoranthene led to relatively severe biological effects, benzo[*k*]fluoranthene induced minimal effects and no effects were observed for benzo[*mno*]fluoranthene (Wynder and Hoffmann 1959 a). Dibenzo[*a,h*]anthracene, dibenzo[*a,i*]pyrene and other PAH were investigated in a further study (Wynder and Hoffman 1959 b). The relative carcinogenic effect was specified in a table with +++ for benzo[*a*]pyrene and dibenzo[*a,h*]anthracene, ++ for benzo[*b*]fluoranthene and benzo[*j*]fluoranthene, + for benzo[*a*]anthracene and chrysene and ± for benzo[*e*]pyrene, benzo[*k*]fluoranthene and benzo[*ghi*]perylene (Wynder and Hoffmann 1959 b).

Benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene, cyclopenta[*cd*]pyrene and coronene were investigated in the epicutaneous drip test on mouse skin. Three doses of the compounds dissolved in acetone were dripped onto the shaved dorsal skin of groups of 40 female NMRI mice (age: 10 weeks) twice a week per dose group; the doses ranged between 1.7 and 9.2 pg/animal, and between 1.7 and 27.2 pg/animal for cyclopenta[*cd*]pyrene. Coronene (dissolved in DMSO) was applied 4 times per week. The animals were observed for the rest of their lives; afterwards, the skin was examined histologically. The control groups included 80 animals (no other details). Benzo[*a*]pyrene and benzo[*b*]fluoranthene produced a clear carcinogenic effect, benzo[*j*]fluoranthene and cyclopenta[*cd*]pyrene induced a weak carcinogenic effect related to the number of tumours. Coronene, benzo[*k*]fluoranthene and indeno[1,2,3-*cd*]pyrene were classified as non-carcinogenic. After statistical evaluation of the data, a relative effect of 0.27 was obtained for benzo[*b*]fluoranthene as compared with benzo[*a*]pyrene and a value of 0.05 for cyclopenta[*cd*]pyrene (Habs et al. 1980).

Benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene were investigated in an initiation-promotion test in groups of 20 female CD-1 mice per dose group. For initiation, 10 doses were administered (total dose of 10 to 100 µg in acetone, benzo[*a*]pyrene dose 30 µg). Ten days after initiation, TPA was administered 3 times a week for 20 weeks. The initiating dose of 30 µg induced 4.9 tumours/animal after benzo[*a*]pyrene, 2.3 tumours/animal after benzo[*b*]fluoranthene, 0.6 tumours/animal after benzo[*j*]fluoranthene and 0.1 tumours/animal after benzo[*k*]fluoranthene. The animals of the control group (administration of acetone) revealed no abnormalities (LaVoie et al. 1982).

Benzo[*a*]pyrene and dibenzo[*a,h*]anthracene were applied once subcutaneously to mice, which were investigated after 114 weeks. A comparison of the activity of benzo[*a*]pyrene with that of dibenzo[*a,h*]anthracene showed that the tumour rates

of 2.35 and 4.7 µg dibenzo[*a,h*]anthracene were higher than those of 3.12 and 6.25 µg benzo[*a*]pyrene. The animals treated with benzo[*a*]pyrene had 9 and 35 sarcomas, whereas 37 and 39 sarcomas were found in the animals treated with dibenzo[*a,h*]anthracene (incidences refer to 100 animals in each case) (Pfeiffer 1977).

Solutions (1 µmol/animal) of <sup>3</sup>H-labelled benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*a*]anthracene and dibenzo[*a,c*]anthracene were applied to the shaved back of male C57BL mice (15 animals per group). After the animals had been sacrificed, the DNA of the treated skin area was isolated, hydrolyzed enzymatically and the hydrolysate was separated via column chromatography. DNA binding rates of 27 pmol/mg DNA were obtained for benzo[*a*]pyrene, 15 pmol/mg DNA for dibenzo[*a,h*]anthracene, 10 pmol/mg DNA for dibenzo[*a,c*]anthracene and 2 pmol/mg DNA for benzo[*a*]anthracene (Phillips et al. 1979).

In studies with male Parkes mice (6 to 8 weeks old), 1.0 µmol benzo[*ghi*]perylene per mouse was applied dermally, groups of 4 mice were sacrificed 6 hours, 1 day and 2, 4, 7, 21 and 84 days after treatment and the DNA of the treated area of skin was isolated. Mice were treated with 0.25 µmol substance/mouse of benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]fluoranthene, benzo[*ghi*]perylene, benzo[*b*]naphtho[2,1-*d*]thiophene and indeno[1,2,3-*cd*]pyrene, and the DNA of the treated area of skin was isolated. The DNA adduct rates after <sup>32</sup>P-labelling cannot be compared with a corresponding effect of benzo[*a*]pyrene (Hughes and Phillips 1993).

A study carried out in female Swiss mice with dibenzo[*a,l*]pyrene synthesized by the authors (Wynder and Hoffmann 1961) cannot be used since the identification of the substance is questionable (melting point specified to be 224 to 226°C; according to WHO 1998, the melting point of dibenzo[*a,l*]pyrene is 162°C). The Office of Environmental Health in California (OEHHA 1994) calculated equivalency factors based on the data obtained by Cavalieri et al. (1989, 1991) for dibenzo[*a,l*]pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene and dibenzo[*a,i*]pyrene. Cavalieri et al. (1989, 1991) provided evidence of the tumourigenic activity of dibenzo[*a,l*]pyrene and its relation to the dose in a study of four dibenzo[*a*]pyrenes in the mouse skin and rat mammary gland. Benzo[*a*]pyrene was used as a reference substance in some tests. Dibenzo[*a,l*]pyrene was shown to be the substance with the highest carcinogenic potential. Several doses were tested. A comparison of the results after dermal application of 33.3 nmol dibenzo[*a,l*]pyrene as an initiator (phorbol ester as a promotor) with those after application of the same amount of benzo[*a*]pyrene showed that dibenzo[*a,l*]pyrene induced tumours in 23 of 24 animals whereas benzo[*a*]pyrene led to tumours in 10 of 23 animals. The result was a relative potency of 5.8. In a second test, 4 nmol per substance was compared. Tumours were observed in 22 of the 24 animals treated with dibenzo[*a,l*]pyrene and in 1 of 24 animals treated with benzo[*a*]pyrene. This resulted in a relative potency of 25. In a third test, 100 nmol of the two substances were compared without promotion. Seven of the 24 animals of the dibenzo[*a,l*]pyrene group and 1 animal of the benzo[*a*]pyrene group had tumours; the relative potency was 4. Direct injection of 0.25

and 1  $\mu\text{mol}$  dibenzo[*a,l*]pyrene into the mammary gland induced tumours in all treated rats (19 and 20 animals, respectively), whereas only one of the animals treated with 0.25  $\mu\text{mol}$  benzo[*a*]pyrene had a tumour. This resulted in a relative potency of 100. An equivalency factor of 10 has been attributed to dibenzo[*a,l*]pyrene on the basis of its considerably higher tumour-initiating activity. The tumourigenicity of dibenzo[*a,h*]pyrene is comparable with that of dibenzo[*a,l*]pyrene. For example, tumours were observed in 18 of 24 treated mice after dermal application of dibenzo[*a,h*]pyrene as compared with 22 of 24 animals treated with dibenzo[*a,l*]pyrene. Skin tumours occurred in 2 of 23 control mice. An equivalency factor of 10 has been assigned to dibenzo[*a,h*]pyrene.

The effect of dibenzo[*a,i*]pyrene is equated with that of dibenzo[*a,l*]pyrene. For example, tumours were observed in 15 of 24 treated mice after dermal application of dibenzo[*a,i*]pyrene as compared with 22 of 24 animals treated with dibenzo[*a,l*]pyrene. Skin tumours were observed in 2 of 23 animals of the control group. An equivalency factor of 10 has been assigned to dibenzo[*a,i*]pyrene.

Dibenzo[*a,e*]pyrene was least active among the four compounds (Cavalieri et al. 1989, 1991). The effect of dibenzo[*a,l*]pyrene was 10 to 20 times higher than that of dibenzo[*a,e*]pyrene. An equivalency factor of 1 has been assigned to dibenzo[*a,e*]pyrene.

Table 12 shows an example of the use of potency equivalents. Here, the concentrations of the “used engine oil” mixture (random sample) were multiplied with the specific potency equivalents and the sum was equated with 100%; however, previously unknown, possibly carcinogenic PAH were of course not taken into account. The percentage of the effect contributed by benzo[*a*]pyrene was calculated to be about 14% for this case. A potency balance analysis carried out by Grimmer et al. (1982) with a used engine oil yielded a relative contribution to the effect of 18% for benzo[*a*]pyrene. If the overestimation discussed for the epicutaneous application of this component is taken into account (see Section 7), quite similar results are obtained with this calculation.

To date there have been a number of proposals on toxic equivalency factors for PAH based on different carcinogenicity studies (see Table 13; (Chu and Chen 1984; Clement 1988; Collins et al. 1998; Malcolm and Dobsen 1994; Nisbet and LaGoy 1992; Petry et al. 1996; Thorslund and Farrer 1991; USEPA 1984). The original proposal of the U.S. Environmental Protection Agency divided the PAH into two groups, one including those with a carcinogenic effect and the other those with a non-carcinogenic effect (USEPA 1984). A value of 1 was assigned to all PAH with a carcinogenic effect, whereas zero was assigned to all non-carcinogenic PAH (Table 13).

Since the effect of most carcinogenic PAH considered in the EPA list is weaker than that of benzo[*a*]pyrene, this concept inevitably results in overrating the biological activity of a mixture. This was later considered by Chu and Chen (1984), Thorslund and Farrer (1991) and Clement (1988); these authors thus obtained more accurate values (Table 13). Nisbet and LaGoy (1992) then assigned an equivalency factor to all PAH of the EPA list based on the available carcinogenicity data



**Table 12** Analysis of a used mineral oil and estimate of the contribution of individual PAH to the total carcinogenic effect

PAH	Concentration [µg/kg] (c) <sup>a</sup>	Potency equivalent (f) <sup>i</sup>	c × f	% of the total effect <sup>k</sup>	Effect relative to benzo[a]pyrene
phenanthrene	11200	0.001 <sup>b</sup>	1.2	2.5	0.2
anthracene	24.0	–	–	–	–
fluoranthene	42.8	0.001	0.04	0.1	–
pyrene	255	0.001	0.26	0.5	– <sup>g</sup>
benzo[b]naphtho[2,1-d]thiophene	314	0.01 <sup>b</sup>	3.14	6.6	0.5
benzo[ghi]fluoranthene	1.37	–	–	–	–
benzo[c]phenanthrene	3.92	–	–	–	–
cyclopenta[cd]pyrene	0.18	0.1	0.02	– <sup>g</sup>	– <sup>g</sup>
benzo[a]anthracene	27.6	0.1 <sup>d</sup>	2.76	5.8	0.4
chrysene/triphenylene	313	0.01 <sup>b</sup>	3.13	6.6	0.5
benzo[b+j+k]fluoranthene	18.9	0.1 <sup>c</sup>	1.89	4.0	0.3
benzo[e]pyrene	50.1	0.01 <sup>c</sup>	0.50	1.1	0.1
<b>benzo[a]pyrene</b>	<b>6.75</b>	<b>1.0</b>	<b>6.75</b>	<b>14.2</b>	<b>1.0</b>
perylene	2.57	0.001	–	–	– <sup>g</sup>
indeno[1,2,3-cd]pyrene	1.56	0.1	0.16	0.3	– <sup>g</sup>
dibenzo[a,h]anthracene	4.67	1.0 <sup>b</sup>	4.67	9.8	0.7
benzo[ghi]perylene	228	0.01 <sup>b</sup>	2.28	4.8	0.3
anthanthrene	4.70	0.1 <sup>c</sup>	0.47	1.0	0.1
coronene	11.5	0.001	0.01	– <sup>g</sup>	– <sup>g</sup>
dibenzo[a,l]pyrene	0.20	100.0 <sup>e</sup>	20.0	42.1	3.0
dibenzo[a,e]pyrene	0.14	1.0 <sup>f</sup>	0.14	0.3	– <sup>g</sup>
dibenzo[a,i]pyrene	0.53	0.1 <sup>f</sup>	0.05	0.1	– <sup>g</sup>
dibenzo[a,h]pyrene	n.n. <sup>h</sup>	1.0 <sup>e</sup>	–	–	–

<sup>a</sup> (Jacob 2007 b); <sup>b</sup> (Wenzel-Hartung et al. 1990); <sup>c</sup> (Deutsch-Wenzel et al. 1983 b); <sup>d</sup> (Jacob 2007 b); (Malcolm and Dobsen 1994); <sup>e</sup> (Muller et al. 1995; Flowers et al. 2002); <sup>f</sup> (McClure 2004). <sup>g</sup> < 0.1; <sup>h</sup> not detectable;

<sup>i</sup> rounded to the base 10; <sup>k</sup> total effect defined as the sum of the calculated effects of the listed PAH

and simplified the values established by other authors since they regarded them as unreasonably precise in relation to uncertainties in the assessment of PAH mixtures (Table 13). However, as Nisbet and LaGoy (1992) explained, it cannot be ruled out that different PAH mixtures may induce altered activities because of differences in bioavailability, competition at critical binding sites, co-carcinogenic effects

**Table 13** Suggested carcinogenicity equivalency factors for the individual PAH of the EPA list

PAH	USEPA (1984)	Chu and Chen (1984)	Clement (1988)	Thorslund and Farrer (1991)	Nisbet and LaGoy 1992
naphthalene	0	–	–	–	0.001
acenaphthylene	0	–	–	–	0.001
acenaphthene	0	–	–	–	0.001
fluorene	0	–	–	–	0.001
phenanthrene	0	–	–	–	0.001
anthracene	0	–	0.32	–	0.01
fluoranthene	0	–	–	–	0.001
pyrene	0	–	0.081	–	0.001
benzo[a]anthracene	1	0.013	0.145	0.145	0.1
chrysene	1	0.001	0.0044	0.0044	0.01
benzo[j+b]fluoranthene	1	0.08	0.14	0.12	0.1
benzo[k]fluoranthene	1	0.004	0.066	0.052	0.1
benzo[a]pyrene	1	1	1	1	1
indeno[1,2,3-cd]pyrene	1	0.017	0.232	0.278	0.1
dibenzo[a,h]anthracene	1	0.69	1.1	1.11	1
benzo[ghi]perylene	0	–	0.022	0.021	0.1

or metabolism. Apart from these arguments, Petry et al. (1996) regarded the application of equivalency factors as not very useful. In their opinion, too low an improvement in the risk assessment of a PAH mixture was achieved by means of equivalency factors with a factor of 2 to 3, given the uncertainties associated with these values as compared with an assessment based only on benzo[a]pyrene as the lead component.

The result of a risk assessment of a PAH mixture depends not only on the validity of the equivalency factors but also decisively on the number of considered carcinogenic PAH that determine the specific PAH profile of their carcinogenic effect. Collins et al. (1998) of the Californian Office of Environmental Health support the concept of equivalency factors and point out that, in contrast to the list of equivalency factors established by Nisbet and LaGoy (1992), only those of known carcinogenic PAH should be included to be able to identify the most important representatives for a risk assessment. Certain dibenzopyrenes, in particular dibenzo[a,l]pyrene, must definitely be included on account of their severe carcinogenic effect mentioned previously. Table 14 shows the example of a calculation of the benzo[a]pyrene equivalents of PAH from tobacco smoke condensate and from an indoor air measurement of kitchens with coal firing in China, including four isomeric dibenzopyr-

**Table 14** Calculation of benzo[*a*]pyrene equivalents based on selected PAH profiles

PAH	Tobacco smoke condensate <sup>a</sup>					Indoor air analysis				
	lowest amount found			highest amount found		particle-bound PAH <sup>b</sup>				% of total effect
	c [ng/cig.]	f <sup>c</sup>	c × f	% of total effect	c [ng/cig.]	f <sup>c</sup>	c [ng/m <sup>3</sup> ]	f <sup>c</sup>	c × f	
phenanthrene	2	0.001	0.002	<0.1	22	0.001	53289	0.001	533	0.6
fluoranthene	10	0.001	0.01	<0.1	272	0.001	32381	0.001	324	0.3
pyrene	50	0.001	0.05	0.2	270	0.001	26741	0.001	267	0.3
benzo[ <i>a</i> ]anthracene	4	0.1	0.4	1.4	76	0.1	25082	0.1	2508	2.7
chrysene	6	0.01	0.06	0.2	96	0.01	26111	0.01	261	0.3
benzo[ <i>b</i> + <i>j</i> + <i>k</i> ]fluoranthene	14	0.1	1.4	4.9	54	0.1	22649	0.1	2265	2.4
benzo[ <i>a</i> ]pyrene	5	1.0	5.0	17.5	40	1.0	14650	1.0	14650	15.6
indeno[1,2,3- <i>cd</i> ]pyrene	4	0.1	0.4	1.4	20	0.1	8368	0.1	837	0.9
dibenzo[ <i>a,h</i> ]anthracene	4	1.0	4.0	14.0	4	1.0	6530	1.0	6530	7.0
benzo[ <i>ghi</i> ]perylene	3	0.01	0.03	0.1	39	0.01	19323	0.01	193	0.2
anthanthrene	2	0.1	0.2	0.7	22	0.1	n.d.	0.1	–	–
dibenzo[ <i>a,l</i> ]pyrene	1.7	10	17	59.6	3.2	10	4936	10	49360	52.4
dibenzo[ <i>a,e</i> ]pyrene	–	1.0	–	–	–	1.0	11702	1.0	11702	12.5
dibenzo[ <i>a,i</i> ]pyrene	–	10	–	–	–	10	448	10	4480	4.8
dibenzo[ <i>a,h</i> ]pyrene	–	10	–	–	–	10	–	10	–	–

<sup>a</sup> The ranges of amounts of PAH in tobacco smoke condensate were taken from data in the literature (Hoffmann and Hoffmann 1998; IARC 1986 a). <sup>b</sup> Air analyses in homes with considerable indoor air exposure from coal firing according to Mumford et al. (1995) <sup>c</sup> Potency equivalents f for dibenzopyrenes according to (Collins et al. 1998)

**Table 15** PAH analytes and equivalency factors of the (USEPA 1984)

Compounds	Equivalency factors
acenaphthene	0
acenaphthylene	0
anthracene	0
benzo[ <i>a</i> ]anthracene	1
benzo[ <i>b</i> ]fluoranthene	1
benzo[ <i>k</i> ]fluoranthene	1
benzo[ <i>ghi</i> ]perylene	0
benzo[ <i>a</i> ]pyrene	1
chrysene	1
dibenzo[ <i>a,h</i> ]anthracene	1
fluoranthene	0
fluorene	0
indeno[1,2,3- <i>cd</i> ]pyrene	1
naphthalene	0
phenanthrene	0
pyrene	0

enes. The relative contribution of dibenzopyrenes to the effect was between 34% and 70% in this case. If the assessment of the tobacco smoke condensate had been based only on the lead component benzo[*a*]pyrene, it would have been too low by about a factor of 6 as compared with the sum parameter. The calculation of the indoor measurement levels also led to an assessment that was too low by this factor with reference to benzo[*a*]pyrene as the lead component. This explicitly illustrates the importance of the relative contribution of dibenzopyrenes to the effect.

Tables 15 and 16 include the PAH suggested for routine determination; the equivalency factors proposed for the assessment of PAH mixtures are also listed. The authors rounded these equivalency factors because an accurate specification would not have made sense. As in the case of benzo[*a*]pyrene, an equivalency factor of 1 was specified for the seven compounds that were listed by the USEPA and regarded as potential human carcinogens. If this equivalency factor, which would have led to an overestimation of the carcinogenic effect of these compounds, is replaced by the equivalency factors specified in recent literature, a more realistic assessment of the contribution of five of these compounds (benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene (indicated as benzo[*j+b*]fluoranthene sum in the EPA list), dibenzo[*a,h*]pyrene and indeno[1,2,3-*cd*]pyrene) to the total carcinogenic activity of the PAH mixture can be made. Introducing equivalency

**Table 16** Relevant PAH analytes suggested for routine determination

Compounds	Equivalency factors (updated proposal)	References
anthanthrene	0.1	Deutsch-Wenzel et al. 1983 b
benzo[a]anthracene	0.1	Nisbet and LaGoy 1992
benzo[b]fluoranthene	0.1	Nisbet and LaGoy 1992
benzo[j]fluoranthene	0.1	Nisbet and LaGoy 1992
benzo[k]fluoranthene	0.1	Nisbet and LaGoy 1992
benzo[b]naphtho[2,1-d]thiophene	0.01	Wenzel-Hartung et al. 1990
benzo[a]pyrene	1	Nisbet and LaGoy 1992
chrysene	0.01	Nisbet and LaGoy 1992
cyclopenta[cd]pyrene	0.1	Malcolm and Dobsen 1994
dibenzo[a,h]anthracene	1	Nisbet and LaGoy 1992
dibenzo[a,l]pyrene	10	Collins et al. 1998
dibenzo[a,e]pyrene	1	Collins et al. 1998
dibenzo[a,h]pyrene	10	Collins et al. 1998
dibenzo[a,i]pyrene	10	Collins et al. 1998
indeno[1,2,3-cd]pyrene	0.1	Nisbet and LaGoy 1992
naphthalene	0.001	Nisbet and LaGoy 1992
phenanthrene	0.001	Nisbet and LaGoy 1992
pyrene	0.001	Nisbet and LaGoy 1992
1-methylpyrene	0.1	Rice et al. 1987 <sup>a)</sup>

<sup>a)</sup> On the basis of these data, an equivalency factor of 0.1 could be attributed to 1-methylpyrene with the restrictions that 1-methylpyrene and benzo[a]pyrene were tested at only one dose level and that newborn mice express relatively few sulfotransferases.

factors for the other PAH more realistically reflects their contribution to the total activity, which would otherwise have been underestimated.

## 10 Manifesto (MAK value/classification)

The table on the first page shows the classifications of the individual components of PAH for their carcinogenicity and germ cell mutagenicity. Anthanthrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[b]naphtho[2,1-d]thiophene, chrysene, dibenzo[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 1-methylpyrene are classified in Carcinogen Category 2 since they

were carcinogenic in animal studies. Phenanthrene and pyrene are not classified in a carcinogen category since negative results were obtained in animal studies on carcinogenicity.

Benzo[*a*]anthracene and dibenzo[*a,h*]anthracene are classified in Germ Cell Mutagen Category 3A since *in vivo* data are available in somatic cells, these substances have been shown to reach the germ cells, or there are *in vivo* data in germ cells. Benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*b*]naphtha[2,1-*d*]thiophene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene and dibenzo[*a,l*]pyrene are classified in Germ Cell Mutagen Category 3B since either *in vitro* data are available and there is an analogy to a germ cell mutagen or there are *in vivo* data in somatic cells. Phenanthrene and pyrene are not classified in any of the germ cell mutagen categories on the basis of the available data nor are anthanthrene, chrysene, indeno[1,2,3-*cd*]pyrene and 1-methylpyrene because of the inadequate database.

The classification of benzo[*a*]pyrene into Germ Cell Mutagen and Carcinogen Category 2 and of cyclopenta[*cd*]pyrene and naphthalene into Germ Cell Mutagen Category 3B and Carcinogen Category 2 has been confirmed (2001 documentation "Benzo[*a*]pyrene"; 2007 documentation "Cyclopenta[*cd*]pyrene"; 2001 documentation "Naphthalene"; all in present volume).

The ready skin penetration of individual PAH has been demonstrated in humans and animal studies. There is no reason to assume that there is any substantial difference in the penetration behaviour of PAH. Since absorption via the skin at the workplace is the main contributor to the body burden, PAH are designated with an "H".

No data are available for sensitization in humans for PAH. Although findings from animal studies indicate contact sensitization induced by benzo[*a*]pyrene, it is not designated with "Sh" since no cases of allergic contact eczema have been reported to date despite skin contact with this substance. There are no data or no valid findings in animals for the remaining PAH, so they are also not designated with "Sh". Neither are the PAH designated with "Sa" since there are no data available for respiratory tract sensitization.

There are hardly any data on the reproductive toxicity of PAH except for benzo[*a*]pyrene. Two reports of transplacental poisoning in humans are available for naphthalene. Naphthalene, benzo[*a*]anthracene and dibenzo[*a,h*]anthracene induced vaginal and intraplacental haemorrhages in rats after subcutaneous treatment. These compounds as well as chrysene led to the death of fetuses in the F1 generation (rats and mice) and to the induction of benzo[*a*]pyrene hydroxylase in the liver. Benzo[*a*]pyrene had a severe embryotoxic effect in mice and rats and impaired fertility in mice.

Based on its high carcinogenic potential and on analytical experience, benzo[*a*]pyrene was previously used as a reference or lead component for the carcinogenicity of a mixture and for the assessment of general and particularly occupationally induced health risks. Accordingly, a number of countries established legally binding threshold values and recommended levels. However, because of the variable effective fraction, this component can not be used without reservation as a lead

component for various types of emissions; at best it can be used for a particular mixture provided there is a constant PAH profile for the mixture. The PAH profiles determined at various workplaces (manufacture of graphite electrodes; manufacture and processing of fireproof materials) also show considerably different relative benzo[*a*]pyrene levels with variations by a factor of 5. The different PAH profiles can easily explain why the contribution of benzo[*a*]pyrene to the total carcinogenic potential depends on the mixture.

Because of (1) the varying contribution of benzo[*a*]pyrene to the total carcinogenic potential related to the fraction of PAH with 4 or more rings and (2) the variability of PAH profiles and therefore of the relative contribution of benzo[*a*]pyrene to the total PAH, today's practise of restricting a toxicologically validated threshold value to a single component seems to be questionable or at least in need of improvement. The carcinogenic effects of numerous PAH can be compared on the basis of the available levels. However, it must be pointed out that some of these are based on different test systems (intrapulmonary injection in rats and epicutaneous application to mice). Since the relative effects do not differ too much, they do not lead to any major misinterpretations. The results from studies of intrapulmonary and epicutaneous treatment of rats and mice were compared with each other and the potential effects of various PAH listed. For the assessment of the carcinogenic potency of a PAH mixture, a sum parameter should be calculated in the form of a dose-addition model on the basis of potency equivalents from carcinogenicity studies in rodents to take into account the variable fraction of benzo[*a*]pyrene in the mixture in question. It cannot yet be decided whether the carcinogenic potencies of individual PAH actually add up to a total potential or whether synergistic effects such as the induction of certain CYP enzymes by PAH that are not carcinogenic themselves or are only weakly carcinogenic, such as benzo[*k*]fluoranthene, lead to superadditive effects caused by the increased formation of proximal and ultimate carcinogens.

Proportionality of external and internal exposure is an important prerequisite for the suggested procedure. In a first approximation, this can be assumed for carcinogenic PAH with 4 to 6 rings. Moreover, the responsible modes of action should be comparable. Besides the differences in bioavailability and pharmacokinetics, the diversity of the modes of action is an important point of criticism particularly in the discussion of toxic equivalency factors for polyhalogenated aromatics. Nor is a comparable mode of action assumed for nitro amino aromatic compounds on account of diverse organotropy and sex-linked differences. As a consequence of the findings discussed here, routine determination of the individual compounds listed in Table 16 is recommended for future PAH analysis. Several widespread carcinogenic PAH have been taken into account in the present proposal as compared with those analytes that are currently often selected (EPA list; Table 15). Individual PAH were selected on the basis of (1) their carcinogenic effect, as far as they have been tested in the animal model to date, and (2) their high concentration at the workplace. Moreover, phenanthrene and pyrene should be determined since their metabolites are often used today for human biomonitoring. A procedure has been developed for their determination. Evidence of a carcinogenic potential of naphtha-

lene was also provided in rodents after inhalation. Whereas neoplasms were seen in the nasal epithelium of female and male rats, alveolar and bronchial adenomas developed in the lungs of female mice. If also the generally high concentration of naphthalene in the environment is considered, its routine determination would likewise be desirable. Since no reliable potency factors have been established so far for lead components of substances with a methylene or methyl bridge or alkyl substituents (methylpyrene and 4*H*-cyclopenta[*def*]chrysene) and for naphthalene, there is urgent need for research for their determination. The lung implantation model should preferably be used on account of the required comparability. As soon as specific data are available, the existing values can be supplemented or corrected, if necessary.

The effect level of the carcinogenicity of PAH based on a sum parameter would have to be assessed higher by about a factor of 10 than the value based on benzo[*a*]pyrene since benzo[*a*]pyrene accounts for only about one tenth of the total carcinogenic effect in most mixtures. For mixtures in which benzo[*a*]pyrene contributes less than 10% to the effect, the threshold value listed above must, however, be corrected downwards accordingly.

The PAH suggested or selected here (see Table 16) must be regarded as provisional since the carcinogenic effect of many occurring PAH has not been examined to date. There may be further highly active components among them that might contribute considerably to the total effect of the PAH fraction (e.g. PAH with molecular weights = 302; the group of dibenzopyrenes). Representatives from the group of methylene-bridged PAH, e.g. 4*H*-cyclopenta[*def*]chrysene, and of methyl-substituted PAH, e.g. 5-methylchrysene and 6- and 11-methylbenzo[*a*]pyrene, might also be involved. Since the carcinogenic effects of these compounds have been thoroughly investigated, they can be recommended as lead components. However, their occurrence in relevant matrices must still be investigated.

The Commission suggests that the PAH listed in Table 16 under "updated proposal" should provisionally be given priority for determining the potency of PAH mixtures based on the corresponding equivalency factors listed there.

## Annex

The Annex contains tables from EHC Volume 202 "Selected non-heterocyclic polycyclic aromatic hydrocarbons" (WHO 1998). Since it can be assumed that the data listed in the tables were verified by the WHO, they were not re-examined once again on the basis of the specified literature.



**Table A1** Acute toxicity of PAH

Compound	Species	Type of administration	LD <sub>50</sub> (mg/kg b.w.) or LC <sub>50</sub> (mg/m <sup>3</sup> )	References
benzo[a]pyrene	mouse	oral	< 1600	Awogi and Sato 1989
	mouse	i.p.	about 250	Salamone 1981; Salamone et al. 1981
	mouse	i.p.	< 1600	Awogi and Sato 1989
	rat	s.c.	50	Montizaan et al. 1989
chrysene	mouse	i.p.	< 320	Simmon et al. 1979
naphthalene	rat	inhalation	> 500 mg/m <sup>3</sup> (8 h)	USEPA 1978
	rat	oral	1250	Sax and Lewis 1984
	rat (♂)	oral	2200	Gaines 1969
	rat (♀)	oral	2400	Gaines 1969
	rat	oral	9430	USEPA 1978
	rat	oral	1110	Montizaan et al. 1989
	rat	oral	490	Montizaan et al. 1989
	rat	oral	1800	Montizaan et al. 1989
	rat (♂)	dermal	< 2500	Gaines 1969
	rat (♀)	dermal	> 2500	Gaines 1969
	rat	i.p.	about 1000	Bolonova 1967
	rat	i.p.	about 1600	Plopper et al. 1992
	mouse (♀)	oral	354	Plasterer et al. 1985
	mouse (♂)	oral	533	Shopp et al. 1984
	mouse (♀)	oral	710	Shopp et al. 1984
	mouse	s.c.	5100	Sandmeyer 1981
	mouse	s.c.	5100	Shopp et al. 1984
	mouse	s.c.	969	Sax and Lewis 1984
	mouse	i.p.	150	Sax and Lewis 1984
	mouse	i.p.	380	Warren et al. 1982
	mouse (♀)	i.p.	about 400	Plopper et al. 1992
	mouse	i.v.	100	Sax and Lewis 1984
	hamster (♂)	i.p.	about 800	Plopper et al. 1992
	guinea pig	oral	1200	Sax and Lewis 1984
phenanthrene	mouse	oral	700	Montizaan et al. 1989
	mouse	oral	1000	Montizaan et al. 1989
	mouse	i.p.	700	Simmon et al. 1979
	mouse	i.v.	56	Montizaan et al. 1989
pyrene	mouse	i.p.	514 (7 d)	Salamone 1981; Salamone et al. 1981
	mouse	i.p.	678 (4 d)	Salamone 1981; Salamone et al. 1981

b.w.: body weight; i.p.: intraperitoneal injection; i.v.: intravenous injection s.c.: subcutaneous injection

Table A2 Acute toxicity of naphthalene

Species, strain, No./group	Exposure	Findings	References
mouse (no other details)	0.1 mg/l; 4 h; inhalation	bronchiolar necrosis	Buckpitt and Franklin 1989
rabbit (no other details)	1000–3000 and 3000 mg/kg b.w.; oral	<b>1000–3000 mg/kg b.w.:</b> corneal clouding <b>3000 mg/kg b.w.:</b> death after 24 h	Flury and Zernik 1935
cat (no other details)	1000–3000 mg/kg b.w.; oral	<b>1000–3000 mg/kg b.w.:</b> lethal	Flury and Zernik 1935
dog, 1	400 and 1800 mg/kg b.w.; oral	<b>400 mg/kg b.w.:</b> weakness; severe anaemia <b>1800 mg/kg b.w.:</b> weakness, vomiting, diarrhoea, slight anaemia; complete recovery within 1–2 weeks	Zuelzer and Apt 1949
dog (no other details)	1000–2000, 4000 or 5000 mg/animal; oral	<b>1000–2000 mg/animal:</b> light diarrhoea <b>4000 mg/animal:</b> heavy diarrhoea	Flury and Zernik 1935
mouse, Swiss Webster, 4–35, ♂	50, 100, 200, 300 and 400 mg/kg b.w.; i.p.	dose-dependent bronchiolar necrosis <b>300 mg/kg b.w.:</b> swollen cells in trachea <b>400 mg/kg b.w.:</b> cytotoxicity in olfactory epithelium	Plopper et al. 1992

b.w.: body weight; i.p.: intraperitoneal injection

**Table A3** Subacute and subchronic toxicity of naphthalene

Species, strain, No. per group	Exposure	Findings	References
mouse, Swiss Webster, ♂	7 days; 50, 100 or 200 mg/kg b.w. and day; i.p.	no significant alterations in lung morphology; tolerance to 300 mg/kg b.w. on day 8	Buckpitt and Franklin 1989; O'Brien et al. 1989
mouse, CD-1, 40–112, ♂/♀	14 days; 27, 53 or 267 mg/ kg b.w. and day; 7 d/w; oral	in all groups, slight alterations in haematological parameters; humoral immune response not affected <b>27 and 53 mg/kg b.w.:</b> no significant effects <b>267 mg/kg b.w.:</b> 5–10% mortality ♂/♀; significantly decreased terminal body weight ♂/♀; 30% decrease in thymus weight ♂; significant decrease in weight of spleen ♀; increase in lung weight ♀ (no other details)	Shopp et al. 1984
mouse, CD-1, ♂/♀	90 days; 5.3, 53 or 133 mg/ kg b.w. and day; 7 d/w; oral	no obvious pulmonary effects or immu- notoxicity; significantly decreased relative spleen weights ♀	Shopp et al. 1984
rat (no other details)	46–60 days; 1 g/kg diet	observation of cataracts	USEPA 1984
rat (no other details)	11 weeks; 150 mg/kg b.w. and day (the first 3 weeks), 200–220 mg/kg b.w. and day (the following 11 weeks); oral	reduced body weight gain and feed consumption; enlarged liver with cellular oedema and damage to the liver paren- chyma; signs of inflammation in the kidneys	Kawai 1979
rat (no other details)	100 days; 2 g/kg diet	growth inhibition; enlarged livers with fatty degeneration	White and White 1939
rabbit (no other details)	46–60 days; 1 g/kg diet	observation of cataracts	USEPA 1984
dog, 1	7 days; 0.22 g/kg b.w. and day	diarrhoea, weakness, lack of appetite, ataxia and very severe anaemia; complete recovery within 1–2 weeks	Zuelzer and Apt 1949

b.w.: body weight; i.p.: intraperitoneal injection

**Table A4** Embryotoxicity of PAH in animal studies

Species, strain, Exposure No. per group	Findings	References
<b>benzo[A]anthracene</b>		
rat, 2 (no other details)	GD 1–11 or 1–15, 5 mg/animal and day; s.c. <b>F0:</b> GD 10 and GD 12 severe vaginal haemorrhage; GD 14: intraplacental haemorrhage <b>F1:</b> foetal death and resorption up to GD 18	Wolfe and Bryan 1939
rat, Sprague Dawley	GD 19; 60 mg/kg b.w.; gavage <b>F1:</b> Induction of benzo[a]pyrene hydroxylase in liver (12 versus < 0.2 units in controls)	Welch et al. 1972
<b>benzo[a]pyrene</b>		
mouse, White Swiss, 9	GD 5 or GD 10 until littering, 150 mg/kg b.w.; diet <b>F1:</b> no malformations	Rigdon and Neal 1965
mouse, C57Bl/6N, AKR/J, 6–17	GD 2–GD 10, 120 mg/kg b.w.; diet <b>F1:</b> increased intrauterine toxicity and malformations in Ah <sup>d</sup> /Ah <sup>d</sup> embryos compared with Ah <sup>b</sup> /Ah <sup>d</sup> embryos in pregnant Ah <sup>d</sup> /Ah <sup>d</sup> mice (effect not observed in pregnant Ah <sup>b</sup> /Ah <sup>d</sup> mice)	Legraverend et al. 1984
mouse, C57Bl/6, AKR, 5–30	GD 7, GD 10 or GD 12, 50–300 mg/kg b. w.; i.p. <b>200 mg/kg b.w.:</b> <b>F1</b> increase in still- births, resorptions and malformations (4-fold higher in pregnant C57Bl than in AKR mice)	Shum et al. 1979
mouse, C57Bl/6, DBA/2, 20	GD8, 150 and 300 mg/ kg b.w.; i.p. <b>150 and 300 mg/kg b.w.:</b> in <b>F0</b> , increased foetal mortality (except DBA/2 × DBA/2 offspring); reduced foetal body weight; increased number of cervical ribs <b>300 mg/kg b.w.:</b> in <b>F1</b> , increased malformations (C57Bl/6 × C57Bl/6)	Hoshino et al. 1981
mouse, CD-1	GD 7–16, 10, 40, 160 mg/kg b.w.; gavage <b>F0:</b> no toxicity <b>F1:</b> no toxicity	MacKenzie and Angevine 1981
rat, 17	GD 1–11 or GD 16, 5 mg/animal; s.c. <b>F0:</b> GD 10 and 12: profuse vaginal haemorrhage; day 14: intraplacental haemorrhage <b>F1:</b> foetal death and resorption up to GD 18	Wolfe and Bryan 1939

Table A4 (Continued)

Species, strain, No. per group	Exposure	Findings	References
rat, Sprague Dawley	GD 19; 60 mg/kg b.w.; gavage	F1: Induction of benzo[a]pyrene hydroxylase in liver (20 versus < 0.2 units in controls)	Welch et al. 1972
rat, Sprague Dawley, 10–15	GD 6–8 and GD 6–11, 50 mg/kg b.w.; s.c.	F1: significant increase in number of resorptions and foetal wastage; foetal weight reduced	Bui et al. 1986
chrysene rat, Sprague Dawley	GD 19; 60 mg/kg b.w.; gavage	F1: Induction of benzo[a]pyrene hydroxylase in liver (6 versus < 0.2 units in controls)	Welch et al. 1972
dibenzo[a,h]anthracene rat, Sprague Dawley	GD 19; 60 mg/kg b.w.; gavage	F1: Induction of benzo[a]pyrene hydroxylase in liver (15 versus < 0.2 units in controls)	Welch et al. 1972
rat (no other details)	GD 1–8 and GD 1–18, 5 mg/animal; s.c.	F0: GD 10 and GD 12 profuse vaginal haemorrhage; GD 14: intraplacental haemorrhage F1: foetal death and resorption up to GD 18	Wolfe and Bryan 1939
naphthalene mouse, CD-1, 50	GD 7–14, 300 mg/kg b.w.; gavage	F0: significant 15% increase in mortality; significant reduction in body weight gain F1: significant reduction in number of live offspring; no malformations	Plasterer et al. 1985
mouse, CD-1	GD 6–13, 300 mg/kg b.w.; gavage	F0: increased mortality 10/50 (control: 0/50); significant reduction in body weight gain F1: significant reduction in the number of live offspring per litter	Hardin et al. 1987
rat, Sprague Dawley, 10–15	GD 1–15, 395 mg/kg b.w.; i.p.	F0: no toxicity F1: no toxicity	Hardin et al. 1981

b.w.: body weight; GD: gestation day; i.p.: intraperitoneal injection; s.c.: subcutaneous injection

**Table A5** Effects of benzo[a]pyrene on fertility in animal studies (WHO 1998)

Species, strain, No. per group	Exposure	Findings	References
mouse, White Swiss, 5, ♂	up to 30 days before mating; 37.5, 75 or 150 mg/kg b.w. and day; diet	150 mg/kg b.w.: NOEL; parameters: sperm in lumen of testes; number of offspring; no effect on fertility	Rigdon and Neal 1965
mouse, White Swiss, 5–65, ♀	20 days before mating; 37.5, 75 or 150 mg/kg b.w. and day; diet	150 mg/kg b.w. NOEL; parameter: number of offspring; no effect on fertility	Rigdon and Neal 1965
mouse, DBA/2N, 15	day 14 before mating; 10, 100, 200 or 500 mg/kg b.w.; once, i.p.	3.4 mg/kg b.w.: threshold (extrapolated from dose-response curve) 10 and 100 mg/kg b.w.: dose-dependent decrease in number of pups 25.5 mg/kg b.w.: 50% effect dose 200 and 500 mg/kg b.w.: completely infertile	Mattison et al. 1980 b
mouse, DBA/2N, ♀	day 21 before sacrifice; 5, 10, 50, 100 or 500 mg/kg b.w.; once, i.p.	dose-dependent increase in primordial oocyte destruction 2.7 mg/kg b.w.: threshold (extrapolated from dose-response curve) 24.5 mg/kg b.w.: 50% effect dose 500 mg/kg b.w.: 100% destruction	Mattison et al. 1980 a
mouse, B6N and D2N, 5, ♀	day 13 before sacrifice; 100 mg/kg b.w.; once, i.p.	100 mg/kg b.w.: significant increase in primordial oocyte destruction in both genotypes; effects in B6N mice greater than in D2N mice	Mattison and Nightingale 1980 a
mouse, C57Bl/6N (B6), DBA/2N (D2), (B6 × D2)F1 (F1; heterozygous crossing), ♀	day 14 before sacrifice; 10 µg/right ovary; once, i.o.	10 µg/ovary: decreased ovarian weight (D2); decreased ovarian volume (D2 and F1); decreased antral follicles (2.5 mm in diameter) (F1); decreased number of small follicles (D2 and F1)	Mattison et al. 1989

Table A5 (Continued)

Species, strain, No. per group	Exposure	Findings	References
mouse, C57Bl/6N, 5, ♀	1, 2, 3 and 4 weeks before sacrifice; 1, 5, 10, 50, 100 and 500 mg/kg b.w.; once, i.p.	1 mg/kg b.w.: NOEL number of corpora lutea/ovary 1.6 mg/kg b.w.: ED <sub>50</sub> number of corpora lutea/ovary 1–500 mg/kg b.w.: dose- and time-dependent decrease in ovarian volume, total volume and number of corpora lutea/ovary (for last parameter, ED <sub>50</sub> was 1.6 mg/kg b.w. after 1 week); effect transitory in low dose groups below 100 mg/kg b.w. (reversible in 3 weeks), but not reversible in two high dose groups (100 and 500 mg/kg b.w.) by 4 weeks 500 mg/kg b.w.: 35% mortality	Swartz and Mattison 1985; Miller et al. 1992

b.w.: body weight; i.o.: intra-ovarian injection; i.p.: intraperitoneal injection

Table A6 Effects of benzo[a]pyrene on postnatal development in animal studies (WHO 1998)

Species, strain, No. per group	Exposure	Findings	References
mouse, non-inbred, ♀	entire gestation period, 1 drop of a 0.5% solution twice per week; F1–F4 treated with benzo[a]pyrene, ♂ once per week, ♀ twice per week; dermal	F1–F4: earlier development of papillomas and carcinomas in the offspring compared with the control animals with a maximum in the second generation; increased incidence of papillomas and carcinomas in all generations compared with animals not treated <i>in utero</i>	Andrianova 1971
mouse, C3H/Anf, 25, ♀	GD 11–13 and GD 16–18, 100 and 150 mg/kg b.w.; i.p.	F1: no difference in birth rate, litter size of offspring as compared with controls; severe suppression of anti-SRBC (sheep red blood cells) PFC (plaque forming cell) response up to 78 weeks of life; 11- to 13-fold increase in tumour incidences (liver, lungs and ovaries) after 56–78 weeks	Urso and Gengozian 1980
mouse, CD-1, ♀	GD 7–16, 10, 40, 160 mg/kg b.w. and day; gavage	10 mg/kg b.w.: in F1, markedly impaired fertility (by 20%) and reduced testis weight (by 40%); 34% sterility of females 40 and 160 mg/kg b.w.: fertility impaired in almost all animals, testis weight reduced and sterility of females	MacKenzie and Angevine 1981

b.w.: body weight; GD: gestation day; i.o.: intra-ovarian injection; i.p.: intraperitoneal injection

**Table A7** Mutagenicity of PAH in Salmonella (WHO 1998)

Strain	Result with metabolic activation	References
<b>anthanthrene</b>		
TA98	+	Hermann 1981
TA100	+	LaVoie et al. 1979
		Andrews et al. 1978
TA98	–	Tokiwa et al. 1977
TM677	+	Kaden et al. 1979
<b>benzo[a]anthracene</b>		
TA100	+	Epler et al. 1979
		Bartsch et al. 1980
TA98, TA100	+	McCann et al. 1975 a
		Coombs et al. 1976
		Simmon 1979
		Salamone et al. 1979 a, 1979 b
TA1535, TA1538	–	Rosenkranz and Poirier 1979
TA100	+	Pahlman and Pelkonen 1987
TA98, TA100	+	Hermann 1981
		Carver et al. 1986
TA100	+	Bartsch et al. 1980
TM677	+	Kaden et al. 1979
TA100	+	Baker et al. 1980
TA98, TA100	+	Bos et al. 1988
TA98, TA100, TA1535, TA1537	+	Probst et al. 1981
TA98, TA100, TA1537, TA1538	±	Dunkel et al. 1984
TA1535	–	Dunkel et al. 1984
TA98, TA100	+	Florin et al. 1980
TA1537, TA1538	–	Teranishi et al. 1975
TA98	+	Tokiwa et al. 1977
<b>benzo[b]fluoranthene</b>		
TA98	+	Hermann 1981
TA100	+	LaVoie et al. 1979
		Hecht et al. 1980
TA100	+	Amin et al. 1985
TA98, TA100	–	Mossanda et al. 1979
<b>benzo[j]fluoranthene</b>		
TA100	+	LaVoie et al. 1980
		Hecht et al. 1980
TM677	+	Kaden et al. 1979
<b>benzo[k]fluoranthene</b>		
TA100	+	LaVoie et al. 1980
		Hecht et al. 1980
TA98	+	Hermann et al. 1980
<b>benzo[b]naphtho[2,1-d]thiophene</b>		
TA98, TA100	+	Grimmer et al. 1989



Table A7 (Continued)

Strain	Result with metabolic activation	References
TA98, TA100, E. coli PKM101 (modified Ames test)	–	Karcher et al. 1981
TA100	+	Misra and Amin 1990
TA100 (1,2-dihydrodiol form)	–	Misra and Amin 1990
TA100 (3,4-dihydrodiol form)	+	Misra and Amin 1990
TA98 (2,1-d isomer)	+	Pool et al. 1989
TA98 (2,3-d isomer)	+	Pool et al. 1989
TA98 (1,2-d isomer)	–	Pool et al. 1989
<b>benzo[a]pyrene</b>		
TA98	+	Epler et al. 1979
TA100	+	Andrews et al. 1978
TA98, TA100	+	LaVoie et al. 1979
TA98, TA100, TA1537, TA1538	+	McCann et al. 1975 a, 1975 b
TM677	+	Kaden et al. 1979
TM677	+	Rastetter et al. 1982
TM677	+	Babson et al. 1986
TA97, TA98, TA100	+	Sakai et al. 1985
TA98, TA100	+	Prasana et al. 1987; Simmon 1979; Glatt et al. 1987
TA1535, TA1538	+	Rosenkranz and Poirier 1979
TA100	+	Norpoth et al. 1984; Alzieu et al. 1987; Carver et al. 1986; Bos et al. 1988; Hermann 1981; Bruce and Heddle 1979; Marino 1987; Alfheim and Ramdahl 1984
TA98	+	Lee and Lin 1988
TA100	+	Pahlman and Pelkonen 1987
TA97, TA98, TA100	+	Marino 1987
TA97, TA98, TA100	+	Sakai et al. 1985
TA98, TA100	+	Devanesan et al. 1990
TM677	+	Skopek and Thilly 1983
TA98, TA100, TA1535, TA1537, TA1538	+	Dunkel et al. 1984
Table TA98, TA100	+	Löfroth et al. 1984
TA98, TA100	+	Florin et al. 1980
TA98	+	Tokiwa et al. 1977
<b>chrysene</b>		
TA100	+	McCann et al. 1975 a; LaVoie et al. 1979
TA98	+	McCann et al. 1975 a
TA100	+	Wood et al. 1977 a
TA100	+	Epler et al. 1979; LaVoie et al. 1979

Table A7 (Continued)

Strain	Result with metabolic activation	References
TA100	+	Salamone et al. 1979 a, 1979 b
TA1535, TA1536, TA1537, TA1538	–	Simmon 1979
TA98, TA100	+	Bhatia et al. 1987
TM677	+	Kaden et al. 1979
TA1535, TA1538	–	Rosenkranz and Poirier 1979
TA97, TA100	+	Sakai et al. 1985
TA98, TA100	+	Bos et al. 1988
TA98	+	Hermann 1981
TA100	+	Carver et al. 1986
TA100	+	Pahlman and Pelkonen 1987
TA100	+	Florin et al. 1980
TA98	+	Tokiwa et al. 1977
<b>cyclopenta[cd]pyrene</b>		
TA98	+	Wood et al. 1980
TA98, TA100, TA1537, TA1538	+	Eisenstadt and Gold 1978
TM677	+	Kaden et al. 1979; Cavalieri et al. 1981 a
TA98	+	Reed et al. 1988
<b>dibenzo[a,h]anthracene</b>		
TA100	+	Andrews et al. 1978; Epler et al. 1979; McCann et al. 1975 a, 1975 b
TA100	+	Salamone et al. 1979 a, 1979 b
TA98	+	Baker et al. 1980
TA98	+	Hermann 1981
TM677	+	Kaden et al. 1979
TA100	+	Wood et al. 1978
TA100	+	Pahlman and Pelkonen 1987; Carver et al. 1986
TA98, TA100, TA1537, TA1538	+	Probst et al. 1981
TA100	+	Platt et al. 1990
TA100	+	Lecoq et al. 1989
TA1537, TA1538	–	Teranishi et al. 1975
<b>dibenzo[a,e]pyrene</b>		
TA100	+	LaVoie et al. 1979
TA1537, TA1538	+	Teranishi et al. 1975
TA98 bzw. TA100	+ or ±	Devanesan et al. 1990
<b>dibenzo[a,h]pyrene</b>		
TA100	±	LaVoie et al. 1979
TA98, TA100	+	Wood et al. 1981
<b>dibenzo[a,l]pyrene</b>		
TA98, TA100	+	Karcher et al. 1984
TA98	+	Hermann 1981

Table A7 (Continued)

Strain	Result with metabolic activation	References
TA98 bzw. TA100	+ or ±	Devanesan et al. 1990
<b>indeno[1,2,3-<i>cd</i>]pyrene</b>		
TA98	+	Hermann et al. 1980
TA100	+	LaVoie et al. 1979
TA100	+	Rice et al. 1985
<b>naphthalene</b>		
TA98, TA100, TA1535, TA1537	–	Florin et al. 1980
TA98, TA100, TA1535, TA1537, TA1538	–	McCann et al. 1975 a
TA98, TA100, TA1535, TA1538	–	Purchase et al. 1976
TA98	–	Ho et al. 1981
TM677	–	Kaden et al. 1979
G46, <i>E. coli</i> K 12	–	Krämer et al. 1974
TA98, TA100	–	Epler et al. 1979
TA98, TA100	–	Mamber et al. 1984
TA97, TA98, TA100	–	Sakai et al. 1985
TA100	–	Pahlman and Pelkonen 1987
TA98, TA100	–	Bos et al. 1988
<b>phenanthrene</b>		
TA100	+	Oesch et al. 1981
TA100	–	Wood et al. 1979
TA98	+	Epler et al. 1979
Table TA98	–	LaVoie et al. 1979, 1980
TA100	–	LaVoie et al. 1981
TA98, TA100	–	Probst et al. 1981
TA100	–	LaVoie et al. 1979, 1980; Gelboin and Ts'o 1978; McCann et al. 1975 a
TA98, TA100, TA1535, TA1537		McCann et al. 1975 a
TA100	+	Carver et al. 1986
TM677	–	Kaden et al. 1979
TA97	+	Sakai et al. 1985
TA98, TA100	±	Bos et al. 1988
TA1535, TA1536, TA1537, TA1538	–	Simmon 1979
TA1535, TA1538		Rosenkranz and Poirier 1979
TA100	–	Pahlman and Pelkonen 1987
TA98, TA100, TA1535, TA1537, TA1538		Dunkel et al. 1984
TA98, TA100	–	Florin et al. 1980
<b>pyrene</b>		
TA98	–	Ho et al. 1981; Rice et al. 1988 a
TA98, TA100,	–	McCann et al. 1975 a;
		LaVoie et al. 1979
TA1535, TA1537	–	Ho et al. 1981

**Table A7** (Continued)

Strain	Result with metabolic activation	References
TA1537	+	Bridges et al. 1981
TA98, TA100	–	Salamone et al. 1979 a, 1979 b
TA98, TA100	–	Probst et al. 1981
TA1537	+	Epler et al. 1979
TM677	+	Kaden et al. 1979
TA97	+	Sakai et al. 1985
TA98, TA100	±	Bos et al. 1988
TA100	–	Carver et al. 1986; Hermann 1981
TA98, TA100	+	Bhatia et al. 1987
TA98, TA100, TA1535, TA1537, TA1538	–	Dunkel et al. 1984
TA100	–	Pahlman and Pelkonen 1987
TA98, TA100	–	Florin et al. 1980

Studies of DNA damage caused by PAH in prokaryotes are listed in Table 82 of the WHO monograph (WHO 1998).

**Table A8** DNA damage induced by PAH in eukaryotes (WHO 1998)

Test substance, test system	End point	Metabolic activation <sup>a</sup>	Result <sup>b</sup>	References
<b>benzo[a]anthracene</b>				
primary rat hepatocytes	UDS	–	+	Probst et al. 1981
primary rat hepatocytes	R	–	+	Tong et al. 1983
HeLa cells	UDS	+/-	+	Martin et al. 1978
rat or human mammary epithelial cells	DS	–	±	Mane et al. 1990
hamster buccal pouch epithelial cells	DS	–	–	Nagabhushan et al. 1990
human peripheral blood lymphocytes	DA	–	+	Gupta et al. 1988
<b>benzo[b]fluoranthene</b>				
rat buccal mucosa epithelial cells	DA	–	+	Autrup and Autrup 1986
human leukocytes	DA	+	+	Roggeband et al. 1994 a
<b>benzo[j]fluoranthene</b>				
rat buccal mucosa epithelial cells	DA	–	+	Autrup and Autrup 1986
<b>benzo[k]fluoranthene</b>				
human leukocytes	DA	+	+	Roggeband et al. 1994 a

Table A8 (Continued)

Test substance, test system	End point	Metabolic activation <sup>a</sup>	Result <sup>b</sup>	References
<b>benzo[<i>b</i>]naphtho[2,1-<i>d</i>]thiophene</b>				
(not listed by WHO 1998 in the corresponding table)				
primary rat hepatocytes (1,2-d isomer, 2,1-d isomer and 2,3-d isomer) (DNA strand breaks)				Pool et al. 1989
Chinese hamster cell line (DNA strand breaks) (1,2-d isomer, 2,1-d isomer and 2,3-d isomer)				Pool et al. 1989
<b>benzo[<i>a</i>]pyrene</b>				
primary rat hepatocytes	UDS	–	+	Probst et al. 1981
primary rat hepatocytes	R	–	+	Williams et al. 1982
C3H/10T1/2 mouse clone 8 (DNA strand breaks)			±	Lubet et al. 1983
human leukocytes	DA	+	+	Roggeband et al. 1994 a
hamster or rat tracheal epithelial cells	DA, UDS	–	+	Roggeband et al. 1994 b
HeLa cells	UDS	+/-	+	Martin et al. 1978
human skin fibroblasts	R	–	+	Milo et al. 1978
human mammary epithelial cells (oxidative DNA damage)		–	+	Leadon et al. 1988
human fibroblasts	UDS	+	+	Agrelo and Arnos 1981
human WI-38 fibroblasts	UDS	+/-	+	Robinson and Mitchell 1981
rat or human mammary epithelial cells	R	–	+	Mane et al. 1990
	DA	–	+	Harris et al. 1984
Syrian hamster embryo cells	R	–	+	Casto 1979
hamster buccal pouch epithelial cells (inhibition of DNA synthesis)		–	+	Nagabhushan et al. 1990
rat buccal mucosa epithelial cells	DA	–	+	Autrup and Autrup 1986
human peripheral blood lymphocytes	DA	–	+	Gupta et al. 1988
primary rat hepatocytes	DA	–	+	Monteith and Gupta 1992
primary human hepatocytes	DA	–	+	Monteith and Gupta 1992
calf thymus DNA	DA	–	+	Bryla and Weyand 1991
primary mouse epidermal keratinocytes	DA, UDS	–	+	Gill et al. 1991

Table A8 (Continued)

Test substance, test system	End point	Metabolic activation <sup>a</sup>	Result <sup>b</sup>	References
primary rat hepatocytes (SCE)	R	–	+	Tong et al. 1983
<b>chrysene</b>				
primary rat hepatocytes	R	–	–	Tong et al. 1983
human leukocytes	DA	+	–	Roggeband et al. 1994 a
<b>cyclopenta[cd]pyrene</b>				
rat liver or lung tissue	DA	–	+	Beach and Gupta 1991
calf thymus DNA	DA	+	+	Beach and Gupta 1994
<b>dibenzo[a,h]anthracene</b>				
primary human foreskin epithelial cells	UDS	–	+	Lake et al. 1978
HeLa cells	UDS	+/-	+	Martin et al. 1978
Syrian hamster embryo cells	R	–	–	Casto 1979
primary rat hepatocytes	UDS	–	+	Probst et al. 1981
mouse liver DNA	DA	+	+	Lecoq et al. 1991
human bronchial cells	DA	–	+	Harris et al. 1984
hamster embryo cells	B	B	+	Kuroki and Heidelberg 1972
C3H10T1/2 mouse clone 8 cells	DA	–	+	Nesnow et al. 1994
<b>phenanthrene</b>				
Syrian hamster embryo cells	R	–	–	Casto 1979
human foreskin epithelial cells	UDS	–	–	Lake et al. 1978
primary rat hepatocytes	UDS	–	–	Probst et al. 1981
human skin fibroblasts	R	–	–	Milo et al. 1978
<b>pyrene</b>				
Syrian hamster embryo cells	R	–	–	Casto 1979
human foreskin epithelial cells	UDS	–	–	Lake et al. 1978
primary rat hepatocytes	UDS	–	–	Probst et al. 1981
HeLa cells	UDS	+/-	–	Martin et al. 1978
human fibroblast WI-38 cell line	UDS	+/-	+	Robinson and Mitchell 1981
primary rat hepatocytes	R	–	–	Williams et al. 1982
human skin fibroblasts	R	C	–	Milo et al. 1978
human skin fibroblasts	UDS	+	–	Agrelo and Arnos 1981
primary rat hepatocytes	R	–	–	Tong et al. 1983
human peripheral blood lymphocytes	DA	–	–	Gupta et al. 1988

R: DNA repair; DA: DNA adducts; UDS: unscheduled DNA synthesis; SCE: sister chromatid exchange; B: study of the binding of the epoxide to the h protein; binding factor 8 higher as compared with parent substance; C: study without metabolic activation; increase in arylhydrocarbon hydroxylase activity and cell proliferation; DNA damage by intermediary metabolites metabolic activation

<sup>a</sup> +: with metabolic activation; –: without metabolic activation; +/-: with and without metabolic activation results

<sup>b</sup> +: positive; –: negative; ±: inconclusive. It is pointed out in the specific table in WHO 1998 that positive results are shown if positive only with metabolic activation.

**Table A9** Effect of PAH in *Drosophila melanogaster* (WHO 1998)

Substance	End point	Result	References
benzo[a]anthracene	SLRL	+	Fahmy and Fahmy 1973
	somatic mutation	–	Fahmy and Fahmy 1980
	SLRL	–	Zijlstra and Vogel 1984
	SMART	+	Frölich and Würgler 1990
	R	+	Fujikawa et al. 1993
benzo[a]pyrene	SLRL	±	Vogel et al. 1983
	somatic mutation	+	Fahmy and Fahmy 1980
	SLRL	–	Zijlstra and Vogel 1984
	SLRL	–	Valencia and Houtchens 1981
	somatic mutation	+	Batiste-Alentorn et al. 1991
	SMART	+	Frölich and Würgler 1990
	SLRL	–	Valencia and Houtchens 1981
	R	+	Fujikawa et al. 1993
pyrene	R	±	Fujikawa et al. 1993

somatic mutation: spontaneous change of eye pigmentation by effects on a locus in the stem cell line; SLRL: sex-linked recessive lethal mutation; SMART: somatic mutation and recombination test; R: DNA repair

**Table A10** Chromosomal effects of PAH in mammalian cell systems *in vivo* including DNA binding and adducts as well as sperm abnormalities (WHO 1998)

Test substance, test system	Result	References
<b>benzo[a]anthracene</b>		
Chinese hamster, bone marrow SCE	+	Roszinsky-Köcher et al. 1979
Chinese hamster, bone marrow: CA	–	Roszinsky-Köcher et al. 1979
Long-Evans rat, bone marrow: CA	–	Sugiyama 1973
Chinese hamster, bone marrow: MN, CA	+	Péter et al. 1979
NMRI mouse, metaphase II oocytes: CA	+	Péter et al. 1979
mouse, gastrointestinal epithelial cells: nuclear anomalies	–	Reddy et al. 1991
rats, lungs: DNA adducts, SCE, MN	+	Whong et al. 1992
mouse, skin: DNA binding	+	Reddy et al. 1984
rat, bone marrow and spleen cells: MN	+	Zhong et al. 1995
<b>benzo[b]fluoranthene</b>		
Chinese hamster, bone marrow: SCE	+	Roszinsky-Köcher et al. 1979
Chinese hamster, bone marrow: CA	–	Roszinsky-Köcher et al. 1979

Table A10 (Continued)

Test substance, test system	Result	References
mouse, skin: DNA binding	+	Weyand et al. 1987
rat, lung, liver and peripheral lymphocytes: DNA adducts	+	Ross et al. 1991; Ross et al. 1992
rat, lung, liver and peripheral blood lymphocytes, whole blood cultures: SCE	+	Ross et al. 1991; Ross et al. 1992
mouse, gastrointestinal epithelial cells: nuclear anomalies	+	Reddy et al. 1991
rat, peripheral blood lymphocytes: SCE, MN	+	Bryant et al. 1991
mouse, gastrointestinal epithelial cells: nuclear anomalies	+	Reddy et al. 1991
mouse, skin: DNA binding	+	Amin et al. 1991
mouse, skin: DNA adducts, MN, UDS	+	Winker et al. 1995
<b>benzo[j]fluoranthene</b>		
mouse, lung and liver cells: DNA adducts	+	Weyand and LaVoie 1988
mouse, skin: DNA adducts	+	Weyand et al. 1993
<b>benzo[k]fluoranthene</b>		
mouse, skin: DNA binding	+	Weyand et al. 1987
mouse, lung and liver cells: DNA adducts	+	Weyand and LaVoie 1988
<b>benzo[b]naphtho[2,1-d]thiophene</b>		
not listed by WHO 1998 in the corresponding table		
mouse, bone marrow: MN	+	Grimmer et al. 1989
mouse, skin: DNA adducts	+	Hughes and Phillips 1993
<b>benzo[a]pyrene</b>		
mouse, dominant lethal test	+	Epstein 1968
mouse, dominant lethal test	+	Generoso et al. 1982
mouse, mammalian spot test	+	Russell 1977
mouse, mammalian spot test	+	Davidson and Dawson 1976
rats, hepatocytes: UDS	–	Miralis et al. 1982
mouse, germ cells: UDS	–	Sega 1979
mouse, skin: DNA binding	+	Weyand et al. 1987; Rice et al. 1984
Chinese hamster, bone marrow cells: CA or SCE	+ or +	Roszinsky-Köcher et al. 1979
Chinese hamster, bone marrow cells: CA or SCE	± or +	Bayer 1978
mouse, CA, heritable translocations	–	Generoso et al. 1982
mouse, bone marrow: MN	+	Salamone 1981; Salamone et al. 1981
mouse, bone marrow: MN	–	Bruce and Heddle 1979



Table A10 (Continued)

Test substance, test system	Result	References
Chinese hamster, bone marrow cells: MN	–	Bayer 1978
mouse, sperm anomalies:	+	Topham 1980
mouse, sperm anomalies:	+	Bruce and Heddle 1979
Chinese hamster as host for V79: SCE	+	Sirianni and Huang 1978
mouse, epidermal cells: DNA adducts	+	Albert et al. 1991 a, 1991 b
mouse, bone marrow: MN	+	Shimada et al. 1990
mouse, keratinocytes: MN	+	He and Baker 1991
mouse, lung cells and hepatocytes: DNA adducts	+	Weyand and LaVoie 1988
mouse, liver, lungs and stomach: DNA adducts	+	Cummings et al. 1991
rat, peripheral lymphocytes: SCE	+	Li et al. 1991
mouse, bone marrow: MN	+	Mavourin et al. 1990
mouse, bone marrow: MN	+	Kliesch et al. 1982
mouse, bone marrow: MN	+	Harper and Legator 1987
mouse, peripheral blood cells: MN	±	Oshiro et al. 1992
mouse, gastrointestinal epithelial cells: nuclear anomalies	+	Reddy et al. 1991
mouse, bone marrow: SCE	+	Wielgosz et al. 1991
rat, peripheral blood lymphocytes: SCE, DNA adducts	+	Willems et al. 1991
rat, hepatocytes: DNA adducts	+	Willems et al. 1991
rat, peripheral blood lymphocytes: CA	–	Willems et al. 1991
Chinese hamster, lung cells: CA	+	Matsuoka et al. 1979
mouse, skin epithelial cells: DNA binding	+	Hughes and Phillips 1991
humans, peripheral lymphocytes: DNA adducts	+	Haugen et al. 1986
rat, lungs, liver and peripheral lymphocytes: DNA adducts	+	Ross et al. 1991
mouse, bone marrow: MN	+	Awogi and Sato 1989
mouse, skin: DNA binding	+	Reddy et al. 1984
mouse, skin: DNA adducts	+	Oueslati et al. 1992
mouse, rat, bone marrow: MN	+	Shimada et al. 1992
mouse, bone marrow: CA	+	Adler and Ingwersen 1989
<b>chrysene</b>		
Chinese hamster, bone marrow cells: SCE	+	Roszinsky-Köcher et al. 1979
Chinese hamster, bone marrow cells: CA	–	Roszinsky-Köcher et al. 1979
NMRI mouse, metaphase II oocytes	+	Basler et al. 1977
mouse, keratinocytes: MN	+	He and Baker 1991
mouse, skin: DNA binding	+	Reddy et al. 1984
<b>dibenzo[a,h]anthracene</b>		
Chinese hamster, bone marrow cells: SCE	+	Roszinsky-Köcher et al. 1979
Chinese hamster, bone marrow cells: CA	–	Roszinsky-Köcher et al. 1979
rat, peripheral blood lymphocytes: SCE, MN	–	Bryant et al. 1990
mouse, skin: DNA binding	+	Lecoq et al. 1991
mouse, skin: DNA binding	+	Reddy et al. 1984

Table A10 (Continued)

Test substance, test system	Result	References
rat, bone marrow and spleen cells:	+	Zhong et al. 1995
rat, lung: DNA adducts, MN, SCE	+	Whong et al. 1994
mouse, skin: DNA adducts, MN, UDS:	+	Winker et al. 1995
<b>dibenzo[<i>a,e</i>]pyrene</b>		
mouse, skin epithelial cells: DNA binding	+	Hughes and Phillips 1991
<b>indeno[1,2,3-<i>cd</i>]pyrene</b>		
mouse, skin: DNA binding	+	Weyand et al. 1987
mouse, skin epithelial cells: DNA binding	+	Rice et al. 1990
<b>naphthalene</b>		
mouse, bone marrow cells: MN	–	Harper et al. 1984
<b>phenanthrene</b>		
Chinese hamster, bone marrow cells: CA	–	Bayer 1978; Roszinsky-Köcher et al. 1979
Chinese hamster, bone marrow cells: SCE	+	Bayer 1978; Roszinsky-Köcher et al. 1979
Chinese hamster, bone marrow cells: MN	–	Bayer 1978
<b>pyrene</b>		
mouse, bone marrow cells: SCE	–	Paika et al. 1981
mouse, bone marrow: MN	–	Salamone 1981; Salamone et al. 1981
mouse, bone marrow cells: MN	–	Tsuchimoto and Matter 1981
mouse as host for V79 cells: SCE	–	Sirianni and Huang 1978
mouse, keratinocytes: MN	–	He and Baker 1991
mouse, peripheral blood cells: MN	–	Oshiro et al. 1992
mouse, gastrointestinal epithelial cells: nuclear anomalies	–	Reddy et al. 1991
mouse, sperm abnormalities:	–	Topham 1980
mouse, skin: DNA binding	–	Reddy et al. 1984

SCE: sister chromatid exchange; MN: micronucleus assay; CA: chromosomal aberrations; UDS: unscheduled DNA synthesis. Result: + positive; ± inconclusive; – negative

Table A11 Effect of PAH on morphological transformation of mammalian cells *in vivo*

Test substance, test system	Result	References
<b>benzo[<i>a</i>]pyrene</b>		
hamster embryos, transplacental exposure	+	Quarles et al. 1979
<b>phenanthrene</b>		
hamster embryos, transplacental exposure	–	Quarles et al. 1979

Result: +: positive; –: negative

Table A12 Carcinogenicity of PAH in animal studies (WHO 1998)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
anthranthrene					
mouse, ♀, 30	0.3% in benzene, twice per week, lifetime, dermal	lifetime	1/30 lung adenomas	n no/ls	Badger et al. 1940
mouse, Ha/ICR/Mil, ♀, 20	0.05 or 0.1%, 3 times per week, 12 months, dermal, recrystallized	15 months	0/20 with tumours	n no/val	Hoffmann and Wynder 1966
mouse, Swiss, Ha/ICR/Mil, ♀, 30	25 µg/animal, 10 times over 20 days: initiation experiment, dermal, recrystallized	6 months	2/25 papillomas; promotor only: 2/26	n no/val	Hoffmann and Wynder 1966
mouse, CR/Ha, ♀, 13	0.25 mg/animal, 4 times; initiation experiment, dermal, purified	65 weeks	2/13 papillomas; promotor only: 5/20 papillomas; control acetone: 0/20 papillomas	n no/val	Van Duuren et al. 1968
mouse, Swiss, ♀, 30	43 µg/animal, twice per week, 75 weeks, dermal, recrystallized	≤ 100 weeks	1/30 with skin carcinoma; control: 2/30 with carcinomas	n no/val	Lijinski and Garcia 1972
mouse, Swiss, ♀, 40	109 µg/animal, twice per week, 30 weeks, dermal, 98.65%	70 weeks	47% skin tumour-bearing animals; solvent control: 0%	p no/val	Cavalieri et al. 1977

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, CD-1, ♀, 30	0.69 mg/animal, once; initiation experiment, dermal, TLC purified	35 weeks	18% with papillomas; promotor only: 3%	p no/val	Scribner 1973
mouse, Sencar, ♀, 27	221 µg/animal, once; initiation experiment, dermal, > 99%	26 weeks	11% with papillomas; promotor only: 9%	n yes/val	Cavalieri et al. 1989
mouse, XVII ♂/♀, 7	0.6 mg/animal, once/month, 3 months, s.c.		no local sarcomas observed	n no/ln, ls	Lacassagne et al. 1958
rat, Osborne Mendel, ♀, 35	0.65 and 3.4 mg/kg, once, i.pul., 99.4%	102/88 weeks	1/35 and 19/35 with lung tumours; control: no tumours	p yes/val	Deutsch-Wenzel et al. 1983 b
rat, Sprague Dawley, ♀, 20	1.1 mg/gland, once, 8 glands, i.mam., > 99%	≤ 40 weeks	1/20 with mammary tumours; control: 0/20 or 2/20	n yes/val	Cavalieri et al. 1989
<b>benzo[<i>a</i>]anthracene</b>					
mouse, C57/BL, 8-19	0.5 mg/animal, once, 8 times or 16 times (highest dose), ≤ 2 months, oral	16 months	0/13; 1/19 and 1/8 with papillomas; no carcinomas observed; control: 0/12	q yes/ln	Bock and King 1959

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, B6AF1/J, ♂, 20 or 40	1.5 mg/animal (newborn), 3 times per week, 5 weeks, oral	≤ 547–600 days	100% hepatomas and 95% lung adenomas; solvent only: 10% hepatomas and 35% lung adenomas	p no/val	Klein 1963
mouse, B6AF1/J, ♂, 20	1.5 mg/animal (newborn), once per day, 2 days, oral	≤ 568 days	80% hepatomas and 85% lung adenomas (inadequately reported)	p no/val	Klein 1963
mouse, 30	0.3% in benzene, twice per week, lifetime, dermal, purified	≤ 584 days	1/30 epitheliomas	n no/ls	Barry et al. 1935
mouse, ♂, 75	3 drops of a 0.5% solution once per week, 1 year; initiation experiment, dermal, “pure”	≤ 1 year	after 12 months 9/75 survived; total of 18 tumours; 2 tumours/animal; promotor only: 0.08 tumour/animal	p no/val	Graffi et al. 1953
mouse, albino, ♀, 30	66 mg/animal, twice per week, 20 weeks, dermal, recrystallized	13–15.5 months	no tumours; solvent only: no tumours	n no/val	Miller and Miller 1963
mouse, C3H, 20	0.5% solution, twice per week, 638 days, dermal	638 days	no tumours; solvent only: no tumours	n no/val	Stevenson and von Haam 1965

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, C3H/He, 30–50	0.0001–0.5 mg/animal in n-dodecane or 0.1 mg/animal in toluene, 3 times/ week, 50 weeks, dermal, recrystallized	≤ 88 weeks	dose-dependent increase in malignant tumours; solvent control: no tumours	p yes/val	Bingham and Falk 1969
mouse, Swiss Millerton ICR/Ha, ♀, 20	1 mg/animal, once; initiation experiment, dermal, recrystallized	58–60 weeks	10/20 with papillomas; promotor only: 1/20; solvent control: 0%	p no/val	Van Duuren et al. 1970
mouse, CD-1, ♀, 30	0.5 mg/animal, once; initiation experiment, dermal, TLC purified	35 weeks	62% with papillomas; promotor only: 3%	p no/val	Scribner 1973
mouse, Swiss, ♀, 40	90 µg/animal, twice per week, 30 weeks, dermal, > 99%	70 weeks	2.6% skin tumour-bearing animals; solvent control: 0%	n no/val	Cavalieri et al. 1977
mouse, CD-1, ♀, 30	0.46 mg/animal, once; initiation experiment, dermal, > 99%	26 weeks	57% with papillomas; promotor only: 6%	p no/val	Slaga et al. 1978 a

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, CD-1, ♀, 30	0.1 and 0.57 mg/animal, once; initiation experiment, dermal,	27 weeks	14% and 36% (p < 0.05) with tumours; solvent control: 7%	p yes/val	Levin et al. 1984
mouse, CD-1, ♀, 30	0.23 and 0.57 mg/ animal, once; initiation experiment, dermal,	27 weeks	17% and 38% papillomas; solvent control: 4%	p yes/val	Weyand et al. 1990; Wood et al. 1980
mouse, C57Bl, ♂/♀, 50	5 mg/animal in tricaprylin, once, s.c., spectrometer	≤ 22 months	8/46 sarcomas after 4 months; solvent control: 3/280	p no/val	Steiner and Falk 1951
mouse, C57Bl, ♂/♀, 40-50	0.05, 0.2, 1, 5 or 10 mg/ animal in tricaprylin, once, s.c.	≤ 22-28 months	5/44, 11/45, 15/44, 20/36 and 5/16 sarcomas	p yes/ls	Steiner and Edgcomb 1952; Steiner 1955
mouse, albino, ♀, 30	0.94 mg/animal, once, s.c., recrystallized	≤ 15 months	no sarcomas; solvent control: no tumours	n no/val	Miller and Miller 1963
mouse, C3H, 20	5 mg in tricaprylin, once, s.c.	638 days	no tumours; control: no tumours	n no/val	Stevenson and von Haam 1965

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, C57BL, ♂/♀, 10	1 mg/animal, once per week, 10 weeks, s.c.	60–80 weeks	8/10 ♂ and 6/10 ♀ with sarcomas; control: 0/20 ♂ and 0/20 ♀	p no/val	Boyland and Sims 1967
mouse, Swiss, ♂/♀, 87	0.2 mg/animal (newborn) in polyethylene glycol on days 0, 1 and 2 after birth, s.c.	70–75 weeks	70 weeks: 15/15 ♂ and 2/18 ♀ with liver tumours, 4/15 ♂ and 10/18 ♀ with lung tumour; corrected control data: 4/22 ♂ and 1/23 ♀ with liver tumours and 3/22 ♂ and 1/23 ♀ with lung tumours	p no/val	Grover et al. 1975
mouse, Swiss Webster BLU:Ha(ICR), ♂/♀, 140	9.1, 18.2 and 36.4 µg/ animal (newborn) on days 1, 8 and 15 after birth, i.p.	26 weeks	10/47 ♂ and 4/38 ♀ with lung tumours; solvent control: 7/43 and 2/24	n no/val	Wislocki et al. 1979
mouse, A, 11	10 mg/kg, once, i.v.	20 weeks	18% lung tumours; control: 21%	n no/val	Shimkin and Stoner 1975
mouse, C57×IF F <sub>1</sub> hybrid, 52	about 2 mg/animal, once, bladder implant (pellets)	≥ 40 weeks	17/52 bladder carcinomas and 1/52 papillomas; control: 4/89	p yes/val	Clayson et al. 1968



Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, Sprague Dawley, ♀, 10	200 mg/kg rat, once, oral	60 days	no tumours in treated animals; control: 8/164 after 310 days	n no/ln, ld	Huggins and Yang 1962
rat, Donyu, ♂, 25	saturated solution in acetone, dropped onto an area of 2 cm <sup>2</sup> twice per week, 5 months, dermal	≤ 18 months	no tumours	n no/ls	Tawfic 1965
rat, Holtzman, ♂, 20	1.88 mg/animal, once, dermal, recrystallized	≥ 4 months	no sarcomas; solvent control: no tumours	n no/val	Miller and Miller 1963
rat, Sprague Dawley, ♀, 28	2 mg/animal (=13 mg/kg), on days 50, 53 and 56 of age, i.v., TLC purified	98 days	no mammary gland tumours	n no/ls	Pataki and Huggins 1969
rat, Long Evans, ♂, 8	2.5 mg/animal into hind leg, once on day 25 of age, i.m., TLC purified	98 days	no sarcomas; control: no spontaneous sarcomas	n no/ln	Pataki and Huggins 1969

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, Sprague Dawley, ♀, 20	0.91 and 3.7 mg into 5th mammary gland, once, intramammary injection, > 99%	20 weeks	no mammary tumours; control: no tumours	n no/val	Cavallieri et al. 1988 a
Syrian golden hamster, ♂/♀, 5	8 drops of a 0.5% solution in acetone, twice per week, 10 weeks, dermal, control by column chromatography	≤ 85/61 weeks	no tumours	n no/ln, ls	Shubik et al. 1960
Syrian golden hamster, ♂, 5 or 26	20 mmol/l solution, painting twice per week, 5 or 20 weeks, dermal (buccal pouch)	≤ 44 weeks	no tumours; control: no tumours	n no/val	Solt et al. 1987
Syrian golden hamster, ♂, 47 or 33	0.5 or 3 mg/animal per week, 30 or 15 weeks, i.tr., purity more than 99% (thin layer chromatography)	≤ 110 weeks	no tracheal tumours; control: no tumours	n yes/val	Sellakumar and Shubik 1974
<b>benzo[<i>b</i>]fluoranthene</b> mouse, Swiss Millerton, ♀, 20	0.01, 0.1 and 0.5% in acetone, 3 times per week, lifetime, dermal, purity verified via melting point, paper chromatography and UV spectrum	≤ 14, 12 and 8 months	0.01%: 5% papillomas after 14 months; 0.1%: 65% papillomas and 85% carcinomas after 12 months; 0.5%: 100% carcinomas after 5 months	p no/ls	Wynder and Hoffmann 1959 a

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Swiss ICR/Ha, ♀, 20	1 mg once; initiation experiment, dermal,	63 weeks	18/20 papillomas, 5/20 carcinomas; promotor only: 5/20, 1/20	p no/ls	Van Duuren et al. 1966
mouse, NMRI, ♀, 40	3.4, 5.6, 9.2 µg/animal, twice per week, lifetime, dermal, > 96%	≤ 2 years	5/15/54% with local tumours; control: no tumours	p yes/val	Habs et al. 1980
mouse, CD-1	10–100 mg/animal; initiation experiment, dermal	20 weeks	dose-related skin tumour incidence	p yes/val	LaVoie et al. 1982
mouse, CrI:CD1 (ICR)BR, ♀, 20	4 and 10 nmol/animal, 10 times every day; initiation experiment, dermal, > 99%	34 weeks	45 and 95% tumour incidence; solvent control: 5%	p yes/val	Amin et al. 1985
mouse, CD-1, ♀, 20	0.025 and 0.1 mg/animal, 10 times every other day; initiation experiment, dermal	24 weeks	100 and 100% tumour incidence; solvent control: 10%	p yes/val	Weyand et al. 1990
mouse, CrI:CD1 (ICR)BR, ♀, 20	3 and 10 µg/animal, 10 times; initiation experiment, dermal	34 weeks	65 and 100% with tumours; solvent control: 15%	p yes/val	Amin et al. 1991

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, XVII nc/Z, ♂/♀, 16/14	0.6 mg/animal, once/month, 3 months, s.c.	about 200 days	8/16 ♂ and 10/14 ♀ with local sarcomas;	p no/l	Lacassagne et al. 1963 a
mouse, CD-1, ♂/♀, 15/17	126 µg/animal (newborn) in DMSO on days 1, 8 and 15 after birth (total dose), i.p.	≤ 52 weeks	53% hepatic, 18% lung tumours; control: 6% hepatic tumours, no lung tumours	p yes/val	LaVoie et al. 1987
rat, Osborne Mendel, ♀, 35	0.1, 0.3 and 1 mg/animal, once, i.pul., 99.5%	110/113/112 weeks	0/35, 1/35 and 9/35 pulmonary carcinomas; 1/35, 2/35 and 4/35 pleomorphic sarcomas; control: no tumours	p yes/val	Deutsch-Wenzel et al. 1983 b
Syrian golden hamster, ♂, 47	0.5 and 0.05 mg/animal per week, 30 weeks, i.tr., purity more than 99% (thin layer chromatography)	≤ 110 weeks	1/47 (0.5 mg/animal) and 0/47 (0.05 mg/animal) tracheal tumours; control: no tumours	n yes/val	Sellakumar and Shubik 1974
<b>benzo[<i>j</i>]fluoranthene</b> mouse, Swiss, ♀, 20	0.1 and 0.5% in acetone, 3 times per week, lifetime, dermal, highly purified (purity verified via melting point, paper chromatography and UV spectrum)	≤ 9 and 7 months	100%/85% with skin carcinomas	p no/l	Wynder and Hoffmann 1959 a

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, NMRI, ♀, 40	3, 4, 5, 6, 9.2 µg/animal, twice per week, lifetime, dermal, 96%	≤ 2 years	3, 3 and 5% with local tumours; control: 0%	q yes/val	Habs et al. 1980
mouse, Crl:CD1 (ICR)BR, ♀, 20	3, 10, 100 µg, 10 times over 20 days; initiation experiment, dermal, > 99%	24 weeks	30, 35 and 95% with tumours (papillomas; keratinizing lesions); 1 malignant lymphoma	p yes/val	LaVoie et al. 1982
mouse, CD-1, ♂/♀, 21/18	278 µg/animal (newborn) in DMSO on days 1, 8 and 15 after birth (total dose), i.p., 99%	≤ 52 weeks	81% males and 22% females with liver and lung tumours; control animals: 6%; 0%	p yes/val	LaVoie et al. 1987
rat, Osborne Mendel, ♀, 35	0.8, 4 and 20 mg/kg b.w., once, i.pul., 99.9%	100/117/89 weeks	1/35, 3/35 and 18/35 pulmonary carcinomas; control: no tumours	p yes/val	Deutsch- Wenzel et al. 1983 b
<b>benzo[k]fluoranthene</b> mouse, Swiss, ♀, 20	0.1 and 0.5% in acetone, 3 times per week, lifetime, dermal, highly purified (purity verified via melting point, paper chromatography and UV spectrum)	≤ 13 months	0/20 and 2/20 skin papillomas	q no/ls	Wynder and Hoffmann 1959 a

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, NMRI, ♂, 25	1 mg/animal, (total dose) in 50 aliquots, dermal	≤ 2 years	no skin tumours; spontaneous tumours: 10%	n no/val	Mohr 1969
mouse, NMRI, ♂, 40	3.4, 5.6, 9.2 µg/animal, twice per week, lifetime, dermal, > 96%	≤ 2 years	3, 0 and 0% with local tumours; control: no tumours	n yes/val	Habs et al. 1980
mouse, CrI:CD1 (ICR)BR, ♀, 20	3, 10, 100 µg, 10 times over 20 days; initiation experiment, dermal, > 99%	24 weeks	5, 25 and 75% with tumours (papillomas/keratinizing lesions)	p yes/val	LaVoie et al. 1982
mouse, XVII nc/Z, ♂/♀, 16/14	0.6 mg/animal, once per month, 3 months, s.c.	about 200 days	8/16 ♂ and 5/14 ♀ with local sarcomas:	p no/ls	Lacassagne et al. 1963 a
mouse, CD-1, ♂/♀, 16/18	530 µg/animal (newborn) in DMSO on days 1, 8 and 15 after birth (total dose), i.p., > 99%	≤ 52 weeks	19% males and 17% females with tumours; control: 6% and 0% liver and lung tumours	q yes/val	LaVoie et al. 1987

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, Osborne Mendel, ♀, 27–35	0.65, 3.4 and 17 mg/kg b.w., once, i.pul., 99.5%	114/95/98 weeks	0/35, 3/31 and 12/27 pulmonary carcinomas; control: no tumours	p yes/val	Deutsch- Wenzel et al. 1983 b
<b>benzo[a]pyrene</b> mouse, A/HeJ, ♀, 15	3 mg/animal in sesame oil, twice, oral	30 weeks	increased number of tumours 16.6; control: 0.3 (number of adenomas per animal)	p yes/val	Wattenberg and Leong 1970
mouse, A/J, ♀, 15	2 mg/animal, 3 times, every 2 weeks, oral	26 weeks	15/15 with forestomach tumours and 15/15 with pulmonary adenomas; no control	p yes/val	Sparmins et al. 1986
mouse, CFW, ♂/♀, 25–73	0.004–1 mg/animal/day, ≤ 110–165 days, oral (diet)	140–200 days	dose-dependent gastric tumours (0–90%); control: no tumours	p no/val	Neal and Rigdon 1967
mouse, CFW, ♂/♀, 9–26	1–20 mg/animal/day, ≤ 1–30 days, oral (diet)	150–300 days	dose-dependent gastric tumours (0–100%); control: no tumours	p no/val	Neal and Rigdon 1967

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, White Swiss, ♂/♀, 60–175	0.25 and 1 mg/g diet, ≤ 34 weeks, oral (diet)	≤ 34 weeks	33 and 61% with stomach tumours; 53 and 20% with lung tumours, control: 1 and 21%	p no/val	Rigdon and Neal 1966
mouse, Swiss, ♀, 20–30	0.001, 0.005 and 0.01%, 3 times per week, lifetime, dermal	≤ 21, 14 and 11 months	3% skin carcinomas and 43% papillomas in the animals treated with 0.001% after 21 months; 63% skin carcinomas and 67% papillomas in the animals treated with 0.005% after 14 months; 95% skin carcino- mas and 95% papillomas in the animals treated with 0.01% after 11 months	p no/ls	Wynder and Hoffmann 1959 b
mouse, Swiss (Millerton), ♀, 20	0.01, 0.05 and 0.5%, 3 times per week, lifetime, dermal, purity verified via melting point, paper chromatography and UV spectrum	≤ 12, 6 and 6 months	85, 95 and 75% with skin carcinomas	p no/ls	Wynder and Hoffmann 1959 a
mouse, Swiss Ha/CR/Mil, ♀, 20	0.05 and 0.1%, 3 times per week, 12 months, dermal, recrystallized	15 months	17/20 and 19/20 skin tumours; solvent control: no tumours	p no/val	Hoffmann and Wynder 1966



Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Swiss Ha/CR/Mil, ♀, 30	25 µg/animal, 10 times over 28 days; initiation experiment, dermal	6 months	24/30 papillomas; promotor only: 2/30	p no/val	Hoffmann and Wynder 1966
mouse, NMRI, ♀, 50	20 and 200 µg/animal, twice per week, 25 weeks, dermal	≤ 22.5 months	50/50 and 50/50 with skin tumours; vehicle control: 7/50	p no/val	Müller 1968
mouse, C3H/He, 20–30	(a) 0.00002% in n-dodecane/decalin, (b) 0.02% in decalin, 3 times per week, 50 weeks, dermal		(a) 21% malignant tumours; (b) 50% tumours (three orders of magnitude difference in dose)	p no/val	Bingham and Falk 1969
mouse, Swiss, Ha/ICR/Mil, ♀, 30	5 µg/animal, 10 times over 20 days; initiation experiment, dermal	24 weeks	19/29 animals with 67 skin tumours; control: 1/30	p no/val	Hoffmann et al. 1972
mouse, Swiss ICR, ♀, 20	0.05 and 0.1 mg/animal; 60 times, dermal	6 months	13 and 18 animals with skin tumours; no solvent control	p no/val	Masuda and Kagawa 1972

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Swiss, Ha/ICR/Mil, ♀, 20	5 µg/animal, 3 times per week, 72 weeks, dermal,	≤ 72 weeks	13/20 with 22 skin tumours; 4/20 with 4 carcinomas; solvent control: no tumours	p no/val	Hecht et al. 1974
mouse, Swiss ICR/Ha, ♀, 50	5 µg/animal, 3 times per week, lifetime, dermal,	440 days	16 animals with 26 tumours; control: no tumours	p no/val	Van Duuren and Goldschmidt 1976
mouse, Swiss Ha/ICR, ♀, 20	5 and 10 µg/animal, 3 times per week, 62 weeks, dermal	62 weeks	low dose: 10/20 with 19 skin tumours, 7/20 with 8 carcinomas, high dose: 18/20 with 70 skin tumours, 14/20 with 16 carcinomas; solvent control: no tumours	p yes/val	Hecht et al. 1976
mouse, Swiss, ♀, 40	100 µg/animal, twice per week, 30 weeks, dermal, 99.9%	70 weeks	79% skin tumour-bearing animals; solvent control: no tumours	p no/val	Cavalieri et al. 1977
mouse, NMRI, ♀, 40	1.7, 2.8, 4.6 µg/animal, twice per week, lifetime, dermal	≤ 2 years	24, 69 and 61% with local tumours (high rate of systemic tumours); control: no tumours	p yes/val	Habs et al. 1980

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Swiss Ha/ICR, ♀, 20	30 µg/animal, 10 times on alternate days; initiation experiment, dermal, > 99.5%	24 weeks	93% with tumours; vehicle control: no tumours	p no/val	LaVoie et al. 1981
mouse, Crl:CD1 (ICR)BR, ♀, 20	3 µg, 10 times, 20 days; initiation experiment, dermal	24 weeks	85% with tumours (papillomas/keratinizing lesions)	p yes/val	LaVoie et al. 1982
mouse, NMRI, ♀, 20	2 and 4 µg/animal, twice per week, lifetime, dermal, > 96%	648 and 528 days (mean)	45% (10% papillomas/35% carcinomas) and 85% (0%/85%) with skin tumours; control: no tumours	p yes/val	Habs et al. 1984
mouse, CH3/HeJ, ♂, 50	12.5 µg/animal, twice per week, 99 weeks, dermal, 99.5%	≤ 99 weeks	94% with malignant skin tumours; solvent control: no tumours; untreated control: no tumours	p no/val	Warshawsky and Barkley 1987
mouse, Sencar, ♀, 24	0.8 µmol/mouse, once; initiation experiment, dermal	24 weeks	enhanced incidence of skin tumours (80–90%)	p no/val	Cavalieri et al. 1988 b
mouse, Swiss, ♂, 12	1.2 mg/animal, 6 days/week, 19 weeks, dermal	≤ 27 weeks	multiple tumours; squamous-cell carcinomas	p no/ln	Shubik and Della Porta 1957

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, CD-1, ♀, 20	2.5 µg/animal, 10 times, 20 days; initiation experiment, dermal, > 99%	24 weeks	89% tumours; 5.5 skin tumours per animal; control: 5%	p yes/val	Rice et al. 1988 b
mouse, CD-1, ♀, 25	2.5 µg/animal, 10 times, 20 days; initiation experiment, dermal, > 99%	23 weeks	96% tumours; 3.4 skin tumours per animal; control: no tumours	p yes/val	Rice et al. 1990
mouse, Sencar, ♀, 23–24	8.4, 25.2 and 75.7 µg/animal, once; initiation experiment, dermal, chromatography purified	15 weeks	10/23, 17/24 and 21/23 with tumours; control: no tumours	p yes/val	Cavalieri et al. 1991
mouse, Sencar, ♀, 24	1, 5 and 25 µg/animal, once; initiation experiment, dermal, chromatography purified	27 weeks	1/24, 10/24 and 22/24 with tumours; control: no tumours	p yes/val	Cavalieri et al. 1991
mouse, Sencar, ♀, 24	25 µg/animal, once; experiment without promotion, dermal, chromatography purified	27 weeks	1/24 with tumours	p yes/val	Cavalieri et al. 1991

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, ICR/Harlan, ♀, 43–50	16, 32 or 64 µg/animal, once per week, 29 weeks, dermal, HPLC purified	≤ 35 weeks	1, 1.5 and 7.5 tumours/animal after 35 weeks	p no/val	Albert et al. 1991 a
mouse, Balb/c, ♂, 20	100 µg/animal, twice per week, 3 weeks to 5 months, dermal		tumours from week 15	p no/val	Andrews et al. 1991
mouse, C57Bl, ♂/♀, 40–50	0.09 mg/animal in tricaprylin, once, s.c.	≤ 22–28 months	16/21 sarcomas after 5 months	p yes/ls	Steiner 1955
mouse, XVII ♂/♀, 14/16	0.6 mg/animal, once/month, 3 months, s.c.	> 129/160 days	13/14 ♂ and 8/16 ♀ with local sarcomas	p no/ld	Lacassagne et al. 1958
mouse, XVII nc/Z, ♂/♀, 154/152	0.6 mg/animal, once/month, 3 months, s.c.	about 110/150 days	154/154 ♂ and 112/162 ♀ with local sarcomas	p no/ls	Lacassagne et al. 1963 a
mouse, NMRI, 20	0.1, 1 and 10 mg/animal, once/2 weeks, 20 weeks, s.c.	17, 7 and 6 months	all animals with sarcomas at injection site	p no/val	Müller 1968

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, NMRI, ♀, 90	25, 50, 100, 200 and 400 µg/animal, once, s.c.	≤ 16 months	25, 50, 55, 75 and 65% with tumours; solvent control: < 5%	p no/val	Pott et al. 1973
mouse, ♂/♀, 31–38	0.01 and 0.1 mg/animal (newborn), once, s.c.	30 weeks	16 and 64% with lung tumours; control: 13% with lung tumours	p no/val	Rippe and Pott 1989
mouse, CD-1, ♂/♀, 17/14	278 µg/animal (newborn) in DMSO on days 1, 8 and 15 after birth (total dose), i.p., > 99%	≤ 52 weeks	76% hepatic and 64% lung tumours; control: 6% hepatic tumours, no lung tumours	p yes/val	La Voie et al. 1987
mouse, Swiss Webster BLU:Ha(1CR), ♂/♀, 28/27	59.5 µg/animal (newborn) on days 1, 8 and 15 after birth (total dose), i.p., > 99%	26 weeks	46 ♂ and 70 ♀ with lung tumours; vehicle control: 14 ♂, 7 ♀	p yes/val	Busby et al. 1989
mouse, A, 10	10 mg/kg, once, i.v.	20 weeks	100% lung tumours; control: 21%	p no/val	Shimkin and Stoner 1975
mouse, NMRI, ♀, 19–22	0.05 and 0.15 mg/ animal, 20 times, i.tr.		27 and 42% with carcinomas in the respiratory tract; control: 9%	p no/val	Pott et al. 1978

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, ICR/Ha, ♀, 45; 60 control animals	1 mg/animal, once per week, 14 weeks, i.col., 99%	≤ 18 months	no colonic tumours; 73% lung tumours, 94% forestomach tumours, 7% subcutaneous sarcomas, 23% mammary tumours; control: 25% lung tumours, 20% forestomach tumours, 9% mammary tumours; no subcutaneous tumours or colonic tumours	p no/val	Anderson et al. 1983
mouse, C57Bl/6; ♀, 38; 45 control animals	1 mg/animal, once per week, 14 weeks, i.col.	≤ 18 months	no colonic tumours, 94% forestomach tumours, 16% peritoneal sarcomas; 28% lymphomas; control: 21% forestomach tumours, no sarcomas, lymphomas or colonic tumours	p yes/val	Anderson et al. 1983
rat, Sprague Dawley, ♀, 9	100 mg/kg b.w., once, oral	60 days	8/9 with mammary tumours; control: 8/164 in 310 days	p no/ln, ld	Huggins and Yang 1962
rat, LEW/Mai, ♀, 20	6.25 mg/animal, once per week, 8 times; 50 mg/animal, once, oral	90 weeks	67–77% with mammary tumours; control: 30%	p yes/val	McCormick et al. 1981

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, Wistar, ♀, 50	33, 100, 900 and 2700 µg/animal, once, s.c.	≤ 16 months	10, 15, 70 and 75% with tumours; solvent only: < 5%	p no/val	Pott et al. 1973
rat, Wistar, ♀, 37	5 mg/animal; once, in (a) bees' wax/tricaprylin 25/75 or (b) physiological saline, i.p.	2 years	(a) 89% abdominal tumours (mesotheliomas, sarcomas); (b) 50%: vehicle control: (a) 7%; (b) 3%	p no/val	Roller et al. 1992
rat, Wistar, ♀, 13–17	0.5, 1 or 2 mg/animal in infusion; once/2 weeks, 18 times, i.tr.	lifetime	7, 65 and 92% with lung tumours; control: no tumours	p yes/val	Davis et al. 1975
rat, Wistar, ♂/♀, 15/15	1 mg/animal, once per week, 15 times, i.tr.	lifetime (mean: 491/540 days)	3/13 ♂ and 3/14 ♀ with malignant lung tumours; (mean: 22.2%); vehicle control: 0%	p no/val	Ishinishi et al. 1976
rat, Wistar, ♀, 36–40	1 mg/animal, 20 times, i.tr.		19% with lung tumours; control: no tumours	p no/val	Pott et al. 1987
rat, Sprague Dawley, ♂/♀, 20/20	7 mg/kg b.w., every 14 days, 22 times (total dose: 154 mg/kg b.w.), i.tr.	≤ 781 days	19/20 ♂ and 18/20 ♀ with lung tumours; vehicle control: 0%	p no/val	Steinhoff et al. 1991



Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, Osborne Mendel, ♀, 35	0.1, 0.3 or 1 mg/animal, once, i.pul., 99.1%	111/77/54 weeks	4/35, 21/35 and 33/35 pulmonary carcinomas; 6/35, 2/35 and 0/35 pleomorphic sarcomas; control: no tumours	p yes/val	Deutsch- Wenzel et al. 1983 b
rat, Osborne Mendel, ♀, 35	0.05, 0.1 or 0.2 mg/ animal, once, i.pul.		11, 17 and 46% with tumours; control: no tumours	p yes/val	Grimmer et al. 1987
rat, Osborne Mendel, ♀, 35	0.03, 0.1 or 0.3 mg/ animal, once, i.pul., 99.6%	≤ 135 weeks	8, 6, 31.4 and 77.1% tumour incidence; control: no tumours	p yes/val	Wenzel- Hartung et al. 1990
rat, Fischer344/Du Crj, ♂, 14–15	50, 100 or 200 µg/ animal, i.pul.	≤ 100 weeks	0/10, 3/10 and 4/9 lung tumours; control: no tumours	p no/val	Horikawa et al. 1991
rat, 94	about 3 to 5 mg/animal, once, i.bro.	about 5 months	carcinoma incidence: 17%	p no/ld	Laskin et al. 1970

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, Sprague Dawley, ♀, 20	1 and 4 mg into 5th mammary gland, once, i.mam., > 99%	20 weeks	50 and 80% with mammary tumours; control: no tumours	p no/val	Cavalieri et al. 1988 a
rat, Sprague Dawley, ♀, 20	63 and 252 µg/gland, once, 8 glands, i.mam.	≤ 24 weeks	7/20 and 9/20 with mammary tumours; control: 1/18	p yes/val	Cavalieri et al. 1991
rat, Fischer 344, ♀, 20	0.5 and 1 mg, once (implanted into tracheal transplants), pellet implan- tation, recrystallized	28 months	12 and 65% with carcinomas in tracheal transplants	p yes/val	Topping et al. 1981
Syrian golden hamster, ♂/♀, 13	2.5 mg/animal and day, 4 days per week, ≤ 14 months, oral (diet)	≤ 14 months	9/13 with forestomach carcinomas; 2/13 with papillomas	p no/val	Chu and Malmgren 1965
Syrian golden hamster, ♂/♀, 15/15	4 drops of a 0.8% solution in mineral oil, once per week, 8 weeks including a 30-week interval, dermal, control by column chromatography	≤ 99/68 weeks	♂: 1 small nodular melanotic lesion; 2 malignant lymphomas; ♀: no tumours	q no/ln, ls	Shubik et al. 1960

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
Syrian golden hamster, ♂/♀, 5/5	6 drops of a 0.01% solution in acetone, twice per week, 40 weeks, dermal, control by column chromatography	≤ 70 weeks	no skin tumours	n no/ln, ls	Shubik et al. 1960
Syrian golden hamster, ♂, 5 or 28	20 mmol/l solution, painting twice per week, 5 or 20 weeks, dermal	≤ 44 weeks	10% buccal pouch carcinomas after 40 weeks; control: no tumours	p no/val	Solt et al. 1987
Syrian golden hamster, ♂, 10	4.5 h/day, 5 days/week, 9.8 mg/m <sup>3</sup> 16 weeks or 44.8 mg/m <sup>3</sup> 10 weeks, inhalation	lifetime	no tumours	n no/ls	Thyssen et al. 1980
Syrian golden hamster, ♂, 24	2.2, 9.5 or 46.5 mg/m <sup>3</sup> , 4.5 h/day in the first 10 weeks, thereafter 3 h/day, 109 weeks, inhalation	109 weeks	dose-dependent tumours in nasal cavity, pharynx, larynx and trachea; also in oesophagus and forestomach (papillomas, polyps, squamous-cell carcinomas); no lung tumours; larynx most affected with 0, 31% and 52% incidence, control: no tumours	p no/val	Thyssen et al. 1981
Syrian golden hamster, ♂/♀, 30/30	3 mg/animal, once per week, 15 weeks, (mixed with inert dust of haematite (ferric oxide) i.tr.	≤ 45/60 weeks	14/19 and 21/21 with tumours in respiratory tract; control: no tumours	p no/val	Saffiotti et al. 1968

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
Syrian golden hamster, ♂, 30	3 mg/animal, once per week, 14 weeks, i.tr.	≤ 74 weeks	all with bronchoalveolar metaplasia, 5/19 squamous-cell carcinomas, 3/19 adenomas, 1/19 tracheal tumours	p no/val	Crocker et al. 1970
Syrian golden hamster, ♂/♀, 30-50	0.25, 0.5, 1 or 2 mg/animal, once per week, 30 weeks, (mixed with inert dust of ferric oxide), i.tr.	lifetime	dose-related increase in respiratory tract tumours; control: no tumours	p no/val	Saffioti et al. 1972
Syrian golden hamster, ♂/♂, 25/25	0.9 mg/animal and week, 30 weeks, i.tr.	≤ 100 weeks	17% (8/46) tumours in respiratory tract; control: no tumours	p no/val	Henry et al. 1975
Syrian golden hamster	0.3 or 0.9 mg/animal, once per week, 20 weeks, i.tr.	≤ 2 years	17 and 68% with tumours	p no/ld	Pott et al. 1978
Syrian golden hamster, ♂, 29	0.125, 0.25, 0.5 or 1 mg/animal, once per week, lifetime, i.tr.	lifetime	31, 83, 66 and 31% tumours in respiratory tract; control: no tumours	p no/val	Ketkar et al. 1979
Syrian golden hamster, ♂, 30	5, 20 or 40 µg/animal, every 2 weeks, lifetime, i.tr.	lifetime	4/28, 5/27 and 7/28 with metaplasia in respiratory tract, malignant neoplasm and 1 adenoma in high-dose group; control: 1/29 or 3/30	q no/val	Künstler 1983
hamster, 97	3 to 5 mg, i.bro.		63/97 with lung cancer	p no/val	Laskin et al. 1970

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
Syrian golden hamster	about 0.83 mg/animal, 3 times per week, 1 year, tracheal instillation		tracheal papillomas and carcinomas	p no/val	Mohr 1971
Syrian golden hamster	bronchial implants (no other details)	150 days	> 90% with focal carcinomas	p no/ld	Benfield and Hammond 1992
dog	parenchymal implants (no other details)	> 8 months	first parenchymal carcinoma after 8 months; 7/12 dogs with tumours	p no/ld	Benfield and Hammond 1992
pig, German Edelland, ♂/♀, 1/1	4.8 mg/kg b.w., once, 6 months later 2.1 mg/kg b.w., once, i.m.	12 months	no sarcomas	n no/val	Kallistratos and Pfau 1971
pig, mini, ♂/♀, 1/1	6.3 mg/kg b.w., once, 6 months later 1.9 mg/kg b.w., once, i.m.	12 months	no sarcomas	n no/val	Kallistratos and Pfau 1971
cattle, German black/white, ♂/♀, 1/1	0.95 mg/kg b.w., once, 6 months later 0.75 mg/kg b.w., once, i.m.	29 months	no sarcomas	n no/val	Kallistratos and Pfau 1971

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
monkey, (a) <i>Saguinus oedipus</i> (cotton-top tamarin) (b) <i>Saguinus fuscicollis</i> (saddle-back tamarin), on the other side), ♂/♀, 1/1	10 mg/animal, once (co-administration with 10 mg 7,12-dime- thylbenzo[ <i>a</i> ]anthracene on the other side), s.c.	(a) > 18 months (b) ≤ 5 months	(a) 1/2 with local tumours (b) death within 5 weeks	q no/ln	Noyes 1969
monkey, <i>Galago crassicaudatus</i> (thick- tailed bush-baby) old world (no other details), 17	once (not specified), s.c. 30–90 mg/kg, multiple application (not specified), s.c.	≤ 18 years	fibrosarcomas  no tumours observed; survival rate: 9/17	p no/ld n no/ld	Adamson and Sieber 1983  Adamson and Sieber 1983
monkey, <i>Galago crassicaudata</i> , ♂/♀, 4/2	3–15 mg, once per week (with ferric oxide), up to 69 weeks, i. tr.	67–69 weeks	bronchoalveolar metaplasia; 2/3 squamous carcinomas arising from bronchi	p no/val	Crocker et al. 1970
<b>chrysene</b> mouse, 100	1% in 90% benzene, dermal	≤ 11 months	no tumours	n no/lis, ld	Kennaway 1924

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse	7.5% in liquid paraffin or oleic acid, 5 times/week, 78 or 50 weeks, dermal, purified	78 or 50 weeks	6 or 18 benign, 1 or 9 malignant tumours	q no/ld	Bottomley and Twort 1934
mouse, 100, 20	(a) 0.3% in benzene or (b) 0.3% in mouse fat, twice per week, lifetime, dermal, doubtful purity	≤ 704 days	(a) 1/100 papilloma and 1/100 epithelioma, (b) no tumours	n no/ls	Barry et al. 1935
mouse, 20	0.3%, twice per week, 440 days, dermal, "synthesized"	440 days	no tumours	n no/ld, ls	Barry et al. 1935
mouse, 50, 100	(a) 0.3% in benzene or (b) 7.5% in oleic acid, twice per week, lifetime, dermal	≤ 797 days	(a) 2/50 papillomas (b) no tumours	n no/ld, ls	Barry et al. 1935
mouse, 50	in benzene, twice per week, 276 days, dermal, "pure" (obtained synthetically and purified by chromatography, compa- rison of melting point, solubility and crystal form with data from other authors)	≤ 276 days	after 276 days, no tumours in 11 of 50 survivors	n no/ld, ls	Schürch and Winterstein 1935

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, CF1, ♂/♀, 10/10	40 µg/animal, twice per week, 31 weeks, dermal	31 weeks	1/15 carcinomas	n no/ls	Riegel et al. 1951
mouse, Swiss, ♀, 20	1%, 3 times per week, lifetime, dermal	≤ 12 months	9/20 papillomas, 8/20 carcinomas; no solvent control	p no/ls	Wynder and Hoffmann 1959 b
mouse, Swiss ICR/Ha, ♀, 20	1 mg, once; initiation experiment, dermal	63 weeks	16/20 papillomas, 2/20 carcinomas; with promotor only: 5/20, 1/20	p no/val	Van Duuren et al. 1966
mouse, CD-1, ♀, 30	1 mg/animal, once; initiation experiment, dermal, TLC purified	35 weeks	73% with papillomas; with promotor only: 3%	p no/val	Scribner 1973
mouse, C3H, ♂, 20	75 µg/animal in decalin, twice per week, 82 weeks, dermal	82 weeks	1/20 papillomas; solvent control: 2/13 papillomas	n no/ls	Horton and Christian 1974



Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, C3H, ♂, 20	75 µg/animal in decalin/dodecane 50/50, twice per week, 82 weeks, co-carcinogenicity experiment, dermal	82 weeks	5/19 papillomas, 12/19 carcinomas; solvent control: 2/13 papillomas	p no/val	Horton and Christian 1974
mouse, Swiss, Ha/ICR/Mil, ♀, 20	0.1 mg/animal on alternate days, 10 times; initiation experiment, dermal, > 99.9%	22 weeks	11/18 papillomas/carcinomas; chrysene only: 4/11 after 72 weeks; solvent control: no tumours	p no/val	Hecht et al. 1974
mouse, CD-1, ♀, 30	0.09, 0.29 and 0.91 mg/animal, once; initiation experiment, dermal	26 weeks	25, 43 and 52% papillomas; promotor only: 7%	p no/val	Levin et al. 1978
mouse, CD-1, ♀, 30	0.46 mg/animal, twice; initiation experiment, dermal, 95%	26 weeks	21/30 papillomas; promotor only: 1/30	p no/val	Wood et al. 1979
mouse, CD-1, ♀, 30	0.57 mg/animal, once; initiation experiment, dermal, 98%	27 weeks	80% papillomas; promotor only: 4%	p yes/val	Wood et al. 1980

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Sencar, ♀, 30	0.46 mg/animal, once; initiation experiment, dermal, > 95%	15 weeks	21/29 papillomas; promotor only: 3/30	p no/val	Slaga et al. 1980, 1981
mouse, CD-1, ♀, 30	0.09 and 0.274 mg/animal, once; initiation experiment, dermal	26 weeks	43%, 43% (or 39%) with skin papillomas; vehicle control: 10%	p yes/val	Chang et al. 1983
mouse, CD-1, ♀, 20	3.4, 11.4 and 34 µg/animal, 10 times, 20 days; initiation experiment, dermal, > 99%	24 weeks	25, 90 and 95% with tumours; 0.5, 3 and 4.5 skin tumours/animal; control: 20%	p yes/val	Rice et al. 1988 b
mouse, CD-1, ♀, 20	7.5 µg/animal, once; initiation experiment, dermal	21 weeks	10% with malignant skin tumours; solvent control: 10%	n yes/val	Amin et al. 1990
mouse, Sencar, ♂/♀, 16/16	365 µg/animal, once; initiation experiment, dermal	≤ 100 weeks	no skin tumours; solvent control: no tumours	n no/val	Bhatt and Coombs 1990
mouse, 50	2 mg/animal, once, s.c., purified	≤ 35 weeks	no tumours	n no/ld	Bottomley and Twort 1934

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Jackson A, 30	10 mg/animal, twice (4-month interval), s.c., purified	15 months	no tumours	n no/l's	Shear and Leister 1941
mouse, C57Bl, ♂/♀, 50	5 mg/animal in tricapylin, once, s.c., spectrometer control	≤ 22 months	4/39 sarcomas after 4 months; solvent control: 3/280	p no/val	Steiner and Falk 1951
mouse, C57Bl, ♂/♀, 40–50	5 mg/animal in tricapylin, once, s.c.	≤ 22–28 months	5/22 sarcomas after 5 months	p yes/l's	Steiner 1955
mouse, C57Bl	1 mg/animal in arachis oil, once per week, 10 weeks, s.c.	60–80 weeks	2/20 injection site tumours; control: no tumours	p no/val	Boyland and Sims 1967
mouse, Swiss, ♂/♀, 104	0.1 mg/animal (newborn) in poly- ethylene glycol on days 1, 2 and 3 after birth, s.c.	70–75 weeks	70 weeks: 13/27 ♂ liver, 1/27 ♂ and 1/21 ♀ lung tumours; vehicle control: 9/30 ♂ liver, 3/30 ♂ and 1/15 ♀ lung tumours	q no/val	Grover et al. 1975
mouse, 10	1 mg, weekly; later 2 mg at longer intervals, s.c.	350 days	no tumours; control: no tumours	n no/l'n, l's	Barry and Cook 1934

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, 50	2 mg/animal, once, i.p., purified	≤ 45 weeks	no tumours	n no/ld	Bottomley and Twort 1934
mouse, Swiss Webster BLU:Ha(ICR), ♂/♀, 100	total dose 0.32 mg/animal (newborn) in DMSO, days 1, 8 and 15 after birth, i.p., TLC control	38–42 weeks	5/24 ♂ and 2/11 ♀ pulmonary tumours; 6/24 ♂ liver tumours; 1/24 ♂ lymphosarcoma; control: 2/21 ♂ and 7/38 ♀ lung tumours;	q yes/val	Buening et al. 1979
mouse, Swiss Webster BLU:Ha(ICR), ♂/♀, 80	0.045, 0.09 and 0.18 mg/animal (newborn) in DMSO on days 1, 8 and 15 after birth, i.p., repurified, melting point 256°C	39–41 weeks	♂: 4/27 lung and 6/27 liver tumours; ♀: 1/11 lung and 0/11 liver tumours; vehicle control: no tumours	p yes/val	Chang et al. 1983
mouse, Swiss Webster BLU:Ha(ICR), 10	2 mg/animal (newborn), weekly, later 6 mg at longer intervals, s.c., > 98%	≤ 626 days	4/10 tumours; control: 2/10 sarcomas	p no/ln, ls	Barry and Cook 1934
rat, 10	1 mg/animal, weekly 103 weeks, s.c., purified	≤ 103 weeks	no tumours	n no/ln, ls	Boyland and Burrows 1935

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, Wistar, 5	5 mg/animal, 7 to 9 times, s.c.	10 months	no tumours	n no/ln	Pollia 1941
rat, Osborne Mendel, ♀, 35	1 and 3 mg/animal, once, i.pul., 99.6%	≤ 135 weeks	14.3% and 28.6% tumour incidence; control: no tumours	p no/val	Wenzel-Hartung et al. 1990
<b>cyclopenta[cd]pyrene</b>					
mouse, NMRI, ♀, 40	1.7, 6.8 and 27.2 µg/animal, twice per week, 112 weeks, dermal, > 96%	112 weeks	low dose: no tumours; high dose: 2/38 skin carcinomas, 1/38 sarcomas; control: no tumours	q yes/val	Habs et al. 1980
mouse, CD-1, ♀, 30	23, 91, 226, 566 µg/animal, once; initiation experiment, dermal, > 98%	27 weeks	10, 21, 30 and 37% papillomas; promotor only: 4%	p yes/val	Wood et al. 1980
mouse, Swiss, ♀, 30	45, 136 and 407 µg/animal, twice per week, 30 weeks, dermal, > 99.9%	57 weeks	low dose: 17; middle dose: 11; high dose: 7 skin tumours; control: no tumours	p no/val	Cavalieri et al. 1981 b

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, CD-1, ♀, 30	4.5, 14 and 41 µg/ animal, every other day for 10 days; initiation experiment, dermal, > 99.9%	44 weeks	low dose: 1/30; middle dose: 9/29; high dose: 6/29 papillomas; promotor only: 3/29	p no/val	Cavalieri et al. 1981 b
mouse, Sencar, ♀, 30	10, 100 and 200 µg/ animal, once; initiation experiment, dermal	26 weeks	low dose: 11%; middle dose: 39%; high dose: 57% papillomas; promotor only: 10%	p no/val	Raveh et al. 1982
mouse, Swiss Webster BLU:Ha(ICR), ♂/♀, 8-14	0.35, 0.7, 1.05, 1.4 and 1.75 mg/ani- mal (newborn) (total dose) in 3 ali- quots on days 1, 8 and 15 after birth, i.p., > 99%	26 weeks	62, 60, 56, 70, 86, 93, 77, 100, and 89, 100% ♂/♀ with lung tumours; vehicle control: 8.8%	p yes/val	Busby et al. 1988
rat, Sprague Dawley, ♀, 20	1.8 and 5.4 mg into 4th mammary gland, once, i.mam., > 99%	≤ 34 weeks	no mammary tumours; control: no tumours	n no/val	Cavalieri et al. 1988 b
<b>dibenz[<i>a,h</i>]anthracene</b> mouse, Swiss, ♂	1.5 mg/animal in PEG-400, once; initiation experiment, oral	30 weeks	21% forestomach papillomas; promotor only: 14%	q no/ld	Berenblum and Haran 1955

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, DBA/2, ♂/♀, 21/21, control: 25/10	0.8 mg/day and animal in olive oil, 8–9 months, drinking water	8–9 months	14/14 ♂ and 13/13 ♀ with pulmonary adenomas; 14/14 ♂ and 10/13 ♀ with alveolar carcinomas; control: 1 mouse with tumour	p no/val	Snell and Stewart 1962
mouse, Swiss Millerton, ♀, 20	0.001, 0.01 and 0.1%, 3 times per week, lifetime, dermal	≤ 21, 13 or 9 months	0.001%: 30% papillomas, 30% carcinomas; 0.01%: 95/90% papillomas/carcinomas; 0.1%: 90%/75% papillomas/carcinomas	p no/ls	Wynder and Hoffmann 1959 b
mouse, Swiss albino, DBA/2Jax ♀, ≤ 50	0.02 and 0.16 µg/animal, once; initiation experiment, dermal	32 weeks	33 and 38% with skin tumours; acetone control: 13%	p yes/val	Klein 1960
mouse, Swiss, ♀, 20	38 µg/animal, twice per week, 44 weeks, dermal, recrystallized by chromatography	≤ 60 weeks	80% with skin tumours; vehicle control: 4%	p no/val	Lijinsky and Saffioti 1965

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, IF/Bcr, ♂/♀, 30/30	♂: 0.3% solution (= 1.5 mg/animal), once per week, 18 weeks; ♀: 0.5% solution (= 1 mg/animal), 8 times, every 2 weeks, dermal	≤ 29/22 weeks	♂: 26% with papillomas after 20 weeks, 100% after 29 weeks; ♀: 100% with breast tumours after 22 weeks	p no/val	Johnson 1968
mouse, NMRI, ♀, 50	1 drop, 3 times per week, 112 weeks; total dose 37.8, 125 and 378 µg/ani- mal, dermal, > 99%	112 weeks	6%, 8% and 32% with skin tumours; control: 2-4%	p no/val	Platt et al. 1990
mouse, NMRI, ♀, 16	83.5 and 167 µg/animal, once; initiation experiment, dermal, > 99%	24 weeks	38 and 93% with skin tumours; vehicle control: no tumours	p no/val	Platt et al. 1990
mouse, 10	0.2 mg/animal, twice per week, 50 weeks, s.c./i.p. alternating	lifetime	3/10 with subcutaneous sarcomas	p no/ln, ls	Boyland and Burrows 1935
mouse, C57Bl, ♂/♀, 50	0.02 mg/animal in tricaprylin, once, s.c., spectrometer control	≤ 22 months	28/48 sarcomas after 4 months; solvent control: 3/280	p no/val	Steiner and Falk 1951



Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, C57Bl, ♂/♀, 40–50	0.02, 0.04 mg/animal in tricaprylin, once, s.c.	≤ 22–28 months	7/21 and 6/18 sarcomas after 6 and 5 months	p yes/ls	Steiner 1955
mouse, C57Bl, ♂/♀, 20/19	1 mg/animal, once per week, 10 weeks, s.c.	60–80 weeks	20/20 ♂ and 17/19 ♀ with sarcomas; p control: no sarcomas	p no/val	Boyland and Sims 1967
mouse, NMRI, ♀, 60	10, 30, 90, 270 and 810 µg/animal, once, s.c.	≤ 16 months	40, 35, 65, 75 and 90% with tumours	p no/val	Pott et al. 1973
mouse, B6, D2, ♂/♀, 30 (60)	0.15 and 0.3 mg/animal, once, s.c.	12 months	B6 mice: 16/30 and 14/30; D2 mice: 1/30 and 0/30 with fibrosarcomas	p no/val	Kouri et al. 1983
mouse, NMRI, ♀, 47–50	10, 30, 86 µg/animal, once, s.c., > 99%	112 weeks	52, 46 and 63% with fibrosarcomas; p control: 2–6%	p no/val	Platt et al. 1990
mouse, NMRI, ♂/♀, 40–50	11.1 and 111 µg/animal (newborn) once on day 2 after birth, s.c., > 99%	40 weeks	12/35 with pulmonary tumours; p control: 2/33 and 4/41	p no/val	Platt et al. 1990

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, A, 10	10 mg/kg b.w., once, i.v.	20 weeks	100% lung tumours; control: 21%	p no/val	Shimkin and Stoner 1975
rat, 2x10 (no other details)	2 mg/animal, weekly; later 6 mg at longer intervals, s.c.	≤ about 200 days	1/10 and 7/10 with tumours; control: 2/10	q no/ln, ls	Barry and Cook 1934
rat, 10 (groups 1–5); 18 (group 6)	1 mg/animal, twice per week, 50 weeks, s.c/i.p. alternating (groups 1–3); s.c. (groups 4–6)	lifetime	3–6/10 (groups 1–5) and 9/18 with subcutaneous sarcomas (group 6)	p no/ln, ls	Boyland and Burrows 1935
rat, Wistar, 5	5 mg/animal, 4–8 times, s.c.	10 months	2 with tumours after 8–9 months	p no/ln	Pollia 1941
rat, Osborne Mendel, ♀, 35	0.1 mg/animal, once, i.pul.	≤ 123 days	57.1% tumour incidences; control: no tumours	p no/val	Wenzel-Hartung et al. 1990
rat, Sprague Dawley, ♀, 20	1.1 and 4.5 mg into 5th mammary gland, once, i.mam., > 99%	20 weeks	no mammary tumours; control: no tumours	n no/val	Cavalieri et al. 1988 a

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
Syrian golden hamster, ♂/♀, 5/5	8 drops of a 0.2% solution, twice per week, 10 weeks, dermal, control by column chromatography	≤ 75 weeks	no tumours	n no/ln, ls	Shubik et al. 1960
Syrian golden hamster, ♂, 46	0.05 and 0.25 mg/animal once per week, 30 weeks, i.tr., purity more than 99% (thin layer chromatography)	≤ 110 weeks	0/46 and 2/46 respiratory tract tumours; control: no tumours	q yes/val	Sellakumar and Shubik 1974
monkey (no other details)	no other details		no tumours	n no/ls, ld	Adamson and Sieber 1983
<b>dibenzo[<i>a,e</i>]pyrene</b>					
mouse, Swiss albino Ha/ICR/Mil, ♀, 40/20	0.05 and 0.1% solution, 3 times per week, 12 months, dermal, recrystallized	15 months	16/40, 9/20 with papillomas and 9/40, 6/20 with epitheliomas; solvent control: none	p no/val	Hoffmann and Wynder 1966
mouse, Swiss albino Ha/ICR/Mil, ♀, 28	25 µg/animal, 10 times over 20 days; initiation experiment, dermal, recrystallized	6 months	10/28 papillomas; promotor only: 2/30	p no/val	Hoffmann and Wynder 1966

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Sencar, ♀, 21	242 µg/animal, once; initiation experiment, dermal, > 99%	26 weeks	24% papillomas; solvent control: 9%	p yes/val	Cavalieri et al. 1989
mouse, XVII nc/Z, ♂/♀, 21/14	0.6 mg/animal, once per month, 3 times, s.c.	≤ 142 days ♂ or 126 days ♀	18/21 ♂ and 14/14 ♀ focal sarcomas; no vehicle control	p no/val	Lacassagne et al. 1963 b
mouse, ♂/♀, 12/15	0.6 mg/animal, once s.c.	≤ 196 days ♂ or 220 days ♀	10/12 ♂ and 10/15 ♀ focal sarcomas; no vehicle control	p no/val	Lacassagne et al. 1963 b
rat, Sprague Dawley, ♀, 19	1.2 mg/gland, once, 8 glands, i.mam., > 99%	≤ 40 weeks	1/19 with mammary tumours; control: 0/21 or 2/20	n yes/val	Cavalieri et al. 1989
<b>dibenzo[a,h]pyrene</b>					
mouse, 74	1 drop of a 0.15% solution on alternate days, 55 or 86 times, dermal	4.5 months	50% with skin tumours	p no/ld	Kleinenberg 1939

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Swiss albino, Ha/ICR/Mil, ♀, 20	0.05 and 0.1% solution, 3 times per week, 12 months, dermal, recrystallized	11, 15 months	16/20, 15/20 with papillomas and 13/20, 15/20 with epitheliomas; solvent control: no tumours	p no/val	Hoffmann and Wynder 1966
mouse, Swiss albino, Ha/ICR/Mil, ♀, 29	25 µg/animal, 10 times over 20 days; initiation experiment, dermal, recrystallized	6 months	21/29 papillomas; promotor only: 2/30	p no/val	Hoffmann and Wynder 1966
mouse, Swiss, ♀, 40	120 µg/animal, twice per week, 30 weeks, dermal, 96.6%	70 weeks	90% tumour incidence; solvent control: no tumours	p no/val	Cavalieri et al. 1977
mouse, CD-1, ♀, 30	15.1, 60.5 and 181.4 µg/ animal, once; initiation experiment, dermal, pure	17 weeks	55, 79 and 72% with skin tumours; control: 0–10%	p yes/val	Chang et al. 1982
mouse, Sencar, ♀, 24	242 µg/animal, once; initiation experiment, dermal, > 99%	26 weeks	75% papillomas; solvent control: 9%	p yes/val	Cavalieri et al. 1989

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, XVII ♂/♀, 35/10	0.6 mg/animal, once per week, 3 months, s.c.	> 111/128 days (average latency)	34/35 ♂ and 1/10 ♀ with local sarcomas	p no/ls	Lacassagne et al. 1958
mouse, CD-1, ♀, 31	0.2 mg/animal, once; initiation experiment, s.c.	27 weeks	26/28 with tumours; solvent control: 2/32	p no/val	Sardella et al. 1981
mouse, Swiss Webster BLU:Ha(ICR), ♂/♀, 40	3.8, 7.6 and 15.1 µg on days 1, 8 and 15 after birth, i.p.	49–54 weeks	97% with pulmonary and 44% with hepatic tumours; control: pulmonary tumours 27%, no hepatic tumours	p yes/val	Chang et al. 1982
rat, Sprague Dawley, ♀, 20	1.2 mg/gland, once, 8 glands, i.mam., > 99%	≤ 40 weeks	19/20 with mammary tumours; control: 0/20 or 2/20	p yes/val	Cavalieri et al. 1989
<b>dibenz[<i>a,h</i>]pyrene</b>					
mouse, Swiss albino, Ha/ICR/Mil, ♀, 20	0.05 and 1% solution, 3 times per week, 12 months, dermal, recrystallized	11, 14 months	17/20, 18/20 with papillomas and 17/20, 18/20 with epithelomas; solvent control: no findings	p no/val	Hoffmann and Wynder 1966

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Swiss albino Ha/ICR/ Mil, 30	25 µg/animal, 10 times over 20 days; initiation experiment, dermal, recrystallized	6 months	18/30 papillomas, 1/30 epithelio- mas; promotor only: 2/30	p no/val	Hoffmann and Wynder 1966
mouse, Swiss ICR, ♀, 19–21	55, 200, 240, 350 and 700 µg/animal, given in 55, 40, 24, 7 and 7 applica- tions, dermal	6 months	20, 19, 21, 19 and 16 with skin tumours; no solvent control group	p no/val	Masuda and Kagawa 1972
mouse, Sencar, ♀, 24	242 µg/animal, once; initiation experiment, dermal, > 99%	26 weeks	92% papillomas; solvent control: 9%	p yes/val	Cavalieri et al. 1989
mouse, Sencar, ♀, 24	10, 30 and 90 µg/animal, once; initiation experiment, dermal, pure (melting point 161–162°C)	15 weeks	23/24, 22/24 and 24/24 with tumours; control: no tumours	p yes/val	Cavalieri et al. 1991
mouse, Sencar, ♀, 24	1.2, 6 and 30 µg/animal, once; initiation experiment, dermal, pure	7 weeks	22/24, 20/24 and 20/24 with tumours; 2 controls; no tumours	p yes/val	Cavalieri et al. 1991

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Sencar, ♀, 24	30 µg/animal, once; initiation experiment, without promotion, dermal, purified by chromatography	27 weeks	7/24 with tumours	p yes/val	Cavalieri et al. 1991
mouse, XV/II nc/ZE, ♂/♀, 12/12	0.6 mg/animal, once per month, 2 months (some animals, 3rd injection after 2 months), s.c.	≤ 7 months	animals with local sarcomas (average latency 120 days); control: no tumours	p no/val	Lacassagne et al. 1968
rat, Sprague Dawley, ♀, 9	1.2 mg/gland, once, 8 glands, i.mam., > 99%	≤ 40 weeks	9/9 with mammary tumours; control: 0/21 or 2/20	p yes/val	Cavalieri et al. 1989
rat, Sprague Dawley, ♀, 20	76 and 302 µg/gland, once, 8 glands, i.mam.	≤ 24 weeks	20/20 and 18/20 with mammary tumours; control: 1/18	p yes/val	Cavalieri et al. 1991



Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
<b>indeno[1,2,3-<i>cd</i>]pyrene</b>					
mouse, Swiss, Ha/ICR/Mil, ♀, 30	25 µg/animal, 10 times over 20 days; initiation experiment, dermal, recrystallized	6 months	5/30 papillomas; promotor: 2/30	q no/val	Hoffmann and Wynder 1966
mouse, Swiss albino, Ha/ICR/Mil, ♀, 20	0.05 and 1% solution, 3 times per week, 12 months, dermal, recrystallized	15 months	dioxane solvent: no tumours; acetone solvent: dose-related tumour increase	q no/val	Hoffmann and Wynder 1966
mouse, NMRI, ♀, 40	3.4, 5.6, 9.2 µg/animal, twice per week, lifetime, dermal, > 96%	≤ 2 years	3, 0, 0% with local tumours; control: no tumours	n yes/val	Habs et al. 1980
mouse, CrI:CD1 (ICR)BR, ♀, 25	100 µg/animal, 10 times over 20 days; initiation experiment, dermal	25 weeks	90% with skin tumours; vehicle control: < 5%	p yes/val	Rice et al. 1986
mouse, CD-1, ♀, 25	110 µg/animal, 10 times over 20 days; initiation experiment, dermal, > 99%	23 weeks	72% tumours, 2.1 skin tumours per animal; control: no tumours	p yes/val	Rice et al. 1990

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, XVII nc/Z, ♂/♀, 14/14	0.6 mg/animal, once per month, 3 months, s.c.	on average 265 days ♂, 145 days ♀	sarcomas: 10/14 ♂ and 1/14 ♀	p no/val	Lacassagne et al. 1963 a
Table mouse, CD-1, ♂/♀, 11/9	580 µg/animal in DMSO on days 1 and 8 after birth (total dose), i.p., > 99%	≤ 52 weeks	9% hepatic or 0% lung tumours; control: 6% hepatic and 0% lung tumours	n yes/val	LaVoie et al. 1987
rat, Osborne Mendel, ♀, 35	0.16, 0.83 and 4.15 mg/animal, once, i.pul., 99.4%	116/109/92 weeks	3/35, 8/35 and 21/35 with lung tumours; control: no tumours	p yes/val	Deutsch-Wenzel et al. 1983 b
<b>naphthalene</b> mouse	several times per week, ≤ 11 months, dermal	≤ 11 months	no skin tumours	n no/ld	Kennaway 1930
mouse, SW inbred, 25; control: 21	0.5% in benzene, 6 times per week for 3 weeks, then twice per week, lifetime, dermal, highly purified	lifetime	4/25 with lymphatic leukaemia; 1/25 lymphosarcoma of thymus; 4/25 with benign tumours; benzene only: 2/21 with sarcomas; 1/21 with lung adenoma	q no/ld, ln	Knake 1956
mouse, Swiss ICR/Ha, ♀, 30	0.25 mg/animal plus 3 µg benzo[a]pyrene, 3 times per week, 78 weeks: co-carcinogenicity test, dermal	78 weeks	5/30 lymphomas; inhibitory effect on skin tumours; naphthalene only: no skin tumours	q no/val	Schmeltz et al. 1978

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, A/I, ♀, 30	0.05 and 0.15 mg/l, 6 h/day, 5 days per week for 6 months, inhalation, 98–99%	6 months	29 and 30% with pulmonary tumours; control: 21% (increase in treated animals not significant)	q yes/val	Adkins et al. 1986
mouse, B6C3F <sub>1</sub> , ♂/♀, 75 (150)	0.053 and 0.16 mg/l, 6 h/day, 5 days per week for 103 weeks, inhalation, > 99%	103 weeks	significantly increased pulmonary alveolar and bronchiolar adenomas in females; no cataracts	q yes/val	Abdo et al. 1992; NTP 1992
mouse, 23	once (dose not specified) bladder implant	7 months	1/23 bladder carcinomas after 1 month; "inert" substance: higher rate of bladder carcinoma	–	Boyland and Sims 1964
rat, BDI/BDI II, inbred, 28	10–20 mg/animal, 6 times per week, 70 weeks, oral (diet)	lifetime	no tumours	n no/ls	Schmähl 1955
rat, BDI/BDI II, inbred, 10	20 mg/animal, once per week, 40 weeks, s.c.	lifetime	no tumours	n no/ln	Schmähl 1955
rat, "white", 38	0.5 g/kg b.w., twice per month, 3.5 months, s.c., 90%	lifetime	5 malignant tumours (4/38 lymphosarcomas, 1/38 uterine sarcoma); 1 benign tumour; vehicle control: 1/38 lymphosarcoma and 1 benign tumour	q no/val	Knake 1956

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
rat, BDI/BDI II, inbred, 10	20 mg/animal, once per week, 40 weeks, i.p.	lifetime	no tumours	n no/ln	Schmähl 1955
rat, F344/N, ♂/♀, 49	0, 10, 30, 60 ml/m <sup>3</sup> , 6 h/d on 5 days per week, inhalation	105 weeks	nose: neoplasms with a positive trend; adenomas with a positive trend	p	NTP 2000
<b>phenanthrene</b>					
mouse, 100	dissolved in 90% benzene, dermal	9 months	no tumours	n no/ls, ld	Kennaway 1924
mouse, white, ♂, 100	3 drops of an about 3% solution, once per week, 1 year; initiation experiment, dermal, "pure"	≤ 1 year	after 12 months 6/100 survived with a total of 1 tumour; 0.16 tumour/animal; promotor only: 0.08 tumour/animal	n no/val	Graffi et al. 1953
mouse, "S", 20	54 mg/animal, 3 times per week, total: 10 times; initiation experiment, dermal	24 weeks	5/20 survivors with 12 papillomas; promotor only: 4/19 survivors/ 4 papillomas	q yes/val	Salaman and Roe 1956

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, "stock albino", ♂/♀, 10/10	0.3 mg, 4 times on days 0, 2, 6, 8; initiation experiment, dermal, highly purified	24 weeks	4/19 papillomas; solvent control: 2/20	q yes/val	Roe 1962
mouse, CD-1, ♀, 30	1.8 mg/animal, once; initiation experiment, dermal, TLC purified	35 weeks	40% with papillomas; promotor only: 3%	p no/val	Scribner 1973
mouse, CD-1, ♀, 30	1.8 mg/animal, once; initiation experiment, dermal, > 98%	36 weeks	5/30 papillomas; solvent control: 2/30	q no/val	Wood et al. 1979
mouse, Swiss Ha/JCR, ♀, 20	100 µg/animal, 10 times over 20 days; initiation experiment, dermal	24 weeks	no skin tumours observed; vehicle control: no tumours	n no/val	LaVoie et al. 1981
mouse, C57Bl	5 mg/animal in tricaprylin, once, s.c.	≤ 22–28 months	no sarcomas after 8 months	n yes/ls	Steiner 1955
mouse, "stock albino"	0.3 mg, 5 times on days 0, 2, 4, 6, 8; initiation experiment, s.c.	24 weeks	3/17 papillomas; solvent control: 2/20	n yes/val	Roe 1962

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, "stock albino", newborn	40 µg/animal, once; administered to neonatal mice, s.c.	≤ 62 weeks	3/49 lung adenomas; control: 8/34 and 5/38	n yes/val	Grant and Roe 1963
mouse, Swiss Webster BLU:Ha(ICR), newborn	35, 70 and 140 µg/animal in DMSO on days 1, 8 and 15 after birth, i.p., > 98%	38–42 weeks	6/35 pulmonary adenomas; DMSO: 9/59	n yes/val	Buening et al. 1979
rat, Sprague Dawley	200 mg/rat, once; oral	60 days	no mammary tumours on day 60; control: 8/164 after 310 days	n no/ln, ld	Huggins and Yang 1962
rat, Osborne Mendel	1, 3 and 10 mg/animal, once, i.pul., 99.9%	≤ 135 weeks	no tumours; control: no tumours	n no/val	Wenzel-Hartung et al. 1990
<b>pyrene</b>					
mouse, 2 × 20	1% in benzene, twice per week, lifetime, dermal	≤ 717 days	1/20 and 1/20 papillomas	n no/ls	Barry et al. 1935
mouse, 40	0.3% in benzene, twice per week, ≤ 680 days, dermal	≤ 680 days	no skin lesions	n no/ls	Badger et al. 1940

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, white, ♂, 150	3 drops of a 0.3% solution, once per week, 1 year; initiation experiment, dermal, "pure"	≤ 1 year	after 6 months 18/150 survived with a total of 1 tumour; 0.06 tumour/animal; promotor only: 0.08 tumour/animal	n no/val	Graffi et al. 1953
mouse, "S"	25 mg/animal, 3 times per week, total: 10 times; initiation experiment, dermal	24 weeks	6/20 mice with 9 papillomas; promotor only: 4/19 mice with 4 papillomas	q yes/val	Salaman and Roe 1956
mouse, Swiss Millerton, ♀, 5	10%, 3 times per week, lifetime, dermal	≤ 18 months	no skin tumours	n no/ls, ln	Wynder and Hoffmann 1959 b
mouse, CD-1, ♀, 30	2 mg/animal, once; initiation experiment, dermal, TLC purified	35 weeks	17% with papillomas; promotor only: 3%	q no/val	Scribner 1973
mouse, C3H, ♂, 20	250 µg/animal in decalin, twice per week, 82 weeks, dermal, highly purified	82 weeks	3/13 papillomas; solvent control: 2/13	q no/val	Horton and Christian 1974

Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Swiss CR/Ha, ♀, 50	12 or 40 µg/animal, 3 times per week, 368 or 440 days, dermal, recrystallized	≤ 440 days	no skin tumours observed; control: no tumours	n no/val	Van Duuren and Gold- schmidt 1976
mouse, Swiss ICR/Ha, ♀, 50	4 and 12 µg/animal plus 5 µg benzo[a]pyrene, 3 times per week, 33 weeks; co-carcinogenicity test, dermal, highly purified	33 weeks	high dose: 13/50 papillomas, 5/50 carcinomas; benzo[a]pyrene only: 6/50 papillomas; pyrene only: no tumours	n no/val	Goldschmidt et al. 1973
mouse, Swiss ICR/Ha, ♀, 50	4, 12 and 40 µg/animal plus 5 µg benzo[a]pyrene, 3 times per week, 368/368/440 days, co-carcinogenicity test, dermal, recrystallized	368 or 440 days	12/26/35 mice with papillomas, 6/20/26 with squamous cell carcino- mas; positive control: 15/11 tumours; solvent control: no tumours	p no/val	Van Duuren and Gold- schmidt 1976
mouse, CD-1, ♀, 30	20.2 and 80.9 µg/animal, once; initiation experiment, dermal, > 98%	27 weeks	14 and 10% with tumours; vehicle control: 10%	n yes/val	Wood et al. 1980



Table A12 (Continued)

Species, strain, No. per group	Exposure, type of administration, substance purity	Study duration	Incidence and type of tumour	Result Stat. method/ validity	References
mouse, Jackson A, ♂/♀, 30	10 mg/animal, twice at a 4-month interval, s.c., crystals	≤ 18 months	no malignant tumours	n no/ls	Shear and Leiter 1941
mouse, Swiss Webster BLU:Ha(ICR), ♂/♀, 23–28	86.1 and 1750 µg/animal (total dose) in 3 aliquots on days 1, 8 and 15 after birth, i.p., recrystallized, HPLC	26 weeks	17.4 and 7.12% ♂/♀ with lung tumours; vehicle control: 14.7% ♂/♀	n no/val	Busby et al. 1989
Syrian golden hamster, ♂, 48	3 mg/animal, once per week, 30 weeks, i.tr., purity more than 99% (thin layer chromatography)	≤ 110 weeks	1/48 tumours of the trachea, 2/48 malignant lymphomas; control: 0/82 and 2/82	n yes/val	Sellakumar and Shubik 1974

i.bro.: intrabronchial injection; i.col.: administration into the colon; i.m.: intramuscular injection; i.mam.: administration into the mammary gland; i.p.: intraperitoneal injection; i.pul.: intrapulmonary injection; i.tr.: intratracheal injection; i.v.: intravenous injection; s.c.: subcutaneous injection

Results: p: positive; n: negative; q: questionable; statistical evaluation: yes or no; val: valid; ld: limited documentation; ln: limited number of animals; ls: limited study design

\* According to Luch and Jacob ("Dibenzo[a]lpyren, ein polycyklischer aromatischer Kohlenwasserstoff mit außergewöhnlichen biologischen Wirkungen" (Dibenzo[a]lpyrene a polycyclic hydrocarbon with unusual biological effects), chapter of DFG 2004), all data published until 1966 are questionable and without scientific relevance because of the synthesis route used earlier.

## References

- Abdo KM, Eustis SL, McDonald M, Jokinen MP, Adkins B, Haseman JK (1992) Naphthalene: A respiratory tract toxicant and carcinogen for mice. *Inhal Toxicol* 4: 393–409
- Adamson RH, Sieber SM (1983) Chemical carcinogenesis studies in nonhuman primates. In: Langenbach R, Nesnow S, Rice JM (Eds) *Organ and Species Specificity in Chemical Carcinogenesis* Plenum Press, New York, 129–156
- Adkins B Jr., van Stee EW, Simmons JE, Eustis SL (1986) Oncogenic response of strain A/J mice to inhaled chemicals. *J Toxicol Environ Health* 17: 311–322
- Adler ID, Ingwersen I (1989) Evaluation of chromosomal aberration in bone marrow of 1C3F1 mice. *Mutat Res* 224: 343–345
- Agrelo C, Arnos H (1981) DNA repair in human fibroblasts. In: de Serres FJ, Ashby J (Eds) *Evaluation of Short-Term Tests for Carcinogens, Report of the International Collaborative Programme*, Elsevier North Holland, New York, 528–532
- Aitio A (1974) Different elimination and effect on mixed function oxidase of 20-methyl-cholanthrene after intragastric and intraperitoneal administration. *Res Commun Chem Pathol Pharmacol* 9: 701–710
- Albert RE, Miller ML, Cody TE, Andringa A, Shukla R, Baxter CS (1991 a) Benzo[a]pyrene-induced skin damage and tumor promotion in the mouse. *Carcinogenesis* 12: 1273–1280
- Albert RE, Miller ML, Cody TE, Barkley W, Shukla R (1991 b) Cell kinetics and benzo[a]pyrene DNA adducts in mouse skin tumorigenesis. *Prog Clin Biol Res* 369: 115–122
- Alexandrie AK, Warholm M, Carstensen U, Axmon A, Hagmar L, Levin LO, Östman C, Rannug A (2000) CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure. *Carcinogenesis* 21: 669–676
- Alfheim I, Ramdahl T (1984) Contribution of wood combustion to indoor air pollution as measured by mutagenicity in *Salmonella* and polycyclic aromatic hydrocarbon concentration. *Environ Mutagen* 6: 121–130
- Alzieu P, Cassand P, Colin C, Grolier P, Narbonne JF (1987) Effect of vitamin A, C and glutathione on the mutagenicity of benzo[a]pyrene mediated by S9 from vitamin A-deficient rats. *Mutat Res* 192: 227–231
- Amin S, Huie K, Hecht SS (1985) Mutagenicity and tumor-initiating activity of methylated benzo[b]fluoranthene. *Carcinogenesis* 6: 1023–1025
- Amin S, Hecht SS, DiRaddo P, Harvey RG (1990) Comparative tumor initiating activities of cyclopentano and methyl derivatives of 5-methylchrysene and chrysene. *Cancer Lett* 51: 17–20
- Amin S, Weyand EH, Huie K, Boger E, Neuber E, Hecht SS, LaVoie EJ (1991) Effects of fluorine substitution on benzo[b]fluoranthene tumorigenicity and DNA adduct formation in mouse skin. In: Cooke M, Loenig K, Merritt J (Eds) *Polynuclear Aromatic Hydrocarbons: Measurements, Means and Metabolism*, Battelle Press, Columbus, OH, USA, 25–35
- Andersen O, Nashed N, Styles JA (1988) The rat peritoneal cell transformation test. In: Ashby J, de Serres FJ, Shelby MD, Margolin BH, Ishidate M Jr., Becking GC (Eds) *Evaluation of Short-Term Tests for Carcinogens, Report of the International Programme on Chemical Safety's Collaborative Study on in vivo Assays, Vol 2*, Cambridge University Press, 288–292
- Anderson LM, Priest LJ, Deschner EE, Budinger JM (1983) Carcinogenic effects of intracolonic benzo[a]pyrene in  $\beta$ -naphthoflavone-induced mice. *Cancer Lett* 20: 117–123
- Andjelkovich DA, Mathew RM, Richardson RB, Levine RJ (1990) Mortality of iron foundry workers. I. Overall findings. *J Occup Med* 32: 529–549

- Andjelkovich DA, Mathew RM, Yu R, Richardson RB, Levine RJ (1992) Mortality of iron foundry workers. II. Analysis by work area. *J Occup Med* 34: 391–401
- Andjelkovich DA, Shy CM, Brown HM, Janszen DB, Levine RJ, Richardson RB (1994) Mortality of iron foundry workers. III. Lung cancer case control study. *J Occup Med* 36: 1301–1309
- Andrews AW, Thibault LH, Lijinsky W (1978) The relationship between carcinogenicity and mutagenicity of some polynuclear hydrocarbons. *Mutat Res* 51: 311–318
- Andrews FJ, Halliday GM, Muller HK (1991) A role for prostaglandins in the suppression of cutaneous cellular immunity and tumor development in benzo[a]pyrene – but not in dimethylbenz[a]anthracene-treated mice. *Clin Exp Immunol* 85: 9–13
- Andrianova MM (1971) Transplacental action of 3-methylcholanthrene and benzo[a]pyrene on four generations of mice. *Bull Exp Biol Med (GUS)* 71: 677–680
- Angerer J, Schaller K-H, Lehnert G (1992) Bio-Monitoring von beruflichen Belastungen gegenüber polycyclischen aromatischen Kohlenwasserstoffen – Hochdruckflüssigkeitschromatographische Bestimmung des Metaboliten 1-Hydroxypyren im Harn (Biomonitoring of occupational exposure to polycyclic aromatic hydrocarbons – high-performance liquid chromatographic determination of the metabolite 1-hydroxypyrene in urine) (German). 4th International Colloquium in Strasbourg, Oct. 22–23 1992, lecture
- Angerer J, Mannschreck C, Gündel J (1997 a) Occupational exposure to polycyclic aromatic hydrocarbons in a graphite-electrode producing plant: biological monitoring of 1-hydroxypyrene and monohydroxylated metabolites of phenanthrene. *Int Arch Occup Environ Health* 69: 323–331
- Angerer J, Mannschreck C, Gündel J (1997 b) Biological monitoring and biochemical effect monitoring of exposure to polycyclic aromatic hydrocarbons. *Int Arch Occup Environ Health* 70: 365–377
- Anziulewicz JA, Dick HJ, Chiarulli EE (1959) Transplacental naphthalene poisoning. *Am J Obstet Gynecol* 9: 519–522
- Arand M, Oesch F (2002) Mammalian xenobiotic epoxide hydrolases. In: Ioannides C (Ed) *Handbook of Enzyme Systems that Metabolise Drugs and Other Xenobiotics*, John Wiley & Sons, Sussex, 459–483
- Arcos JC, Argus MF, Wolf G (1968) *Chemical Induction of Cancer. Structural Bases and Biological Mechanisms*, 2nd edn, Vol 4, Academic Press, New York
- Arcos JC, Woo Y-T, Lai LY (1988) Database on binary combinations of effects of chemical carcinogens. *J Environ Sci Health C6*: 1–150
- Ariese F, Verkaik M, Hoornweg GP, van de Nesse RJ, Jukema-Leenstra SR, Hofstra JW, Gooijer C, Veithorst NH (1994) Trace analysis of 3-hydroxybenzo[a]pyrene in urine for the biomonitoring of human exposure to polycyclic aromatic hydrocarbons. *J Anal Toxicol* 18: 195–204
- Armstrong B, Tremblay C, Baris D, Theriault G (1994) Lung cancer mortality and polynuclear aromatic hydrocarbons: a case-cohort study of aluminium production workers in Arvida, Quebec. *Canada Am J Epidemiol* 139: 250–262
- Armstrong B, Hutchinson E, Fletcher T (2003) *Cancer risk following exposure to polycyclic aromatic hydrocarbons (PAHs): a meta-analysis*. London School of Hygiene and Tropical Medicine for the Health and Safety Executive (ISBN 0 71762604)
- Armstrong B, Hutchinson E, Unwin J, Fletcher T (2004) Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environ Health Perspect* 112: 970–978
- Ashby J, Basketter DA, Paton D, Kimber I (1995) Structure activity relationships in skin sensitization using the murine local lymph node assay. *Toxicology* 103: 177–194

- Atamna H, Cheung I, Ames BN (2000) A method for detecting abasic sites in living cells: age-dependent changes in base excision repair. *Proc Natl Acad Sci USA* 97: 686–691
- Autrup JL, Autrup H (1986) Metabolism of tobacco-specific carcinogens in cultured rat buccal mucosa epithelial cells. *Acta Pharmacol Toxicol* 59: 339–344
- Autrup H (2000) Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response. *Mutat Res* 464: 65–76
- Awogi T, Sato T (1989) Micronucleus test with benzo[a]pyrene using a single peroral administration and intraperitoneal injection in males of the MS/Ae and CD1-1 mouse strains. *Mutat Res* 223: 353–356
- Babson JR, Russo-Rodriguez SE, Wattley RV, Bergstein PL, Rastetter WH, Liber HL, Andon BM, Thilly WG, Wogan GN (1986) Microsomal activation of fluoranthene to mutagenic metabolites. *Toxicol Appl Pharmacol* 85: 355–366
- Backer JM, Weinstein IB (1982) Interaction of benzo[a]pyrene and its dihydrodiol-epoxide derivative with nuclear and mitochondrial DNA in C3H10T1/2 cell cultures. *Cancer Res* 42: 2764–2769
- Badger GM, Cook JW, Hewett CL, Kennaway EL, Kennaway MM, Martin RH, Robinson AM (1940) The production of cancer by pure hydrocarbons. Part V. *Proc R Soc Med* 129: 439–474
- Baird WM, Hooven LA, Mahadevan B (2005) Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. *Environ Mol Mutagen* 45: 106–114
- Band PR, Le ND, MacArthur AC, Fang R, Gallagher RP (2005) Identification of occupational cancer risks in British Columbia: a population-based case-control study of 1129 cases of bladder cancer. *J Occup Environ Med* 47: 854–858
- Baker RSU, Bonin AM, Stupans I, Holder GM (1980) Comparison of rat and guinea pig as sources of the S9 fraction in the *Salmonella*/mammalian microsome mutagenicity test. *Mutat Res* 71: 43–52
- Bakhiya N, Stephani M, Bahn A, Ugele B, Seidel A, Burckhardt G, Glatt G (2006) Uptake of chemically reactive, DNA-damaging sulphuric acid esters into renal cells by human organic anion transporters. *J Am Soc Nephrol* 17: 1414–1421
- Barry G, Cook CW (1934) A comparison of the action of some polycyclic aromatic hydrocarbons in producing tumours of connective tissue. *Am J Cancer* 20: 58–69
- Barry G, Cook JW, Haslewood AD, Hewett CL, Hieger I, Kennaway EL (1935) The production of cancer by pure hydrocarbons. Part III. *Proc R Soc Med* 117: 318–351
- Bartsch H, Malaveille C, Camus AM, Martel-Planche G, Brun G, Hautefeuille A, Sabadic N, Barbin A, Kuroki T, Drevon C, Piccoli C, Montesano R (1980) Validation and comparative studies on 180 chemicals with *S. typhimurium* strains and V79 Chinese hamster cells in the presence of various metabolizing systems. *Mutat Res* 76: 1–50
- Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K (2000) Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol Biomarkers Prev* 9: 3–28
- Basler A, Herbold B, Péter S, Röhrborn G (1977) Mutagenicity of polycyclic hydrocarbons. II. Monitoring genetic hazards of chrysene in vitro and in vivo. *Mutat Res* 48: 249–254
- Batiste-Alentorn M, Xamena N, Creus A, Marcos R (1991) Genotoxicity studies with the unstable zeste-white (UZ) system of *Drosophila melanogaster*. Results with ten carcinogenic compounds. *Environ Mol Mutagen* 18: 120–125
- Bayer U (1978) *In vivo* induction of sister chromatid exchanges by three polyaromatic hydrocarbons. *Carcinogenesis* 3: 423–428

- Beach AC, Gupta RC (1991) Analysis of cyclopenta(CP)-fused and 'pseudo-CP' polycyclic aromatic hydrocarbon (PAH)-DNA adducts by  $^{32}\text{P}$ -postlabelling (Abstract). *Proc Am Assoc Cancer Res* 32: 98
- Beach AC, Gupta RC (1994) DNA adducts of the ubiquitous contaminant cyclopenta[cd]pyrene. *Carcinogenesis* 15: 1065–1072
- Becher G, Bjørseth A (1983) Determination of exposure to polycyclic aromatic hydrocarbons by analysis of human urine. *Cancer Lett* 17: 301–311
- Becher H, Ramsoth H, Ahrens W, Risch A, Schmezer P, Dietz A (2005) Occupation, exposure to polycyclic aromatic hydrocarbons and laryngeal cancer risk. *Int J Cancer* 116: 451–457
- Bell CMJ, Jenkinson CM, Murrells TJ, Skeet RG, Everall JD (1987) Aetiological factors in cutaneous malignant melanoma seen at UK skin clinic. *J Epidemiol Comm Health* 41: 306–311
- Benfield JR, Hammond WG (1992) Bronchial and pulmonary carcinogenesis at focal sites in dogs and hamsters. *Cancer Res* 52: 2687–2693
- Bentsen-Farmen RK, Botnen IV, Notø H, Jacob J, Vrebø S (1999) Detection of polycyclic aromatic hydrocarbon metabolites by high-pressure liquid chromatography after purification on immunoaffinity columns in urine from occupationally exposed workers. *Int Arch Occup Environ Health* 72: 161–168
- Berenblum I, Haran H (1955) The influence of croton oil and of polyethylene glycol-400 on carcinogenesis in the forestomach of the mouse. *Cancer Res* 15: 510–516
- Berrino F, Richiardi L, Boffetta P, Esteve J, Belletti I, Raymond L, Troschel L, Pisani P, Zubiri L, Asuncion N, Guberan E, Tuyns A, Terracini B, Merletti F, Milan JEM, and Working Group (2003) Occupation and larynx and hypopharynx cancer: a job-exposure matrix approach in an international case-control study in France, Italy, Spain and Switzerland. *Cancer Causes Control* 14: 213–223
- BGFA (Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin) (2005) Abschlussbericht zum Forschungsvorhaben des HVBG: PAK-Belastung am Arbeitsplatz – Reduzierung hoher innerer Belastung durch betriebliche Maßnahmen anhand von biochemischem Effekt-Monitoring (Final report on the HVBG research project: PAH exposure at the workplace – reduction of high internal exposure by occupational measures via biochemical effect monitoring) (German), BGFA, Bochum
- Bhatia AL, Tausch H, Stehlik G (1987) Mutagenicity of chlorinated polycyclic aromatic compounds. *Ecotoxicol Environ Saf* 14: 48–55
- Bhatt TS, Coombs MM (1990) The carcinogenicity of cyclopenta[a]phenanthrene and chrysene derivatives in the Sencar mouse. *Polycyclic Aromat Compd* 1: 51–58
- Bingham E, Falk HL (1969) Environmental carcinogens. The modifying effect of cocarcinogens on the threshold response. *Arch Environ Health* 19: 779–783
- Binkova B, Sram RJ (2004) The genotoxic effect of carcinogenic PAHs, their artificial and environmental mixtures (EOM) on human diploid lung fibroblasts. *Mutat Res* 547: 109–121
- Bjørseth A, Bjørseth O, Fjeldstad PE (1978 a) Polycyclic aromatic hydrocarbons in the work atmosphere. I. Determination in an aluminium reduction plant. *Scand J Work Environ Health* 4: 212–223
- Bjørseth A, Bjørseth O, Fjeldstad PE (1978 b) Polycyclic aromatic hydrocarbons in the work atmosphere. II. Determination in a coke plant. *Scand J Work Environ Health* 4: 212–223
- Blome H (1981) Messungen polycyclischer aromatischer Kohlenwasserstoffe an Arbeitsplätzen – Beurteilung der Ergebnisse (Measurement of polycyclic aromatic hydrocarbons at workplaces – assessment of the results) (German). *Staub Reinhalt Luft* 41: 225–229
- Blome H (1983) Polyzyklische aromatische Kohlenwasserstoffe (PAH) am Arbeitsplatz (Polycyclic aromatic hydrocarbons (PAH) at the workplace) (German), BIA Report 3/83

- BMA (Bundesministerium für Arbeit und Soziales) (1989) 23rd TRK-Wert für Benzo[a]pyren (Technical exposure limit (TRK value) for benzo[a]pyrene) (German). *Bundesarbeitsblatt* 10: 58–61
- BMA (1998) Bekanntmachung einer Empfehlung des Ärztlichen Sachverständigenbeirats, Sektion "Berufskrankheiten": "Lungenkrebs durch polyzyklische aromatische Kohlenwasserstoffe bei Nachweis der Einwirkung einer kumulativen Dosis von mindestens 100 Benzo[a]pyren-Jahren [ $(\mu\text{g}/\text{m}^3) \times \text{Jahre}$ ]" (Announcement of a recommendation by the Medical Experts Forum, "Occupational Diseases" Division: "Lung cancer caused by polycyclic aromatic hydrocarbons if there is evidence of an impact of a cumulative dose of at least 100 benzo[a]pyrene years [ $(\mu\text{g}/\text{m}^3) \times \text{years}$ ]" (German). *Bundesarbeitsblatt* 4: 80–87
- Bock FG, Mund R (1958) A survey of compounds for activity in suppression of mouse subacous glands. *Cancer Res* 18: 887–892
- Bock FG, King DW (1959) A study of the sensitivity of the mouse forestomach toward certain polycyclic hydrocarbons. *J Natl Cancer Inst* 23: 833–838
- Boffetta P, Jourenkova N, Gustavsson P (1997) Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Causes Control* 8: 444–472
- Boffetta P, Burstyn I, Partanen T, Kromhout H, Svane O, Langard S, Jarvholm B, Frentzel-Beyme R, Kauppinen T, Stucker I, Shaham J, Heederik D, Ahrens W, Bergdahl IA, Cenee S, Ferro G, Heikkilä P, Hooiveld M, Johansen C, Randem BG, Schill W (2003) Cancer mortality among European asphalt workers: an international epidemiological study. II. Exposure to bitumen fume and other agents. *Am J Ind Med* 43: 28–39
- Bolm-Audorff A (1998) Polycyclische aromatische Kohlenwasserstoffe (Polycyclic aromatic hydrocarbons) (German). In: Konietzko J, Dupuis H (Eds) *Handbuch der Arbeitsmedizin* Ecomed, Landsberg, 1–31
- Bolonova LN (1967) Action of naphthalene and its methyl derivatives on the ammonia content in rat brain (Russian). *Farmakol Toksikol (Moscow)* 30: 484–486
- Boos KS, Lintelmann J, Kettrup A (1992) Coupled-column high-performance liquid chromatographic method for the determination of 1-hydroxypyrene in urine of subjects exposed to polycyclic aromatic hydrocarbons. *J Chromatogr* 600: 189–194
- Bos RP, Theuws JGL, Jongeneelen FJ, Henderson PT (1988) Mutagenicity of bi-, tri- and tetracyclic aromatic hydrocarbons in the 'taped-plate assay' and in the conventional *Salmonella* mutagenicity assay. *Mutat Res* 204: 203–206
- Bosetti C, Boffetta P, La VC (2006) Occupational exposures to polycyclic aromatic hydrocarbons, and respiratory and urinary tract cancers: a quantitative review to 2005. *Ann Oncol* 18: 431–446
- Bottomley AC, Twort CC (1934) The carcinogenicity of chrysene and oleic acid. *Am J Cancer* 21: 781–786
- Bouchard M, Dodd C, Viau C (1994) Improved procedure for the high-performance liquid chromatographic determination of monohydroxylated PAH metabolites in urine. *J Anal Toxicol* 18: 261–264
- Bouchard M, Krishnan K, Viau C (1998) Kinetics of tissue distribution and elimination of pyrene and 1-hydroxypyrene following intravenous administration of [ $^{14}\text{C}$ ]pyrene in rats. *Toxicol Sci* 46: 11–20
- Boutwell RK (1964) Some biological aspects of skin carcinogenesis. *Prog Exp Tumor Res* 4: 207–250

- Bowman ED, Rothman N, Hackl C, Santella RM, Weston A (1997) Interindividual variation in the levels of certain urinary polycyclic aromatic hydrocarbon metabolites following medicinal exposure to coal tar ointment. *Biomarkers* 2: 321–327
- Boyland E, Burrows H (1935) The experimental production of sarcoma in rats and mice by a colloidal aqueous solution of 1:2:5:6-dibenzanthracene. *J Pathol Bacteriol* 41: 231–238
- Boyland E, Wolf G (1950) Metabolism of polycyclic compounds. 6. Conversion of phenanthrene into dihydroxydihydrophenanthrene. *Biochem J* 47: 64–69
- Boyland E, Sims P (1962 a) Metabolism of polycyclic compounds. 20. The metabolism of phenanthrene in rabbits and rats: Mercapturic acids and related compounds. *Biochem J* 84: 564–570
- Boyland E, Sims P (1962 b) Metabolism of polycyclic compounds. 21. The metabolism of phenanthrene in rabbits and rats: Dihydrodihydroxy compounds and related glucuronic acids. *Biochem J* 84: 571–582
- Boyland E, Sims P (1962 c) Metabolism of polycyclic compounds. 22. The metabolism of (±)-trans-9, 10-dihydro-9, 10-dihydroxyphenanthrene in rats. *Biochem J* 84: 583–586
- Boyland E, Sims P (1964) Metabolism of polycyclic compounds. 23. The metabolism of pyrene in rats and rabbits. *Biochem J* 90: 391–398
- Boyland E, Sims P (1967) The carcinogenic activities in mice of compounds related to benzo[a]-anthracene. *Int J Cancer* 2: 500–504
- Braszczyńska Z, Lindscheid D, Osinska R (1978) Evaluation of the influence of new technologies in coke industry on aromatic polycyclic hydrocarbons concentrations in ambient air. *Med Pr* 29: 357–364
- Brescia G, Celotti L, Clonfero E, Neumann HG, Forni A, Foà V, Pisoni M, Ferri GM, Assenato G (1999) The influence of cytochrome P450 1A1 and glutathione S-transferase M1 genotype on biomarker levels in coke-oven workers. *Arch Toxicol* 73: 431–439
- Bridges BA, Zeiger E, McGregor DB (1981) Summary report on the performance of bacterial mutation assays. In: de Serres FJ, Ashby J (Eds) Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Programme, Elsevier North Holland, New York, USA, 49–67
- Broman D, Colmsjö A, Näf C (1987) Characterization of the PAC profile in settling particulates from the urban waters of Stockholm. *Bull Environ Contam Toxicol* 38: 1020–1028
- Brune K, Kalin H, Schmidt R, Hecker E (1978) Inflammatory, tumor initiating and promoting activities of polycyclic aromatic hydrocarbons and diterpene esters in mouse skin as compared with their prostaglandin releasing potency *in vitro*. *Cancer Lett* 4: 333–342
- Bruce WR, Heddle JA (1979) The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella*, and sperm abnormality assays. *Can J Genet Cytol* 21: 319–334
- Bryant MF, Erexson GL, Kwanyuen P, Atwater AL, Kligerman AD (1990) Sister chromatid exchange and micronucleus analyses in rat peripheral blood lymphocytes following *in vivo* exposure to dibenz[a,h]anthracene. *Environ Mol Mutagen* 15, Suppl 17: 10–11
- Bryant MF, Kwanyuen P, Atwater AL, Erexson GL, Kligerman AD (1991) Cytogenetic effects of benzo[b]fluoranthene in Sprague-Dawley rat peripheral blood lymphocytes after *in vivo* exposure. *Environ Mol Mutagen* 17, Suppl 19: 13
- Bryla P, Weyand EH (1991) Role of activated oxygen species in benzo[a]pyrene: DNA adduct formation *in vitro*. *Free Radical Biol Med* 11: 17–24
- Brzeznicki S, Jakubowski M, Czerski B (1997) Elimination of 1-hydroxypyrene after human volunteer exposure to polycyclic aromatic hydrocarbons. *Int Arch Occup Environ Health* 70: 257–260

- Buckley TJ, Lioy PJ (1992) An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. *Br J Ind Med* 49: 113–124
- Buckpitt AR, Franklin RB (1989) Relationship of naphthalene and 2-methylnaphthalene metabolism to pulmonary bronchiolar epithelial cell necrosis. *Pharmacol Ther* 41: 393–410
- Buening MK, Levin W, Karle JM, Yagi H, Jerina DM, Conney AH (1979) Tumorigenicity of bay-region epoxides and other derivatives of chrysene and phenanthrene in newborn mice. *Cancer Res* 39: 5063–5068
- Bui QQ, Tran MB, West WL (1986) A comparative study of the reproductive effects of methadone and benzo[a]pyrene in the pregnant and pseudopregnant rat. *Toxicology* 42: 195–204
- 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: 520–526
- Busby WF Jr., Stevens EK, Kellenbach ER, Cornelisse J, Lugtenburg J (1988) Dose response relationship of the tumorigenicity of cyclopenta[cd]pyrene, benzo[a]pyrene and 6-nitrochrysene in a newborn mouse lung adenoma bioassay. *Carcinogenesis* 9: 741–746
- Busby WF Jr., Stevens EK, Martin CN, Chow FL, Garner RC (1989) Comparative lung tumorigenicity of parent and mononitro-polynuclear aromatic hydrocarbons in the BLU:Ha newborn mouse assay. *Toxicol Appl Pharmacol* 99: 555–563
- Cadez E (1983) Ergebnisse langjähriger Untersuchungen über den Einfluß von Schadstoffen auf die Gesundheit von Schornsteinfegern (Results of many years of examinations on the influence of hazardous substances on the health of chimney sweeps) (German). *Staub Reinhalt Luft* 43: 116–117
- CAREX (2007) Datenbank CAREX,  
URL [www.ttl.fi/Internet/English/Organization/Collaboration/Carex/](http://www.ttl.fi/Internet/English/Organization/Collaboration/Carex/)
- Carmichael PL, Jacob J, Grimmer G, Phillips dh (1990) Analysis of the polycyclic aromatic hydrocarbon content of petrol and diesel engine lubricating oils and determination of DNA adducts in topically treated mice by <sup>32</sup>P-postlabelling. *Carcinogenesis* 11: 2025–2032
- Carver JH, Machado ML, MacGregor JA (1986) Application of modified *Salmonella* microsome prescreen to petroleum-derived complex mixtures and polynuclear aromatic hydrocarbons (PAH). *Mutat Res* 174: 247–253
- Casale GP, Higginbotham S, Johansson SL, Rogan EG, Cavalieri EL (1997) Inflammatory response of mouse skin exposed to the very potent carcinogen dibenzo[a,l]pyrene: a model for tumor promotion. *Fundam Appl Toxicol* 36: 71–78
- Casto BC (1979) Polycyclic hydrocarbons and Syrian hamster embryo cells: Cell transformation, enhancement of viral transformation and analysis of DNA-damage. In: Jones PW, Leber P (Eds) *Polynuclear Aromatic Hydrocarbons*, Ann Arbor Science Publishers, Ann Arbor, Michigan, USA, 51–66
- Caux C, O'Brien C, Viau C (2002) Determination of firefighter exposure to polycyclic aromatic hydrocarbons and benzene during fire fighting using measurement of biological indicators. *Appl Occup Environ Hyg* 17: 379–386
- Cavalieri E, Mailander P, Pelfrence A (1977) Carcinogenic activity of anthanthrene on mouse skin. *Z Krebsforsch* 89: 113–118
- Cavalieri E, Roth R, Grandjean C, Althoff J, Patil K, Liakus S, Marsh S (1978) Carcinogenicity and metabolic profiles of 6-substituted benzo[a]pyrene derivatives on mouse skin. *Chem Biol Interact* 22: 53–67



- Cavalieri E, Rogan E, Toth B, Munhall A (1981 b) Carcinogenicity of the environmental pollutants cyclopenteno[cd]pyrene and cyclopentano[cd]pyrene in mouse skin. *Carcinogenesis* 2: 277–281
- Cavalieri E, Rogan E, Thilly WG (1981 a) Carcinogenicity, mutagenicity and binding studies on the environmental contaminant cyclopenteno[cd]pyrene and some of its derivatives. In: Cooke M, Dennis AJ (Eds) *Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*, Battelle Press, Columbus, OH, USA, 487–499
- Cavalieri E, Rogan E, Smith D (1988 a) Carcinogenicity of aromatic hydrocarbons directly applied to rat mammary gland. *J Cancer Res Clin Oncol* 114: 3–9
- Cavalieri E, Rogan E, Cremonesi P, Higginbotham S, Salmasi S (1988 b) Tumorigenicity of 6–halogenated derivatives of benzo[a]pyrene in mouse skin and rat mammary gland. *J Cancer Res Clin Oncol* 114: 10–15
- Cavalieri EL, Rogan EG, Higginbotham S, Cremonesi P, Salmasi S (1989) Tumor-initiating activity in mouse skin and carcinogenicity in rat mammary gland of dibenzo[a,l]pyrenes: The very potent environmental carcinogen dibenzo[a,l]pyrene. *J Cancer Res Clin Oncol* 115: 67–72
- Cavalieri EL, Higginbotham S, RamaKrishna NVS, Devanesan PD, Todorovic R, Rogan EG, Salmasi S (1991) Comparative dose-response tumorigenicity studies of dibenzo[a,l]pyrene versus 7,12-dimethylbenz[a]anthracene, benzo[a]pyrene and two dibenzo[a,l]pyrene dihydrodiols in mouse skin and rat mammary gland. *Carcinogenesis* 12: 1939–1944
- Cavalieri EL, Rogan EG (1995) Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica* 25: 677–688
- CBEAP (Committee on Biologic Effects of Atmospheric Pollutants) (1972) *Particulate Polycyclic Organic Matter*, National Academy of Sciences, Washington, DC, USA
- CDC (Centers for Disease Control and Prevention) (2003) *Second National Report on Human Exposure to Environmental Chemicals*, National Center for Environmental Health, Atlanta, GA, USA, NCEH Pub. No 02–0716
- CDC (2005) *Third National Report on Human Exposure to Environmental Chemicals*, National Center for Environmental Health, Atlanta, GA, USA, NCEH Pub. No 05–0570
- Chakravarti D, Mailander PC, Cavalieri EL, Rogan EG (2000) Evidence that error-prone DNA repair converts dibenzo[a,l]pyrene-induced depurinating lesions into mutations: formation, clonal proliferation and regression of initiated cells carrying H-ras oncogene mutations in early preneoplasia. *Mutat Res* 456: 17–32
- Chang RL, Levin W, Wood AW, Lehr RE, Kumar S, Yagi H, Jerina DM, Conney AH (1982) Tumorigenicity of bay-region diol-epoxides and other benzo-ring derivatives of dibenzo[a,h]pyrene and dibenzo[a,i]pyrene on mouse skin and newborn mice. *Cancer Res* 42: 25–29
- Chang RL, Levin W, Wood AW, Yagi H, Tada M, Vyas KP, Jerina DM, Conney AH (1983) Tumorigenicity of enantiomers of chrysene 1,2-dihydrodiol and of the diastereomeric bay-region chrysene 1,2-diol-3, 4-epoxides on mouse skin and in newborn mice. *Cancer Res* 43: 192–196
- Chesis PL, Levin DE, Smith MT, Ernster L, Ames BN (1984) Mutagenicity of quinones: pathways of metabolic activation and detoxification. *Proc Natl Acad Sci USA* 81: 1696–1700
- Chu EW, Malmgren RA (1965) An inhibitory effect of vitamin A on the induction of tumors of forestomach and cervix in the Syrian hamster by carcinogenic polycyclic hydrocarbons. *Cancer Res* 25: 884–895
- Chu M, Chen C (1984) Evaluation and estimation of potential carcinogenic risks of polynuclear aromatic hydrocarbons. Paper presented at the Symposium on polycyclic aromatic hydrocarbons in the workplace, Pacific Rim Risk Conference, Honolulu, HI, USA

- Clayson DB, Pringle JAS, Bonser G, Wood M (1968) The technique of bladder implantation: further results and an assessment. *Br J Cancer* 22: 825–832
- Clement (1988) Comparative potency approach for estimating the cancer risk associated with exposure to mixtures of polycyclic aromatic hydrocarbons. Contract USEPA 68-02-4403, ICF-Clement Associates, Fairfax, VA, USA
- Clonfero E, Zordan M, Venier P, Paleologo M, Levis AG, Cottica D, Pozzoli L, Jongeneelen FJ, Bos RP, Anzion RBM (1989) Biological monitoring of human exposure to coal tar. Urinary excretion of total PAH, 1-hydroxypyrene and mutagens in psoriatic patients. *Int Arch Occup Environ Health* 61: 363–369
- Coenen W (1988) Expositionssituation in Gießereien (Exposure situation in foundries) (German). *Staub Reinhalt Luft* 48: 189–195
- Collins JF, Brown JP, Alexeeff GV, Salmon AG (1998) Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. *Regul Toxicol Pharmacol* 28: 45–54
- Conney AH, Chang RL, Cui XX, Schiltz M, Yagi H, Ferina DM, Wei SJ (2001) Dose-dependent differences in the profile of mutations induced by carcinogenic (R,S,S,R) bay- and fjord-region diol epoxides of polycyclic aromatic hydrocarbons. *Adv Exp Med Biol* 500: 697–707
- Constantino JP, Redmond CK, Bearden A (1995) Occupationally related cancer risk among coke oven workers: 30 years of follow-up. *J Occup Environ Med* 37: 597–604
- Cook JW, Hewett C, Hieger I (1932) Coal tar constituents and cancer. *Nature* 130: 926
- Coombs MM, Dixon C, Kissonerghis AM (1976) Evaluation of the mutagenicity of compounds of known carcinogenicity, belonging to the benz[a]anthracene, chrysene, and cyclopenta[a]-phenanthrene series, using Ames' test. *Cancer Res* 36: 4525–4529
- Crabtree HG (1946) Some effects of aromatic hydrocarbons on sulfur metabolism and tumor induction in mice. *Cancer Res* 6: 553–559
- Crocker TT, Chase JE, Wells SA, Nunes LL (1970) Preliminary report on experimental squamous carcinoma of the lung in hamsters and in primate (*Galago Crassicauda* Tus.). Office of County Veterinarian, Symposium Series No. 21, San Diego, California, USA, pp. 317–328
- Cruciani V, Bessi H, Alexandre S, Durand MJ, Rast C, Nguyen-Ba G, Vasseur P (1996) SHE cell transformation assay as a predictive system for carcinogenicity of PAH and non-genotoxic carcinogens. *Polycyclic Aromat Compd* 10: 307–314
- Cummings DA, Lin ELC, Daniel FB, Klaunig JE, Shut HAJ (1991) *In vivo* and *in vitro* binding of benzo[a]pyrene to tissue DNA and circulating macromolecules in the mouse, monkey and human (Abstract). *Proc Am Assoc Cancer Res* 32: 159
- Davis BR, Whitehead JK, Gill ME, Lee PN, Butterworth AD, Roe FJR (1975) Response of rat lung to 3, 4-benzpyrene administered by intratracheal instillation in infusine with or without carbon black. *Br J Cancer* 31: 443–452
- Davis C, Desa D, Amin S, Nesnow S (1999) Comparison of the morphological transforming activities of fjord-region PAHs with dibenzo[a,e]pyrene and benzo[a]pyrene. *Polycyclic, Aromat Compd* 16: 141–149
- Davidson GE, Dawson GWP (1976) Chemically induced presumed somatic mutations in the mouse. *Mutat Res* 38: 151–154
- Degen GH, Vogel C, Abel J (2002) Prostaglandin synthases. In: Ioannides C (Ed) *Handbook of Enzyme Systems that Metabolise Drugs and Other Xenobiotics*, John Wiley & Sons, Sussex, 189–230
- De Jong WH, Kroese ED, Vos JG, Van Loveren H (1999) Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure to rats. *Toxicol Sci* 50: 214–220

- DeMarini DM, Landi S, Tian D, Hanley NM, Li X, Hu F, Roop BC, Mass MJ, Keohavong P, Gao W, Olivier M, Hainaut P, Mumford JL (2001) Lung tumor KRAS and TP53 mutations in non-smokers reflect exposure to PAH-rich coal combustion emissions. *Cancer Res* 61: 6679–6681
- Deutsch-Wenzel RP, Brune H, Grimmer G (1983 a) Experimental studies on the carcinogenicity of five nitrogen containing polynuclear aromatic compounds directly injected into rat lungs. *Cancer Lett* 20: 97–101
- Deutsch-Wenzel R, Brune H, Grimmer G, Dettbarn G, Misfeld J (1983 b) Experimental studies in rat lungs on the carcinogenicity and dose-response relationships of eight frequently occurring environmental polycyclic aromatic hydrocarbons. *J Natl Cancer Inst* 71: 539–544
- Devanesan PD, Cremonesi P, Nunnally JE, Rogan EG, Cavalieri EL (1990) Metabolism and mutagenicity of dibenzo[a,e]pyrene and the very potent environmental carcinogen dibenzo[a,l]pyrene. *Chem Res Toxicol* 3: 580–586
- Devanesan PD, RamaKrishna NVS, Padmavathi NS, Higginbotham S, Rogan EG, Cavalieri EL, Marsch GA, Jankowiak R, Small GJ (1993) Identification and quantitation of 7, 12-dimethylbenz[a]anthracene-DNA adducts formed in mouse skin. *Chem Res Toxicol* 6: 364–371
- DFG (Deutsche Forschungsgemeinschaft) (2004) Forschungsbericht “Polycyclische Aromatische Kohlenwasserstoffe (PAH)” (Research report “Polycyclic aromatic hydrocarbons (PAH)”) (German), Verlag VCH-Wiley, Weinheim
- DFG (Deutsche Forschungsgemeinschaft) (2007) List of MAK and BAT Values 2007. Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 43, Wiley-VCH-Verlag, Weinheim
- Doli R, Fisher REW, Gammon EJ, Gunn W, Hughes GO, Tyrer FH, Wilson W (1972) Mortality of gasworkers – final report of a prospective study. *Br J Ind Med* 29: 394–406
- Dostal L, Guthenberg C, Mannervik B, Bend JR (1988) Stereoselectivity and regioselectivity of purified human glutathione transferases pi, alpha-epsilon, and mu with alkene and polycyclic arene oxide substrates. *Drug Metab Dispos* 16: 420–424
- Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simon VF (1984) Reproducibility of microbial mutagenicity assays: I. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. *Environ Mutagen* 6, Suppl 2: 1–39
- Eckardt RE (1959) Industrial Carcinogens. Modern Monographs in Industrial Medicine, Vol 4: Grune & Stratton, New York, London
- Eisenstadt E, Gold A (1978) Cyclopenta[cd]pyrene: A highly mutagenic polycyclic aromatic hydrocarbon. *Proc Natl Acad Sci USA* 75: 1667–1669
- Elgjo K (1968) Growth kinetics of the mouse epidermis after a single application of 3,4-benzopyrene, croton oil, or 1,2-benzopyrene. *Acta Pathol Microbiol Scand* 73: 183–190
- Elovaara E, Heikkilä P, Pyy L, Mutanen P, Riihimäki V (1995) Significance of dermal and respiratory uptake of creosote workers: exposure to polycyclic aromatic hydrocarbons and urinary excretion of 1-hydroxypyrene. *Occup Environ Med* 52: 196–203
- Engst W, Landsiedel R, Hermersdörfer H, Doehmer J, Glatt H (1999) Benzylic hydroxylation of 1-methylpyrene and 1-ethylpyrene by human and rat cytochromes P450 individually expressed in V79 Chinese hamster cells. *Carcinogenesis* 20: 1777–1785
- Enwald E (1982) How safe is it to work with rubber? *Kautsch Gummi Kunstst* 34: 953
- Epler JL, Rao TK, Guerin MR (1979) Evaluation of feasibility of mutagenic testing of shale oil products and effluents. *Environ Health Perspect* 30: 179–184
- Epstein S (1968) Chemical mutagens in the human environment. *Nature* 219: 385–387

- Fahmy OG, Fahmy MJ (1973) Oxidative activation of benz[*a*]anthracene and methylated derivatives in mutagenesis and carcinogenesis. *Cancer Res* 33: 2354–2361
- Fahmy MJ, Fahmy OG (1980) Altered control of gene activity in the soma by carcinogens. *Mutat Res* 72: 165–172
- Falk HL, Kotin P, Thompson S (1964) Inhibition of carcinogenesis. *Arch Environ Health* 9: 169–179
- Fan R, Dong Y, Zhang W, Wang Y, Yu Z, Sheng G, Fu J (2006) Fast simultaneous determination of urinary 1-hydroxypyrene and 3-hydroxybenzo[*a*]pyrene by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 836: 92–97
- Fanburg SJ (1940) Exfoliative dermatitis due to naphthalene: Report of an eruption resembling mycosis fungoides. *Arch Dermatol Syph* 42: 53–58
- 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, Suppl 1: 175–200
- Fields WR, Desiderio JG, Leonard RM, Burger EE, Brown BG, Doolittle DJ (2004) Differential c-myc expression profiles in normal human bronchial epithelial cells following treatment with benzo[*a*]pyrene, benzo[*a*]pyrene-4,5 epoxide, and benzo[*a*]pyrene-7,8,9,10 diol epoxide. *Mol Carcinog* 40: 79–89
- Finzi C, Daudel P, Prodi G (1968) Interference among polycyclic hydrocarbons in experimental skin carcinogenesis. *Eur J Cancer* 3: 497–501
- Flesher JW, Harvey RG, Sydnor KL (1976) Oncogenicity of K-region epoxides of benzo[*a*]pyrene and 7, 12-dimethylbenz[*a*]anthracene. *Int J Cancer* 18: 351–353
- Flesher JW, Horn J, Lehner AF (1998 a) 9-Sulfooxymethylanthracene is an ultimate electrophilic and carcinogenic form of 9-hydroxymethylanthracene. *Biochem Biophys Res Commun* 251: 239–243
- Flesher JW, Horn J, Lehner AF (1998 b) Carcinogenicity of 1-hydroxy-3-methylcholanthrene and its electrophilic sulfate ester 1-sulfooxy-3-methylcholanthrene in Sprague-Dawley rats. *Biochem Biophys Res Commun* 243: 30–35
- Florin I, Rutberg L, Curvall M, Enzell CR (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology* 18: 219–232
- Flowers L, Rieth SH, Coglian VJ, Foureman GL, Hertzberg R, Hofmann EL, Murphy DL, Nesnow S, Schoeny RS (2002) Health assessment of polycyclic aromatic hydrocarbon mixtures: current practices and future directions. *Polycyclic Aromat Compd* 22: 811–821
- Flowers-Geary L, Blecinski W, Harvey RG, Penning TM (1996) Cytotoxicity and mutagenicity of polycyclic aromatic hydrocarbon o-quinones produced by dihydrodiol dehydrogenase. *Chem Biol Interact* 99: 55–72
- Flury F, Zernik F (1935) List of the toxic and lethal doses for the most common poisons in experimental animals In: Abderhalden E (Ed) Handbook of Biological Methods, Urban Schwarzenberg, Berlin, 12289–14222
- Förster K, Preuss R, Rossbach B, Brüning T, Angerer J, Simon P (2007) 3-Hydroxybenzo[*a*]pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. *Occup Environ Med* accepted for publication
- Friesen MC, Demers PA, Spinelli JJ, Lorenzi MF, Le ND (2007) Comparison of two indices of exposure to polycyclic aromatic hydrocarbons in a retrospective aluminium smelter cohort. *Occup Environ Med* 64: 273–278
- Frölich A, Würzler FE (1990) Drosophila wing-spot test: Improved detectability of genotoxicity of polycyclic aromatic hydrocarbons. *Mutat Res* 234: 71–80

- Fujikawa K, Fort FL, Samejima K, Sakamoto Y (1993) Genotoxic potency in *Drosophila melanogaster* of selected aromatic amines and polycyclic aromatic hydrocarbons as assayed in the DNA repair test. *Mutat Res* 290: 175–182
- Gaertner RR, Theriault GP (2002) Risk of bladder cancer in foundry workers: a meta-analysis. *Occup Environ Med* 59: 655–663
- Gaines TB (1969) Acute toxicity of pesticides. *Toxicol Appl Pharmacol* 14: 515–534
- Geacintov NE, Cosman M, Hingerty BE, Amin S, Broyde S, Patel DJ (1997) NMR solution structures of stereoisomeric covalent polycyclic aromatic carcinogen-DNA adducts: principles, patterns, and diversity. *Chem Res Toxicol* 10: 111–146
- Gelboin HV, Ts'o POP (1978) Polycyclic Hydrocarbons and Cancer. Vol 2: Metabolism and Cell Biology, Academic Press, New York
- Generoso WM, Cain KT, Hellwig CD, Cacheiro NLA (1982) Lack of association between induction of dominant lethal mutations and induction of heritable translocations with benzo[a]pyrene in postmeiotic germ cells of male mice. *Mutat Res* 94: 155–163
- Gerarde HW (1960 a) Toxicology and biochemistry of aromatic hydrocarbons. Toxicological effects of the aromatic hydrocarbons. In: Browning E (Ed) Elsevier Monographs on Toxic Agents Elsevier Publishing, Amsterdam, 225–248
- Gerarde HW (1960 b) Toxicology and biochemistry of aromatic hydrocarbons. Polycyclic aromatic hydrocarbons. In: Browning E (Ed) Elsevier Monographs on Toxic Agents, Elsevier Publishing, Amsterdam, 249–274
- Gibb HJ, Chen CW (1986) Multistage model interpretation of additive and multiplicative carcinogenic effects. *Risk Anal* 6: 167–170
- Gibbs GW (1985) Mortality of aluminium reduction plant workers, 1950 through 1977. *J Occup Med* 27: 761–770
- Gill RD, Butterworth BE, Nettikumara AN, Digiovanni J (1991) Relationship between DNA adduct formation and unscheduled DNA synthesis (UDS) in cultured mouse epidermal keratinocytes. *Environ Mol Mutagen* 18: 200–206
- Glatt H, Vogel K, Bentley P, Oesch F (1979) Reduction of benzo[a]pyrene mutagenicity by dihydrodiol dehydrogenase. *Nature* 277: 319–320
- Glatt H, Cooper CS, Grover PL, Sims P, Bentley P, Merdes M, Waechter F, Vogel K, Guenther TM, Oesch F (1982) Inactivation of a diol-epoxide by dihydrodiol dehydrogenase, but not by two epoxide hydrolases. *Science* 215: 1507–1509
- Glatt H, Friedberg T, Grover PL, Sims P, Oesch F (1983) Inactivation of a diol-epoxide and a K-region epoxide with high efficiency by glutathione transferase X. *Cancer Res* 43: 5713–5717
- Glatt H, Seidel A, Ribeiro O, Kirkby C, Hirom P, Oesch F (1987) Metabolic activation to a mutagen of 3-hydroxy-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene, a secondary metabolite of benzo[a]pyrene. *Carcinogenesis* 8: 1621–1627
- Glatt H, Henschler R, Phillips d h, Blake JW, Steinberg P, Seidel A, Oesch F (1990) Sulfotransferase-mediated chlorination of 1-hydroxymethylpyrene to a mutagen capable of penetrating indicator cells. *Environ Health Perspect* 88: 43–48
- Glatt H, Werle-Schneider G, Seidel A, Schwarz M (1994 a) Initiation and promotion of enzyme-altered foci in rat liver by 1-hydroxymethylpyrene. *Polycyclic Aromat Compd* 7: 67–74
- Glatt H, Seidel A, Harvey RG, Coughtrie MWH (1994 b) Activation of benzylic alcohols to mutagens by human hepatic sulphotransferases. *Mutagenesis* 9: 553–557
- Glatt H, Bartsch I, Christoph S, Coughtrie MWH, Falany CN, Hagen M, Landsiedel R, Pabel U, Phillips d h, Seidel A, Yamazoe Y (1998) Sulfotransferase-mediated activation of mutagens studied using heterologous expression systems. *Chem Biol Interact* 109: 195–219

- Glatt H (2000) Sulfotransferases in the bioactivation of xenobiotics. *Chem Biol Interact* 129: 141–170
- Glatt H, Pabel U, Muckel E, Meinel W (2002) Activation of polycyclic aromatic compounds by cDNA-expressed phase I and phase II enzymes. *Polycyclic Aromat Compd* 22: 955–967
- Glatt H, Meinel W, Kuhlow A, Ma L (2003) Metabolic formation, distribution and toxicological effects of reactive sulphuric acid esters. *Nova Acta Leopold* 329: 151–161
- Glatt H (2004) Genetische Polymorphismen und Suszeptibilität gegenüber der kanzerogenen Wirkung polycyclischer aromatischer Verbindungen: Grundlagen (Genetic polymorphisms and susceptibility to the carcinogenic effect of polycyclic aromatic compounds: basic principles) (German). In: Jacob J, Greim H (Eds) *Polycyclische Aromatische Kohlenwasserstoff – Forschungsbericht*, Wiley-Verlag, Weinheim, 148–182
- Glatt H (2005) Indicator assays for polycyclic aromatic hydrocarbon-induced genotoxicity. In: Luch A (Ed) *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons*, Imperial College Press, London, 283–314
- Glatt H (2007 a) Communication to the secretariat of the Commission, Feb. 7, 2007
- Glatt H (2007 b) Communication to the secretariat of the Commission, June 14, 2007
- Glatt H (2007 c) Sulfotransferase-mediated activation of alkylated and alkanobridged polycyclic aromatic hydrocarbons present in tobacco smoke, and its modulation by alcohol and drugs. Project report, Deutsches Institut für Ernährungsforschung, Potsdam-Rehbrücke, unpublished
- Glatt H (2007 d) Communication to the secretariat of the Commission, June 14, 2007
- Godschalk RW, Ostertag JU, Moonen EJ, Neumann HA, Kleinjans JC, van Schooten FJ (1998) Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar. *Cancer Epidemiol Biomarkers Prev* 7: 767–773
- Gold A, Eisenstadt E (1980) Metabolic activation of cyclopenta[cd]pyrene to 3, 4-epoxycyclopenta[cd]pyrene by rat liver microsomes. *Cancer Res* 40: 3940–3944
- Goldschmidt BM, Katz C, Van Duuren BL (1973) The cocarcinogenic activity of noncarcinogenic aromatic hydrocarbons. *Proc Am Assoc Cancer Res* 14: 84
- Göen T, Gündel J, Schaller K-H, Angerer J (1995) The elimination of 1-hydroxypyrene in the urine of the general population and workers with different occupational exposure to PAH. *Sci Total Environ* 163: 195–201
- Graffi A, Vlamynck E, Hoffmann F, Schulz I (1953) Untersuchungen über die geschwulstauslösende Wirkung verschiedener chemischer Stoffe in der Kombination mit Crotonöl (Investigations into the oncogenic effect of various chemical substances in combination with croton oil) (German). *Arch Geschwulstforsch* 5: 110–126
- Granella M, Clonfero E (1993) Urinary excretion of 1-pyrenol in automotive repair workers. *Int Arch Occup Environ Health* 65: 241–245
- Grant G, Roe FJC (1963) The effect of phenanthrene on tumour induction by 3, 4-benzopyrene administered to newly born mice. *Br J Cancer* 17: 261–265
- Grimmer G, Hildebrandt A, Böhnke H (1973) Investigations on the carcinogenic burden by air pollution in man. 3. Sampling and analytics of polycyclic aromatic hydrocarbons in automobile exhaust gas. 2. Enrichment of the PNA and separation of the mixture of all PNA. *Zentralbl Bakteriol Orig B* 158: 35–49
- Grimmer G (1977) Analysis of automobile exhaust condensates. In: Mohr U, Schmähl D, Tomatis L (Eds) *Air Pollution and Cancer in Man*, IARC Scientific Publications No. 16, Lyon, France, 29–39
- Grimmer G, Dettbarn G, Brune H, Deutsch-Wenzel R, Misfeld J (1982) Quantitation of the carcinogenic effect of polycyclic aromatic hydrocarbons in used engine oil by topical application onto the skin of mice. *Int Arch Occup Environ Health* 50: 95–100

- Grimmer G, Brune H, Deutsch-Wenzel R, Dettbarn G, Misfeld J, Abel U, Timm J (1985) The contribution of polycyclic aromatic hydrocarbon fractions with different boiling ranges to the carcinogenic impact of emission condensate from coal fired residential furnaces as evaluated by topical application to the skin of mice. *Cancer Lett* 28: 203–211
- Grimmer G, Naujack KW, Dettbarn G (1987) Beitrag zur Ursachenforschung exogen bedingter Blasenkarzinome – Profilanalyse aromatischer Amine am Arbeitsplatz (Contribution to the aetiology of exogenously induced bladder carcinomas – profile analysis of aromatic amines at the workplace) (German). Wirtschaftsverlag, Bremerhaven
- Grimmer G, Brune H, Dettbarn G, Heinrich U, Jacob J, Mohtashamipur E, Norpoth K, Pott F, Wenzel-Hartung R (1988 a) Urinary and faecal excretion of chrysene and chrysene metabolites by rat after oral, intraperitoneal, intratracheal or intrapulmonary application. *Arch Toxicol* 62: 401–405
- Grimmer G (1988 b) Polycyclische aromatische Kohlenwasserstoffe. In: Eisenbrand G, Frank HK, Grimmer Hapke HJ, Thier HP, Weigert G (Eds) *Derzeitige Belastung und Trends bei der Belastung der Lebensmittel durch Fremdstoffe* (Current food contamination and trends of food contamination by xenobiotics) (German), Verlag W Kohlhammer GmbH, Karlsruhe, 151–187
- Grimmer G, Jacob J, Schmoltdt A, Pool-Zobel B, Raab G (1989) Die Bedeutung polycyclischer aromatischer Thiophene (Thiaarene) als Umweltcarcinogene (Relevance of polycyclic aromatic thiophenes (thiaarenes) as environmental carcinogens) (German). BMFT-Projekt Förderkennzeichen 0704845B2, Biochemisches Institut für Umweltcarcinogene Großhansdorf, Institut für Rechtsmedizin Hamburg, Deutsches Krebsforschungszentrum Heidelberg
- Grimmer G, Dettbarn G, Naujack KW (1990) Ausscheidung von Hydroxyderivaten polycyclischer aromatischer Kohlenwasserstoffe im Harn von Kokerei- und Straßenbaubeschäftigten (Excretion of hydroxy derivatives of polycyclic aromatic hydrocarbons in the urine of coke oven and road workers) (German). In: Schuckmann F, Schopper-Jochum S (Eds) *Bericht über die 30. Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin eV*, Genter Verlag, Stuttgart, 283–287
- Grimmer G, Dettbarn G, Naujack KW, Jacob J (1991 a) Excretion of hydroxy derivatives of polycyclic aromatic hydrocarbons of the masses 178, 202, 228 and 252 in the urine of coke and road workers. *Int J Environ Anal Chem* 43: 177–186
- Grimmer G, Brune H, Dettbarn G, Jacob J, Mohtashamipur E, Norpoth K, Wenzel-Hartung R (1991 b) Urinary and faecal excretion of phenanthrene and phenanthrols by rats following oral, intraperitoneal, or intrapulmonary application. *Polycyclic Aromat Compd* 2: 39–47
- Grimmer G, Naujack KW, Dettbarn G (1993 a) Bestimmung der Harnmetaboliten polycyclischer aromatischer Kohlenwasserstoffe bei Kokereibesetzten (Determination of urinary metabolites of aromatic hydrocarbons among coke oven workers) (German). In: INRS (Institut National de la Recherche Scientifique), *Maitriser le risque au poste de travail*, Presses Universitaires de Nancy, pp. 227–233
- Grimmer G, Dettbarn G, Jacob J (1993 b) Biomonitoring of polycyclic aromatic hydrocarbons in highly exposed coke plant workers by measurement of urinary phenanthrene and pyrene metabolites (phenols and dihydrodiols). *Int Arch Occup Environ Health* 65: 189–199
- Grimmer G (1993 c) Relevance of polycyclic aromatic hydrocarbons as environmental carcinogens. In: Garrigues P, Lamotte M (Eds) *Polycyclic Aromatic Compounds – Synthesis, Properties, Analytical Measurements, Occurrence and Biological Effects*, Verlag Gordon & Breach, Yverdon, Schweiz, 31–41
- Grimmer G, Dettbarn G, Naujack KW, Jacob J (1994) Relationship between inhaled PAH and urinary excretion of phenanthrene, pyrene and benzo[a]pyrene metabolites in coke plant workers. *Polycyclic Aromat Compd* 5: 269–277

- Grimmer G, Jacob J, Dettbarn G, Naujack KW (1997) Determination of urinary metabolites of polycyclic aromatic hydrocarbons (PAH) for the risk assessment of PAH-exposed workers. *Int Arch Occup Environ Health* 69: 231–239
- Grodstein F, Speizer FE, Hunter DJ (1995) A prospective study of incident squamous cell carcinoma of the skin in the nurses' health study. *J Natl Cancer Inst* 87: 1061–1066
- Grover PL, Sims P, Mitchel BCU, Roe FJ (1975) The carcinogenicity of polycyclic hydrocarbon epoxides in newborn mice. *Br J Cancer* 31: 182–188
- Gündel J, Mannschreck C, Büttner K, Ewers U, Angerer J (1996) Urinary levels of 1-hydroxypyrene, 1-, 2-, 3-, and 4-hydroxyphenanthrene in females living in an industrial area of Germany. *Arch Environ Contam Toxicol* 31: 585–590
- Gündel J, Angerer J (2000) High-performance liquid chromatographic method with fluorescence detection for the determination of 3-hydroxybenzo[a]pyrene and 3-hydroxybenz[a]anthracene in the urine of polycyclic aromatic hydrocarbon-exposed workers. *J Chromatogr B* 738: 47–55
- Gündel J, Schaller KH, Angerer J (2000) Occupational exposure to polycyclic aromatic hydrocarbons in a fireproof stone producing plant: biological monitoring of 1-hydroxy-pyrene, 1-, 2-, 3- and 4-hydroxyphenanthrene, 3-hydroxybenz[a]anthracene and 3-hydroxybenzo[a]pyrene. *Int Arch Occup Environ Health* 73: 270–274
- Guillén MD, Sopelana P (2004) Occurrence of polycyclic aromatic hydrocarbons in smoked cheese. *J Dairy Sci* 87: 556–564
- Guillén MD, Sopelana P, Palencia G (2004) Polycyclic aromatic hydrocarbons and olive pomace oil. *J Agric Food Chem* 52: 2123–2132
- Gunier RB, Reynolds P, Hurley SE, Yerabati S, Hertz A, Strickland P, Horn-Ross PL (2006) Estimating exposure to polycyclic aromatic hydrocarbons: a comparison of survey, biological monitoring, and geographic information system-based methods. *Cancer Epidemiol Biomarkers Prev* 15: 1376–1381
- Gupta RC, Earley K, Sharma S (1988) Use of human peripheral blood lymphocytes to measure DNA binding capacity of chemical carcinogens. *Proc Natl Acad Sci USA* 85: 3513–3517
- Gustavsson P, Gustavsson A, Hogstedt C (1987) Excess mortality among Swedish chimney sweeps. *Br J Ind Med* 44: 738–743
- Gustavsson P, Gustavsson A, Hogstedt C (1988) Excess of cancer in Swedish chimney sweeps. *Br J Ind Med* 45: 777–781
- Gustavsson P, Jakobsson R, Johansson H, Lewin F, Norell S, Rutkvist LE (1998) Occupational exposures and squamous cell carcinoma of the oral cavity, pharynx, larynx and oesophagus: a case-control study in Sweden. *Occup Environ Med* 55: 393–400
- Habs M, Schmähl S, Misfeld J (1980) Local carcinogenicity of some environmentally relevant polycyclic aromatic hydrocarbons after lifelong topical application to mouse skin. *Geschwulstforschung* 50: 266–274
- Habs M, Jahn SAA, Schmähl D (1984) Carcinogenic activity of condensate from colocynth seeds (*Citrullus colocynthis*) after chronic epicutaneous administration to mice. *J Cancer Res Clin Oncol* 108: 154–156
- Haddow A, Scott CM, Scott JD (1937) The influence of certain carcinogenic and other hydrocarbons on body growth in the rat. *Proc R Soc Edinburgh, Sect B* 122: 477–507
- Hammond EC, Selikoff IJ, Lawther PL, Seidman H (1976) Inhalation of benzpyrene and cancer in man. *Ann NY Acad Sci* 271: 116–124
- Hansen ES (1989) Cancer incidence in an occupational cohort exposed to bitumen fumes. *Scand J Work Environ Health* 15: 101–105



- Hardin BD, Bond GP, Sikov MR, Andrew FD, Beliles RR, Niemeier RW (1981) Testing of selected workplace chemicals for teratogenic potential. *Scand J Work Environ Health* 7: 66–75
- Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, Smith KN (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratog Carcinog Mutagen* 7: 29–48
- Harper KH (1957) The metabolism of pyrene. *Br J Cancer* 11: 499–507
- Harper KH (1958) The intermediary metabolism of pyrene. *Br J Cancer* 12: 116–120
- Harper BL, Sadagopa Ramanujam VM, Gad-El-Karim MM, Legator MS (1984) The influence of simple aromatics on benzene clastogenicity. *Mutat Res* 128: 105–114
- Harper BL, Legator MS (1987) Pyridine prevents the clastogenicity of benzene but not of benzo[a]pyrene or cyclophosphamide. *Mutat Res* 179: 23–31
- Harris CC, Grafstrom RC, Shamsuddin AM, Sinopoli NT, Trump BF, Autrup H (1984) Carcinogen metabolism and carcinogen-DNA adducts in human tissues and cells. In: Greim H, Jung R, Kramer M, Marquardt H, Oesch F (Eds) *Biochemical Basis of Chemical Carcinogenesis*, Raven Press, New York, 123–135
- Hashibe M, Boffetta P, Zaridze D, Shangina O, Szeszenia-Dabrowska N, Mates D, Fabianova E, Rudnai P, Brennan P (2007) Contribution of tobacco and alcohol to the high rates of squamous cell carcinoma of the supraglottis and glottis in Central Europe. *Am J Epidemiol* 165: 814–820
- Haugen DA, Peak MJ (1983) Mixtures of polycyclic aromatic compounds inhibit mutagenesis in the *Salmonella*/microsome assay by inhibition of metabolic activation. *Mutat Res* 116: 257–269
- Haugen A, Becher G, Benestad C, Vahakangas K, Trivers GE, Newman MJ, Harris CC (1986) Determination of polycyclic aromatic hydrocarbons in the urine, benzo[a]pyrene diol epoxide-DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. *Cancer Res* 46: 4178–4183
- He S, Baker R (1991) Micronuclei in mouse skin cells following in vivo exposure to benzo[a]pyrene, 7,12-dimethylbenzo[a]anthracene, chrysene, pyrene and urethane. *Environ Mol Mutagen* 17: 163–168
- Hecht SS, Bondinell WE, Hoffmann D (1974) Chrysene and methylchrysenes: Presence in tobacco smoke and carcinogenicity. *J Natl Cancer Inst* 53: 1121–1133
- Hecht SS, Loy M, Hoffmann D (1976) On the structure and carcinogenicity of the methylchrysenes. In: Freudenthal R, Jones PW (Eds) *Carcinogenesis: A Comprehensive Survey*. Vol. 1, Polynuclear Aromatic Hydrocarbons Chemistry, Metabolism, and Carcinogenesis, Raven Press, New York, 325–340
- Hecht SS, LaVoie E, Amin S, Bedenko V, Hoffmann D (1980) On the metabolic activation of the benzo[fluoranthenes]. In: Bjørseth A, Dennis AJ (Eds) *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Battelle Press, Columbus, OH, USA, 417–433
- Hecht SS, Ronai ZA, Dolan L, Desai D, Amin S (1998) Comparative mouse skin tumorigenicity and induction of Ha-ras mutations by bay region diol epoxides of 5-methylchrysene and 5,6-dimethylchrysene. *Carcinogenesis* 19: 157–160
- Hecht SS (2002) Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis* 23: 907–922
- Hecht SS, Chen M, Yagi H, Jerina DM, Carmella SG (2003) r-1,t-2,3,c-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. *Cancer Epidemiol Biomarkers Prev* 12: 1501–1508

- Hecht SS, Chen M, Yoder A, Jensen J, Hatsukami D, Le C, Carmella SG (2005) Longitudinal study of urinary phenanthrene metabolite ratios: effect of smoking on the diol epoxide pathway. *Cancer Epidemiol Biomarkers Prev* 14: 2969–2974
- Hecht SS, Carmella SG, Yoder A, Chen M, Li ZZ, Le C, Dayton R, Jensen J, Hatsukami DK (2006) Comparison of polymorphisms in genes involved in polycyclic aromatic hydrocarbon metabolism with urinary phenanthrene metabolite ratios in smokers. *Cancer Epidemiol Biomarkers Prev* 15: 1805–1811
- Henry SA (1946) *Cancer of the Scrotum in Relation to Occupation*, University Press, London
- Henry MC, Port CD, Kauhman DG (1975) Importance of physical properties of benzo[a]pyrene-ferric oxide mixtures in lung tumor induction. *Cancer Res* 35: 207–217
- Hermann M (1981) Synergistic effects of individual polycyclic aromatic hydrocarbons on the mutagenicity of their mixtures. *Mutat Res* 90: 399–409
- Hermann M, Durand JP, Charpentier JM, Chaude O, Hofnung M, Petroff N, Vandecasteele JP, Weill N (1980) Correlations of mutagenic activity with polynuclear aromatic hydrocarbon content of various mineral oils. In: Cooke M, Dennis AJ (Eds) *Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*, Battelle Press, Columbus, OH, USA, 899–916
- Hermersdörfer H, Ozierenski B, Schmalix WA, Doehmer J, Glatt HR (1997) Cell culture system for the controlled intracellular generation of reactive oxygen species. *Toxicol In vitro* 11: 569–572
- Hessel S (2006) Glutathion-S-Transferase T2 aus der Ratte: Expression in *Salmonella typhimurium* und Charakterisierung (Rat glutathione S-transferase T2: expression in *S. typhimurium* and characterization) (German). Diploma dissertation, Biochemistry Faculty of Potsdam University, Germany
- Hill RH, Head SL, Baker S, Gregg M, Shealy DB, Bailey SL, Williams CC, Sampson EJ, Needham LL (1995) Pesticide residues in urine of adults living in the United States: reference range concentrations. *Environ Res* 71: 99–108
- Hill WT, Stanger DW, Pizzo A, Riegel B, Shubik P, Wartman WB (1952) Inhibition of skin carcinogenesis in mice by mixtures of strong carcinogens. *Cancer Res* 12: 270
- Hiratsuka A, Okada T, Nishiyama T, Fujikawa M, Ogura K, Okuda H, Watabe T, Watabe T (1994) Novel *theta* class glutathione S-transferases  $Y_{rs}-Y_{rs}'$  and  $Y_{rs}'-Y_{rs}'$  in rat liver cytosol: their potent activity toward 5-sulfoxymethylchrysene, a reactive metabolite of the carcinogen 5-hydroxymethylchrysene. *Biochem Biophys Res Commun* 202: 278–284
- Ho CH, Clark BR, Guerin MR, Barkenbus BD, Rao TK, Epler JL (1981) Analytical and biological analyses of test materials from the synthetic fuel technologies: IV. Studies of chemical structure-mutagenic activity relationships of aromatic nitrogen compounds relevant to synfuels. *Mutat Res* 85: 335–345
- Hoch-Ligeti C (1941) Studies on the changes in the lymphoid tissue of mice treated with carcinogenic and noncarcinogenic hydrocarbons. *Cancer Res* 1: 484–488
- Hoepfner I, Dettbarn G, Scherer G, Grimmer G, Adlkofer F (1987) Hydroxyphenanthrenes in the urine of non-smokers. *Toxicol Lett* 35: 67–71
- Hofmann F, Boente L (1933) Beiträge zur Pechchemie (Contributions to pitch chemistry) (German). *Brennstoff-Chemie* 14: 381–382
- Hoffmann D, Wynder EL (1962) Analytical and biological studies on gasoline engine exhaust. *J Natl Cancer Inst* 9: 91
- Hoffmann D, Wynder EL (1966) Beitrag zur carcinogenen Wirkung von Dibenzo[pyren] (Article on the carcinogenic effect of dibenzopyrene) (German). *Z Krebsforsch* 68: 137–149

- Hoffmann D, Rathkamp G, Nesnow S, Wynder EL (1972) Fluoranthenes: Quantitative determination in cigarette smoke, formation by pyrolysis, and tumor-initiating activity. *J Natl Cancer Inst* 49: 1165–1175
- Hoffmann D, Hoffmann I (1998) Tobacco smoke components. *Beitr Tabakforsch* 18: 49–52
- Hogstedt C, Andersson K, Frenning B, Gustavsson A (1982) A cohort study on mortality among long-time employed Swedish chimney sweeps. *Scand J Work Environ Health* 8, Suppl 1: 72–78
- Hollender J, Koch B, Dott W (2000) Biomonitoring of environmental polycyclic aromatic hydrocarbon exposure by simultaneous measurement of urinary phenanthrene, pyrene and benzo[a]pyrene hydroxides. *J Chromatogr B* 739: 225–229
- Hong YC, Leem JH, Park HS, Lee KH, Lee SJ, Lee CK, Kang D (1999) Variations in urinary 1-hydroxypyrene glucuronide in relation to smoking and the modification effects of GSTM1 and GSTT1. *Toxicol Lett* 108: 217–223
- Horikawa K, Sera N, Otofujii T, Murakami K, Tokiwa H, Hwagawa M, Izumi K, Otsuka H (1991) Pulmonary carcinogenicity of 3,9- and 3,7-dinitrofluoranthene, 3-nitrofluoranthene and benzo[a]pyrene in F344 rats. *Carcinogenesis* 12: 1003–1008
- Horn J, Flesher JW, Lehner AF (1996) 1-Sulfooxymethylpyrene is an electrophilic mutagen and ultimate carcinogen of 1-methyl- and 1-hydroxymethylpyrene. *Biochem Biophys Res Commun* 228: 105–109
- Horning MG, Sheng LS, Nowlin JG, Lertratanangkoon K, Horning EC (1987) Analytical methods for the study of urinary thioether metabolites in the rat and guinea pig. *J Chromatogr* 399: 303–319
- Horton AW, Christian GM (1974) Cocarcinogenic versus incomplete carcinogenic activity among aromatic hydrocarbons: Contrast between chrysene and benzo[b]triphenylene. *J Natl Cancer Inst* 53: 1017–1020
- Hoshino K, Hayashi Y, Takehira Y, Kameyama Y (1981) Influence of genetic factors on the teratogenicity of environmental pollutants: Teratogenic susceptibility to benzo[a]pyrene and Ah locus in mice. *Congenit Anom (Kyoto)* 21: 97–103
- Hsu CH, Skipper PL, Tannenbaum SR (1999) DNA adduct formation by secondary metabolites of cyclopenta[cd]pyrene *in vitro*. *Cancer Lett* 136: 137–141
- Hu Y, Zhou Z, Xue X, Li X, Fu J, Cohen B, Melikian AA, Desai M, Tang MS, Huang X, Roy N, Sun J, Nan P, Qu Q (2006) Sensitive biomarker of polycyclic aromatic hydrocarbons (PAHs): urinary 1-hydroxypyrene glucuronide in relation to smoking and low ambient levels of exposure. *Biomarkers* 11: 306–318
- Hueper WC (Ed) (1964) Berufskrebs (Occupational cancer) (German), in Beiträge zur Krebsforschung, Vol 9: Theodor Steinkopf, Dresden and Leipzig, 79–83
- Huggins C, Yang NC (1962) Induction and extinction of mammary cancer. *Science* 137: 257–262
- Hughes MF, Chamulitrat W, Mason RP, Eling TE (1989) Epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene via a hydroperoxide-dependent mechanism catalyzed by lipoxygenases. *Carcinogenesis* 10: 2075–2080
- Hughes NC, Phillips d h (1990) Covalent binding of dibenzpyrenes and benzo[a]pyrene to DNA: evidence for synergistic and inhibitory interactions when applied in combination to mouse skin. *Carcinogenesis* 11: 1611–1619
- Hughes NC, Phillips d h (1991) Dependence on dose of initial and persistent levels of benzo[a]pyrene and dibenzo[a,e]pyrene adducts in mouse tissues. *Proc Am Assoc Cancer Res* 32: 98
- Hughes NC, Phillips d h (1993) <sup>32</sup>P-Postlabelling analysis of the covalent binding of benzo[ghi]perylene to DNA *in vivo* and *in vitro*. *Carcinogenesis* 14: 127–133

- Hurley JF, Archibald RM, Collings PL, Fanning DM, Jacobsen M, Steele RC (1983) The mortality of coke workers in Britain. *Am J Ind Med* 4: 691–704
- IARC (International Agency for Research on Cancer) (1973) Certain polycyclic aromatic hydrocarbons and heterocyclic compounds, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 3: IARC, Lyon, France
- IARC (1983) Polynuclear aromatic compounds, Part 1: Chemical, environment and experimental data, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 32: IARC, Lyon, France
- IARC (1984 a) Polynuclear aromatic compounds, Part 2: Carbon blacks, mineral oils and some nitroarenes, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 33: IARC, Lyon, France
- IARC (1984 b) Polynuclear aromatic compounds, Part 3: Industrial exposures in aluminium production, coal gasification, coke production, and iron and steel founding, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 34: IARC, Lyon, France
- IARC (1985) Polynuclear aromatic compounds, Part 4: Bitumens, coal-tars and derived products, shale-oils and soots, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 35: IARC, Lyon, France
- IARC (1986 a) Tobacco smoking, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 38: IARC, Lyon, France
- IARC (1986 b) Some halogenated hydrocarbons and pesticide exposures, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 41: IARC, Lyon, France
- IARC (1987) Overall evaluations of carcinogenicity: An updating of IARC monographs volumes 1–42, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement 7, Lyon, France
- IARC (2010) Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures, in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 92: IARC, Lyon, France
- Ingram AJ, Phillips JC (1993) The dermal bioavailability of radiolabelled benzo[a]pyrene from acetone or from oils of differing viscosity, assessed by DNA and protein binding. *J Appl Toxicol* 13: 25–32
- IPCS (International Programme on Chemical Safety) (1988) Evaluation of Short-Term Tests for Carcinogens, Cambridge University Press
- Ishinishi N, Kodema Y, Kunitake E, Nobutomo K, Fukushima Y (1976) The carcinogenicity of dusts collected from an open-heart furnace for the smelting of iron: a preliminary experimental study. In: Nordberg GF (Ed) Effects and Dose-Response Relationships of Toxic Metals, Elsevier Scientific Publishers, Amsterdam, 480–488
- Jacob J, Grimmer G, Schmoldt A (1979) Metabolitenprofile von polycyclischen aromatischen Kohlenwasserstoffen nach Vorbehandlung mit verschiedenen Induktoren mikrosomaler Monooxygenasen der Rattenleber (Metabolite profiles of polycyclic aromatic hydrocarbons after pre-treatment with different inducers of microsomal monooxygenases from rat liver) (German). *Hoppe-Seyler's Z Physiol Chem* 360: 1525–1534
- Jacob J, Grimmer G, Raab G, Schmoldt A (1982) The metabolism of pyrene by rat liver microsomes and the influence of various monooxygenase inducers. *Xenobiotica* 12: 45–53
- Jacob J, Brune H, Dettbarn G, Grimmer G, Heinrich U, Mohtashamipur E, Norpoth K, Pott F, Wenzel-Hartung R (1989) Urinary and faecal excretion of pyrene and hydroxypyrene by rats after oral, intraperitoneal, intratracheal or intrapulmonary application. *Cancer Lett* 46: 15–20

- Jacob J (1990) Sulfur analogues of polycyclic aromatic hydrocarbons (thiaarenes), in Cambridge Monographs on Cancer Research, Cambridge University Press, Cambridge
- Jacob J, Brune H, Grimmer G, Heinrich U, Mohtashamipur E, Norpoth K, Pott F, Wenzel-Hartung R (1990) Urinary and faecal excretion of metabolites after various modes of administration of polycyclic aromatic hydrocarbons (PAH) to rats. In: Seemayer NH, Hadnagy W (Eds) *Environmental Hygiene II*, Springer-Verlag, Berlin, 87–90
- Jacob J, Schmoldt A, Augustin C, Raab G, Grimmer G (1991) Rat liver microsomal ring- and S-oxidation of thiaarenes with central or peripheral thiophene rings. *Toxicology* 67: 181–194
- Jacob J (1996) The significance of polycyclic aromatic hydrocarbons as environmental carcinogens. *Pure Appl Chem* 68: 301–308
- Jacob J, Doehmer J, Grimmer G, Soballa V, Raab G, Seidel A, Greim H (1996 a) Metabolism of phenanthrene, benz[a]anthracene, benzo[a]pyrene and benzo[c]phenanthrene by eight cDNA-expressed human and rat cytochromes. *Polycyclic Aromat Compd* 10: 1–9
- Jacob J, Raab G, Soballa V, Holtkamp D, Schmalix W, Grimmer G, Greim H, Doehmer J, Seidel A (1996 b) Cytochrome P450-mediated activation of phenanthrene in genetically engineered V79 Chinese hamster cells. *Environ Toxicol Pharmacol* 1: 1–11
- Jacob J, Grimmer G, Dettbarn G (1999 a) Profile of urinary phenanthrene metabolites in smokers and non-smokers. *Biomarkers* 4: 319–327
- Jacob J, Raab G, Soballa VJ, Luch A, Grimmer G, Greim H, Doehmer J, Morrison HG, Stegeman JJ, Seidel A (1999 b) Species-dependent metabolism of benzo[c]phenanthrene and dibenzo[a,l]pyrene by various CYP450 isoforms. *Polycyclic Aromat Compd* 16: 191–203
- Jacob J, Seidel A (2002) Zwischenbericht zum Verbundvorhaben des HVBG: PAH-Belastung am Arbeitsplatz – Reduzierung hoher innerer Belastung durch betriebliche Maßnahmen anhand von Ambient und Biological Monitoring. Teilprojekt B: Bestimmung mehrfach-hydroxylierter PAH-Metaboliten und der individuellen Suszeptibilität (Interim report on the joint HVBG research project: PAH exposure at the workplace – reduction of high systemic exposure by occupational measures via ambient and biological monitoring. Subproject B: Determination of poly-hydroxylated PAH metabolites and individual susceptibility) (German)
- Jacob J (2007 a) Communication to the secretariat of the Commission, July 15, 2007
- Jacob J (2007 b) Communication to the secretariat of the Commission, Nov. 2, 2007
- Järnholm B, Lavenius B (1987) Mortality and cancer morbidity in workers exposed to cutting fluids. *Arch Environ Health* 42: 36–366
- Jerina DM (1983) The 1982 Bernard B. Brodie award lecture: metabolism of aromatic hydrocarbons by the cytochrome P 450 system and epoxide hydrolase. *Drug Metab Dispos* 11: 1–4
- Jerina DM, Chadha A, Cheh AM, Schurdak ME, Wood AW, Sayer JM (1991) Covalent bonding of bay-region diol epoxides to nucleic acids. In: Witmer CM, Snyder R, Jollow DJ, Kalf GF, Kocsis JJ, Sipes IG (Eds) *Biological Reactive Intermediates IV. Molecular and Cellular Effects and Their Impact on Human Health*, Plenum Press, New York, NY, 533–553
- Jez JM, Penning TM (2001) The aldo-keto reductase (AKR) superfamily: an update. *Chem Biol Interact* 130: 499–525
- Johnson S (1968) Effect of thymectomy on the induction of skin tumours by dibenzanthracene, and of breast tumours by dimethylbenzanthracene in mice of the IF strain. *Br J Cancer* 22: 755–761
- Jongeneelen FJ, Leijdekkers CM, Bos RP, Theuvs JLG, Henderson PT (1985 a) Excretion of 3-hydroxybenzo[a]pyrene and mutagenicity in rat urine after exposure to benzo[a]pyrene. *J Appl Toxicol* 5: 277–282

- Jongeneelen FJ, Bos RP, Anzion RBM, Theuvs JLG, Henderson PT (1986) Biological monitoring of polycyclic aromatic hydrocarbons: metabolites in urine. *Scand J Work Environ Health* 12: 137–143
- Jongeneelen FJ, Anzion RBM, Henderson PT (1987) Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J Chromatogr* 413: 227–232
- Jongeneelen FJ, Scheepers PTJ, Groenedijk A, van Aerts LAGJM, Anzion RBM, Bos RP, Veenstra SJ (1988) Airborne concentrations, skin contamination, urinary metabolite excretion of polycyclic aromatic hydrocarbons among paving workers exposed to coal tar-derived road tars. *Am Ind Hyg Assoc J* 49: 600–607
- Jongeneelen FJ (1992) Biological exposure limit for occupational exposure to coal tar pitch volatiles at cokeovens. *Int Arch Occup Environ Health* 63: 511–516
- Jongeneelen FJ (1997) Methods for routine biological monitoring of carcinogenic PAH-mixtures. *Sci Total Environ* 199: 141–149
- Joseph P, Long DJ, Klein-Szanto AJ, Jaiswal AK (2000) Role of NAD(P)H:quinone oxidoreductase 1 (DT diaphorase) in protection against quinone toxicity. *Biochem Pharmacol* 60: 207–214
- Kadamani S, Asal NR, Nelson RY (1989) Occupational hydrocarbon exposure and risk of renal cell carcinoma. *Am J Ind Med* 15: 131–141
- Kaden DA, Hites RA, Thilly WG (1979) Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. *Cancer Res* 39: 4152–4159
- Kallistratos G, Pfau A (1971) Zur Wirkung von 3,4-Benzpyren bei großen Säugetieren (On the effect of 3,4-benzo[a]pyrene in large mammals) (German). *Naturwissenschaften* 58: 222
- Kang JW, Cho SH, Kim H, Lee CH (2002) Correlation of urinary 1-hydroxypyrene and 2-naphthol with total suspended particulates in ambient air in municipal middle-school students in Korea. *Arch Environ Health* 57: 377–382
- Kao J, Patterson FK, Hall J (1985) Skin penetration and metabolism of topically applied chemicals in six mammalian species, including man: An *in vitro* study with benzo[a]pyrene and testosterone. *Toxicol Appl Pharmacol* 81: 502–516
- Karakaya A, Ates I, Yucesoy B (2004) Effects of occupational polycyclic aromatic hydrocarbon exposure on T-lymphocyte functions and natural killer cell activity in asphalt and coke oven workers. *Hum Exp Toxicol* 23: 317–322
- Karcher W, Nelen A, Depaus R, van Eijk J, Glaude P, Jacob J (1981) New results in the detection, identification and mutagenic testing of heterocyclic polycyclic aromatic hydrocarbons. In: Cooke M, Dennis AJ (Eds) *Chemical Analysis and Biological Fate: Polynuclear Aromatic Hydrocarbons*, Battelle Press, Columbus OH, USA, 317–327
- Karcher W, Dubois J, Fordham RJ, Glaude P, Barale R, Zucconi D (1984) Molecular spectra and mutagenic activity of some nitrogen containing heterocyclic aromatic compounds. In: Cooke M, Dennis AJ (Eds) *Mechanisms, Methods and Metabolism: Polynuclear Aromatic Hydrocarbons*, Battelle Press, Columbus, OH, USA, 688–696
- Karlehagen S, Andersen A, Ohlson CG (1992) Cancer incidence among creosote-exposed workers. *Scand J Work Environ Health* 18: 26–29
- Kawabata TT, White KL (1990) Effects of naphthalene and naphthalene metabolites on the *in vitro* humoral immune response. *J Toxicol Environ Health* 30: 53–67
- Kawai M (1979) Review of toxicological and occupational aspects of naphthalene (Japanese). *Aromatics* 31: 161–181
- Keimig SD, Kirby KW, Morgan DP, Kaiser JE, Hubert TD (1983) Identification of 1-hydroxypyrene as a major metabolite of pyrene in pig urine. *Xenobiotica* 13: 415–420

- Kemp MQ, Liu W, Thorne PA, Kane MD, Selmin O, Romagnolo DF (2006) Induction of the transferrin receptor gene by benzo[a]pyrene in breast cancer MCF-7 cells: potential as a biomarker of PAH exposure. *Environ Mol Mutagen* 47: 518–526
- Kennaway FL (1924) On the cancer-producing factor in tar. *Br Med J* 1: 564–567
- Kennaway EL (1930) Further experiments on cancer-producing substances. *Biochem J* 24: 497–504
- Kennaway NM, Kennaway EL (1936) A study of the incidence of cancer of the lung and larynx. *J Hyg* 36: 236–267
- Kennaway EL, Kennaway NM (1947) A further study of the incidence of cancer of the larynx. *Br J Cancer* 1: 260–298
- Ketkar M, Green V, Schneider P, Mohr U (1979) Investigations on the carcinogenic burden by air pollution in man. Intratracheal instillation studies with benzo[a]pyrene in a mixture of tris buffer and saline in Syrian golden hamsters. *Cancer Lett* 6: 279–284
- Kim YD, Lee CH, Nan HM, Kang JW, Kim H (2003) Effects of genetic polymorphisms in metabolic enzymes on the relationships between 8-hydroxydeoxyguanosine levels in human leukocytes and urinary 1-hydroxypyrene and 2-naphthol concentrations. *J Occup Health* 45: 160–167
- Klein M (1960) A comparison of the initiating and promoting actions of 9,10-dimethyl-1,2-benzanthracene and 1,2,5, 6-dibenzanthracene in skin tumorigenesis. *Cancer Res* 20: 1179–1183
- Klein M (1963) Susceptibility of strain B6AF1/J hybrid infant mice to tumorigenesis with 1,2-benzanthracene, deoxycholic acid, and 3-methylcholanthrene. II. Tumours called forth by painting the skin with dibenzpyrene. *Cancer Res* 23: 1701–1707
- Kleinenberg HE (1939) Investigations on the blastomogenic effect of 3:4:8:9-dibenzpyrene and some of its derivatives (Russian). *Arch Biol Nauk* 56: 19–47
- Klemme JC, Mukhtar H, Elmetts CA (1987) Induction of contact hypersensitivity to dimethylbenz[a]anthracene and benzo[a]pyrene in C3H/HeN mice. *Cancer Res* 47: 6074–6078
- Kliesch U, Roupoval I, Adler ID (1982) Induction of chromosome damage in mouse bone marrow by benzo[a]pyrene. *Mutat Res* 102: 265–273
- Knake E (1956) Über schwache geschwulsterzeugende Wirkung von Naphthalin und Benzol (Weak oncogenic effect of naphthalene and benzene) (German). *Virchows Arch* 329: 141–176
- Knecht U, Elliehausen H, Weitowitz HJ (1986) Gaseous and adsorbed PAH in an iron foundry. *Br J Ind Med* 43: 834–838
- Knecht U, Weitowitz HJ (1989) Risk of cancer from the use of tar bitumen in road works. *Br J Ind Med* 46: 24–30
- Kogevinas M, Mannetje A, Cordier S, Ranft U, Gonzales CA, Vineis P, Chang-Claude J, Lynge E, Wahrendorf J, Tzonou A, Jockel KH, Serra C, Porru S, Hours M, Greiser E, Boffetta P (2003) Occupation and bladder cancer among men in Western Europe. *Cancer Causes Control* 14: 907–914
- Kollock R, Meinel W, Schneider H, Batke M, Frank H, Seidel A, Glatt HR (2008) Efficient oxidation of promutagenic hydroxymethylpyrenes by cDNA-expressed human alcohol dehydrogenase ADH2 and its inhibition by various agents. *Biochem Pharmacol* 75: 527–537
- Konar NR, Roy HK, De MN (1939) Naphthalene poisoning. *Indian Med Gaz* 74: 723–725
- Kondraganti SR, Muthiah K, Jiang W, Barrios R, Moorthy B (2005) Effects of 3-methylcholanthrene on gene expression profiling in the rat using cDNA microarray analyses. *Chem Res Toxicol* 18: 1634–1641
- Kouri RE, Connolly GM, Nebert DW, Lubet RA (1983) Association between susceptibility to dibenzanthracene induced fibrosarcoma formation and the Ah locus. *Int J Cancer* 32: 765–768

- Krämer M, Bimboes D, Greim H (1974) *Salmonella typhimurium* and *E.coli* to detect chemical mutagens. *Arch Pharmacol* 284: R46
- Krewski D, Thorslund T, Withey J (1989) Carcinogenic risk assessment of complex mixtures. *Toxicol Ind Health* 5: 851–867
- Kubasiewicz M, Starzynski Z, Szymzak W (1991) Case-referent study on skin cancer and its relation to occupational exposure to polycyclic aromatic hydrocarbons-II. Study results. *Pol J Occup Med Environ Health* 4: 141–147
- Künstler K (1983) Failure to induce tumors by intratracheal instillation of automobile exhaust condensate and fractions thereof in Syrian golden hamsters. *Cancer Lett* 18: 105–108
- Kuljukkka-Rabb T, Nylund L, Vaaranrinta R, Savela K, Mutanen P, Veidebaum T, Sorsa M, Rannug A, Peltonen K (2002) The effect of relevant genotypes on PAH exposure-related biomarkers. *J Expos Anal Environ Epidemiol* 12: 81–91
- Kulkarni AP (2002) Lipoxygenases. In: Ioannides C (Ed) *Handbook of Enzyme Systems that Metabolise Drugs and Other Xenobiotics*, John Wiley & Sons, Sussex, 231–279
- Kuroki T, Heidelberger C (1972) Determination of the h-protein in transformable and transformed cells in culture. *Biochemistry* 11: 2116–2124
- Kuusimäki L, Peltonen Y, Mutanen P, Peltonen K, Savela K (2004) Urinary hydroxy-metabolites of naphthalene, phenanthrene and pyrene as markers of exposure to diesel exhaust. *Int Arch Occup Environ Health* 77: 23–30
- Lacassagne A, Buu-Hoi NP, Rudali G (1945) Inhibition of the carcinogenic action produced by a weakly carcinogenic hydrocarbon on a highly active carcinogenic hydrocarbon. *Br J Exp Pathol* 26: 5–12
- Lacassagne A, Buu-Hoi NP, Zajdela F (1958) Relation entre structure moléculaire et activité cancérogène dans trois séries d'hydrocarbures aromatique hexacycliques (Relation between molecular structure and carcinogenic activity in three series of hexacyclic aromatic hydrocarbons) (French). *C R Acad Sci Paris* 245: 1477–1481
- Lacassagne A, Buu-Hoi NP, Zajdela F, Lavit-Lamy D, Chalvet O (1963 a) Activité cancérogène d'hydrocarbures aromatiques polycycliques à noyau fluoranthène [Carcinogenic activity of fluoranthene-based polycyclic aromatic hydrocarbons] (French). *Acta Unio Int Contra Cancerum* 19: 490–496
- Lacassagne A, Buu-Hoi NP, Zajdela F, Lavit-Lamy D (1963 b) Activité cancérogène élevée du 1,2:3,4-dibenzopyrène et 1,2:4,5 dibenzopyrène [High carcinogenic activity of 1,2:3,4-dibenzopyrene and 1,2:4,5 dibenzopyrene] (French). *C R Hebd Seances* 256: 2728–2731
- Lacassagne A, Buu-Hoi NP, Zajdela F, Vingiello FA (1968) The true dibenzo[a,l]pyrene, a new, potent carcinogen. *Naturwissenschaften* 55: 43
- Lafontaine M, Attenont H, Hubert G, Taiclet A, Truy S (1990) Emission of polycyclic aromatic hydrocarbons in foundries. *Cah Notes Doc* 141: 799–807
- Lafontaine M, Chapmartin C, Simon P, Delsaut P, Funck-Brentano C (2006) 3-hydroxybenzo[a]pyrene in the urine of smokers and non-smokers. *Toxicol Lett* 162: 181–185
- Lake RS, Kropko ML, Pezzutti MR, Shoemaker RH, Igel HJ (1978) Chemical induction of unscheduled DNA synthesis in human skin epithelial cell cultures. *Water Res* 38: 2091–2098
- Laskin S, Kuschner M, Drew RT (1970) Studies in pulmonary carcinogenesis. In: Hanna MG, Nettesheim P, Gilbert J (Eds) *Inhalation Carcinogenesis*, US Atomic Energy Commission, Division of Technical, Information, AEC Symposium Series No 18, Oak Ridge, Tennessee, 321–351
- Laskin S, Sellakumar AR (1978) Comparison of pulmonary carcinogenicity of known carcinogens with and without added H<sub>2</sub>SO<sub>4</sub> mists, airborne respirable particles, and gases. Final report of progress to the Environmental Protection Agency, Proj. No. 68-02-1750



- Lasnitzki A, Woodhouse DL (1944) The effect of 1:2:5:6-dibenzanthracene on the lymph nodes of the rat. *J Anat* 78: 121–128
- Lau HH, Baird WM (1992) The co-carcinogen benzo[*e*]pyrene increases the binding of a low dose of the carcinogen benzo[*a*]pyrene to DNA in Sencar mouse epidermis. *Cancer Lett* 63: 229–236
- LaVoie EJ, Bedenko V, Hirota N, Hecht SS, Hoffmann D (1979) A comparison of the mutagenicity, tumor-initiating activity and complete carcinogenicity of polynuclear aromatic hydrocarbons. In: Jones PW, Leber P (Eds) *Polynuclear Aromatic Hydrocarbons*, Ann Arbor Science Publishers, Ann Arbor, Michigan, USA, 705–721
- LaVoie EJ, Tulley L, Bedenko V, Hoffmann D (1980) Mutagenicity, tumor initiating activity, and metabolism of tricyclic aromatic hydrocarbons. In: Bjørseth A, Dennis AJ (Eds) *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Battelle Press, Columbus, OH, USA, 1041–1057
- LaVoie EJ, Tulley-Freiler L, Bedenko V, Hoffmann D (1981) Mutagenicity, tumor-initiating activity and metabolism of methylphenanthrenes. *Cancer Res* 41: 3441–3447
- LaVoie EJ, Amin S, Hecht SS, Furuya K, Hoffmann D (1982) Tumor initiating activity of dihydrodiols of benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene. *Carcinogenesis* 3: 49–53
- LaVoie EJ, Braley J, Rice JE, Rivenson A (1987) Tumorigenic activity of nonalternant polynuclear aromatic hydrocarbons in newborn mice. *Cancer Lett* 34: 15–20
- Leadon SA, Stampfer MR, Bartley J (1988) Production of oxidative DNA damage during the metabolic activation of benzo[*a*]pyrene in human mammary epithelial cells correlated with cell killing. *Proc Natl Acad Sci USA* 85: 4365–4368
- Lecoq S, Perin F, Plessis MJ, Strapelias H, Duquesne M (1989) Comparison of the *in vitro* metabolisms and mutagenicities of dibenzo[*a,c*]anthracene, dibenzo[*a,h*]anthracene, and dibenzo[*a,j*]anthracene: Influence of norharman. *Carcinogenesis* 10: 461–469
- Lecoq S, Ni She M, Hewer A, Grover PL, Platt KL, Oesch F, Phillips dh (1991) The metabolic activation of dibenz[*a,h*]anthracene in mouse skin examined by <sup>32</sup>P-postlabelling: Minor contribution of the 3,4-diol 1,2-oxides to DNA binding. *Carcinogenesis* 12: 1079–1083
- Lee H, Lin JY (1988) Antimutagenic activity of extracts from anticancer drugs in Chinese medicine. *Mutat Res* 204: 229–234
- Legraverend C, Harrison DE, Ruscetti FW, Nebert DW (1983) Bone marrow toxicity induced by oral benzo[*a*]pyrene: protection resides at the level of the intestine and liver. *Toxicol Appl Pharmacol* 70: 390–401
- Legraverend C, Guenther TM, Nebert DW (1984) Importance of the route of administration for genetic differences in benzo[*a*]pyrene induced *in utero* toxicity and teratogenicity. *Teratology* 29: 35–47
- Lertratanangkoon K, Horning MG, Middleditch BS, Tsang WS, Griffin GW (1982) Urinary bivalent sulfur metabolites of phenanthrene excreted by the rat and guinea pig. *Drug Metab Dispos* 10: 614–623
- Lesage J, Perrault G, Durand P (1987) Evaluation of worker exposure to polycyclic aromatic hydrocarbons. *Am Ind Hyg Assoc J* 48: 753–759
- Letzel S, Drexler H (1998) Occupationally related tumors in tar refinery workers. *J Am Acad Dermatol* 39: 712–720
- Levin W, Wood AW, Chang RL, Yagi H, Mah HD, Jerina DM, Conney AH (1978) Evidence for bay region activation of chrysene 1, 2-dihydrodiol to an ultimate carcinogen. *Cancer Res* 38: 1831–1834

- Levin W, Chang RL, Wood AW, Yagi H, Thakker DR, Jerina DM, Conney AH (1984) High stereo-selectivity among the optical isomers of the diastereometric bay-region diolepoxides of benzo[*a*]anthracene in the expression of tumorigenic activity in murine tumor models. *Cancer Res* 44: 929–933
- Levin JO, Rhén M, Sikström E (1995 a) Occupational PAH exposure: urinary 1-hydroxypyrene levels of coke oven workers, aluminium smelter pot-room workers, road pavers, and occupationally non-exposed persons in Sweden. *Sci Total Environ* 163: 169–177
- Levin JO (1995 b) First international workshop on hydroxypyrene as a biomarker for PAH exposure in man – summary and conclusions. *Sci Total Environ* 163: 165–168
- Li Y, Xu H, Song Y, Yang X, Li Z (1991) A study on the relationship between urinary BaP and SCE frequency in rats (Chinese). *Zhongguo Huanjing Kexue* 11: 361–364
- Li Z, Romanoff LC, Trinidad DA, Hussain N, Jones RS, Porter EN, Patterson DG Jr., Sjodin A (2006) Measurement of urinary monohydroxy polycyclic aromatic hydrocarbons using automated liquid-liquid extraction and gas chromatography/isotope dilution high-resolution mass spectrometry. *Anal Chem* 78: 5744–5751
- Lijinsky W, Saffiotti U (1965) Relationships between structure and skin tumorigenic activity among hydrogenated derivatives of several polycyclic aromatic hydrocarbons. *Ann It di Derm Clin e Sper* 19: 34–44
- Lijinski W, Garcia H (1972) Skin carcinogenesis tests of hydrogenated derivatives of anthanthrene and other polynuclear hydrocarbons. *Z Krebsforsch* 77: 226–230
- Lilienblum W, Bock-Hennig BS, Bock KW (1985) Protection against toxic redox cycles between benzo[*a*]pyrene-3,6-quinone and its quinol by 3-methylcholanthrene-inducible formation of the quinol mono- and diglucuronide. *Mol Pharmacol* 27: 451–458
- Lind C, Hochstein P, Ernster L (1982) DT-diaphorase as a quinone reductase: a cellular control device against semiquinone and superoxide radical formation. *Arch Biochem Biophys* 216: 178–185
- Lind C (1985) Formation of benzo[*a*]pyrene-3,6-quinol mono- and diglucuronides in rat liver microsomes. *Arch Biochem Biophys* 240: 226–235
- Lindstedt G, Sollenberg J (1982) Polycyclic aromatic hydrocarbons in the occupational environment with special reference to benzo[*a*]pyrene measurements in Swedish industry. *Scand J Work Environ Health* 8: 1–19
- Lintelmann J, Boos KS, Kettrup A (1994 a) Biomonitoring bei PAK-Belastung mit Hilfe einer LC-LC-Kopplung für die Bestimmung von 1-Hydroxypyren im Urin. Teil 1 (Biomonitoring in the case of PAH exposure by means of LC/LC coupling for the determination of 1-hydroxypyrene in urine. Part 1) (German). *BlOforum* 11: 442–445
- Lintelmann J, Boos KS, Kettrup A (1994 b) Biomonitoring bei PAK-Belastung mit Hilfe einer LC-LC-Kopplung für die Bestimmung von 1-Hydroxypyren im Urin. Teil 2 (Biomonitoring in the case of PAH exposure by means of LC/LC coupling for the determination of 1-hydroxypyrene in urine. Part 2) (German). *BlOforum* 11: 514–516
- Löfroth G, Toftgard R, Nilsson L, Aurell E, Gustafsson JA (1984) Short-term bioassays of nitro derivatives of benzo[*a*]pyrene and perylene. *Carcinogenesis* 5: 925–930
- Lubet RA, Kiss E, Gallagher MM, Dively C, Konri R, Schechtman LM (1983) Induction of neoplastic transformation and DNA single strand breaks in C3H/10T1/2 clone 8 cells by polycyclic hydrocarbons and alkylating agents. *J Natl Cancer Inst* 71: 991–997
- Lubet RA, Kouri RE, Curren RA, Putman DL, Schechtman LM (1990) Induction of mutagenesis and transformation in BALB/c-3T3 clone A31-1 cells by diverse chemical carcinogens. *Environ Mol Mutagen* 16: 13–20

- Ludewig G, Dogra S, Setiabudi F, Seidel A, Oesch F, Glatt H (1991) Quinones derived from polycyclic aromatic hydrocarbons: induction of diverse mutagenic and genotoxic effects in mammalian cells. In: Cooke M, Loening K, Merritt J (Eds) Polynuclear Aromatic Hydrocarbons: Measurements, Means, and Metabolism, Battelle Press, Columbus, OH, USA, 545–556
- Ma Q (2001) Induction of CYP1A1. The AhR/DRE paradigm: transcription, receptor regulation, and expanding biological roles. *Curr Drug Metab* 2: 149–164
- Ma L (2002) Mercaptursäuren und Nucleosidaddukte im Harn als Biomarker in 1-Hydroxymethylpyren-exponierten Ratten (Mercapturic acids and nucleoside adducts in the urine as biomarkers in 1-hydroxymethylpyrene-exposed rats) (German). Diploma dissertation, Potsdam University, Germany
- Ma L, Kuhlow A, Glatt H (2002) Ethanol enhances the activation of 1-hydroxymethylpyrene to DNA adduct-forming species in the rat. *Polycyclic Aromat Compd* 22: 933–946
- Mackell JV, Rieders F, Brieger H, Bauer EL (1951) Acute hemolytic anemia due to ingestion of naphthalene mothballs. *Pediatrics* 7: 722–728
- MacKenzie KM, Angevine DM (1981) Infertility in mice exposed *in utero* to benzo[a]pyrene. *Biol Reprod* 24: 183–191
- Madhavan ND, Naidu KA (2000) Purification and partial characterization of peroxidase from human term placenta of non-smokers: metabolism of benzo[a]pyrene-7,8-dihydrodiol. *Placenta* 21: 501–509
- Malaiyandi M, Benedek A, Holko AP, Bancsi JJ (1982) Measurement of potentially hazardous polynuclear aromatic hydrocarbons from occupational exposure during roofing and paving operations. In: Cooke M, Dennios AJ, Fisher GL (Eds) Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry, Battelle-Press, Columbus, OH, USA, 471–489
- Malcolm HM, Dobsen S (1994) The calculation of an environmental assessment level (EAL) for atmospheric PAH using relative potencies. Department of the Environment, Report No DoE/HMIP/RR/94/041, ITE Project TO7061i1 Report to the Her Majesty's Inspectorate of Pollution, London
- Mamber SW, Bryson V, Katz SE (1984) Evaluation of the *Escherichia coli* K 12 inducetest for detection of potential chemical carcinogens. *Mutat Res* 130: 141–151
- Mandel JS, McLaughlin JK, Schlehofer B, Møller A, Helmer U, Lindblad P, McCredie M, Adami HO (1995) International renal-cell cancer study. IV Occupation. *Int J Cancer* 61: 601–605
- Mane SS, Purnell DM, Hsu IC (1990) Genotoxic effects of five polycyclic aromatic hydrocarbons in human and rat mammary epithelial cells. *Environ Mol Mutagen* 15: 78–82
- Mannschreck C, Gündel J, Angerer J (1996) Occupational exposure to PAHs – Biological monitoring of hydroxylated metabolites. *Polycyclic Aromat Compd* 11: 11–18
- Manz A (1984) Krebsrisiko bei Ofenblockarbeitern von Kokereibetrieben (Cancer risk among coke oven workers of coking plants) (German). *Zentralbl Arbeitsmed* 34: 34–41
- Marczynski B, Raulf-Heimsoth M, Preuss R, Kappler M, Schott K, Pesch B, Zoubek G, Hahn JU, Mensing T, Angerer J, Kählerlein 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: 645–651
- Marino DJ (1987) Evaluation of Pluronic® Polyol F 17 as a vehicle for petroleum hydrocarbons in the *Salmonella*/microsomal assay. *Environ Mutagen* 9: 307–316
- Marnett L, Johnson JT, Bienkowski MJ (1979) Arachidonic acid-dependent metabolism of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by ram seminal vesicles. *FEBS Lett* 106: 13–16

- Martin CN, McDermid AC, Garner RC (1978) Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. *Cancer Res* 38: 2621–2627
- Martin F, Hoepfner I, Scherer G, Adlkofer F, Dettbarn G, Grimmer G (1989) Urinary excretion of hydroxyphenanthrenes after intake of polycyclic aromatic hydrocarbons. *Environ Intern* 15: 41–47
- Masuda Y, Kagawa R (1972) A novel synthesis and carcinogenicity of dibenzo[a,l]pyrene. *Chem Pharm Bull* 20: 2736–2737
- Matsuoka A, Hayashi M, Ishidate MJ (1979) Chromosomal aberration tests on 29 chemicals combined with S9 mix *in vitro*. *Mutat Res* 66: 277–290
- Mattison DR, Nightingale MS (1980 a) The biochemical and genetic characteristics of murine ovarian aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity and its relationship to primordial oocyte destruction by polycyclic aromatic hydrocarbons. *Toxicol Appl Pharmacol* 56: 399–408
- Mattison DR, White NB, Nightingale MS (1980 b) The effect of benzo[a]pyrene on fertility, primordial oocyte number, and ovarian response to pregnant mare's serum gonadotropin. *Pediatr Pharmacol* 1: 143–151
- Mattison DR, Singh H, Takizawa K, Thomford PJ (1989) Ovarian toxicity of benzo[a]pyrene and metabolites in mice. *Reprod Toxicol* 3: 115–125
- Mavourin KH, Blakey dh, Cimono MC, Salamone MF, Heddle JA (1990) The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutat Res* 239: 29–80
- McCann J, Choi E, Yamasaki E, Ames BN (1975 a) Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. *Proc Natl Acad Sci USA* 72: 5135–5139
- McCann J, Spingarn NE, Kobori J, Ames BN (1975 b) Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. *Proc Natl Acad Sci USA* 72: 979–983
- McClure P (2004) Communication to the secretariat of the Commission, Nov. 12, 2004
- McCormick DL, Burns FJ, Albert RE (1981) Inhibition of benzo[a]pyrene-induced mammary carcinogenesis by retinyl acetate. *J Natl Cancer Inst* 66: 559–564
- McLaughlin JK, Mandel JS, Blot WJ, Schuman LM, Mehl ES, Fraumeni JF (1984) A population based case-control study of renal cell carcinoma. *J Natl Cancer Inst* 72: 275–284
- McLaughlin JK, Blot WJ, Mehl ES, Steward PA, Venable FS, Fraumeni JF (1985) Petroleum related employment and renal cancer. *J Occup Med* 27: 672–674
- McClean MD, Rinehart RD, Ngo L, Eisen EA, Kelsey KT, Herrick RF (2004) Inhalation and dermal exposure among asphalt paving workers. *Ann Occup Hyg* 48: 663–671
- Mellemgaard A, Engholm G, McLaughlin JK, Olsen JH (1994) Occupational risk factors for renal cell carcinoma in Denmark. *Scand J Work Environ Health* 20: 160–165
- Merlo F, Andreassen A, Weston A, Pan CF, Haugen A, Valerio F, Reggiardo G, Fontana V, Garte S, Puntoni R, Abbondandolo A (1998) Urinary excretion of 1-hydroxypyrene as a marker of exposure to urban air level of polycyclic aromatic hydrocarbons. *Cancer Epidemiol Biomarkers Prev* 7: 147–155
- Miller JA, Miller EC (1963) The carcinogenicities of fluoro derivatives of 10-methyl-1,2-benzanthrene. II. Substitution of the K region and the 3', 6-, and 7-positions. *Cancer Res* 23: 229–239
- Miller MM, Plowchalk DR, Weitzman GA, London SN, Mattison DR (1992) The effect of benzo[a]pyrene on murine ovarian and corpora lutea volumes. *Am J Obstet Gynecol* 166: 1535–1541
- Milo GE, Blakeslee J, Yohn DS, DiPaolo JA (1978) Biochemical activation of aryl hydrocarbon hydroxylase activity, cellular distribution of polynuclear hydrocarbon metabolites, and DNA

- damage by polynuclear hydrocarbon products in human cells *in vitro*. *Cancer Res* 38: 1638–1644
- Miralis JC, Tyson CK, Butterworth BE (1982) Detection of genotoxic carcinogens in the *in vivo-in vitro* hepatocyte DNA repair assay. *Environ Mutagen* 4: 553–562
- Misra B, Amin S (1990) Synthesis and mutagenicity of *trans*-dihydrodiol metabolites of benzo[b]-naphtho[2,1-d]thiophene. *Chem Res Toxicol* 3: 93–97
- Mitchell CE (1982) Distribution and retention of benzo[a]pyrene in rats after inhalation. *Toxicol Lett* 11: 35–42
- Miyata M, Kudo G, Lee YH, Yang TJ, Gelboin HV, Fernandez-Salguero P, Kimura S, Gonzalez FJ (1999) Targeted disruption of the microsomal epoxide hydrolase gene: microsomal epoxide hydrolase is required for the carcinogenic activity of 7,12-dimethylbenzo[a]anthracene. *J Biol Chem* 274: 23963–23968
- Mohr U (1969) Untersuchungen über die kanzerogenen Eigenschaften von 3,4-, 10,11-, 12,13-Tribenzfluoranthren an Mäusen (Studies on the carcinogenic properties of 3, 4-10, 11-, 12, 13-tribenzofluoranthene in mice). *Arch Hyg* 153: 495–510
- Mohr U (1971) Kanzerogenese durch Diäthylnitrosamin [Carcinogenicity of diethylnitrosamine] (German). *Fortschr Med* 89: 251–253
- Monks TJ, Lau SS (1998) The pharmacology and toxicology of polyphenolic-glutathione conjugates. *Annu Rev Pharmacol Toxicol* 38: 229–255
- Monteith DK, Gupta RC (1992) Carcinogen-DNA adducts in cultures of rat and human hepatocytes. *Cancer Lett* 62: 87–93
- Montesano S, Saffiotti U, Shubik PH (1970) The role of topical and systemic factors in experimental respiratory carcinogenesis. In: Hanna MG, Nettesheim P, Gilbert JR (Eds) *Inhalation Carcinogenesis*, US Atomic Commission, National Technical Information, Service, US Department of Commerce, Springfield
- Montizaan GK, Kramers PGH, Janus JA, Posthumus R (1989) Integrated criteria document PAH: Effects of 10 selected compounds. Appendix to Report No. 758474011. Bilthoven, National Institute of Public Health and Environmental Protection, 180 p (Republication in March 1989 of addendum to Report No. 758447007)
- Moss GP (1998) Nomenclature of fused and bridged fused ring systems. (IUPAC recommendations 1998). *Pure Appl Chem* 70: 143–216
- Mossanda K, Poncelet F, Fouassin A, Mercier A (1979) Short papers. Detection of mutagenic polycyclic aromatic hydrocarbons in African smoked fish. *Food Cosmet Toxicol* 17: 141–143
- Müller E (1968) Kanzerogene Substanzen in Wasser und Boden. XX. Untersuchungen über die kanzerogenen Eigenschaften von 1. 12-Benzperylene [Carcinogenic compounds in water and soil. XX. Investigation into the carcinogenic properties of benzo[ghi]perylene] (German). *Arch Hyg* 152: 23–36
- Muller P, Leece B, Raha D (1995) Dose-Response Assessment PAH, Ministry of the Environment and Energy, Ottawa, Ontario
- Muller P, Leece B, Raha D, Li-Muller A (1997) Scientific Criteria Document for Multimedia Standards Development Polycyclic Aromatic Hydrocarbons (PAH). Part 1: Hazards Identification and Dose-Response Assessment, Ministry of Environment and Energy, Standards, Development Branch Ontario, Ontario, Canada
- Mumford JL, Li X, Hu F, Lu XB, Chuang JC (1995) Human exposure and dosimetry of polycyclic aromatic hydrocarbons in urine from Xuan Wei, China with high lung cancer mortality associated with exposure to unvented coal smoke. *Carcinogenesis* 16: 3031–3036

- Nagabhushan M, Hussong J, Polverini PJ, Solt DB (1990) Inhibition of hamster buccal pouch epithelial cell replication during *in vitro* exposure to polycyclic aromatic hydrocarbons. *Proc Am Assoc Cancer Res* 31: 86
- Nakamura J, Swenberg JA (1999) Endogenous apurinic/aprimidinic sites in genomic DNA of mammalian tissues. *Cancer Res* 59: 2522–2526
- Nan HM, Kim H, Lim HS, Choi JK, Kawamoto T, Kang JW, Lee CH, Kim YD, Kwon EH (2001) Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1 and GSTT1 on urinary 1-hydroxypyrene and 2-naphthol concentrations. *Carcinogenesis* 22: 787–793
- Nau H (1994) Toxikokinetik (Toxicokinetics) (German). In: Marquardt H, Schäfer SG (Eds) *Lehrbuch der Toxikologie*, BI-Wissenschaftsverlag, Mannheim, Leipzig, Wien, Zürich, 49
- Neal J, Rigdon RH (1967) Gastric tumors in mice fed benzo[a]pyrene: A quantitative study. *Tex Rep Biol Med* 25: 553–557
- Nemoto N, Gelboin HV (1976) Enzymatic conjugation of benzo[a]pyrene oxides, phenols and dihydrodiols with UDP-glucuronic acid. *Biochem Pharmacol* 25: 1221–1226
- Nelson G, Ross J, Prusiewicz CM, Sangaiah R, Gold A (2002) Identification of stereochemical configurations of cyclopenta[cd]pyrene DNA adducts in strain A/J mouse lung and C3H10T1/2Cl8 cells. *Polycyclic Aromat Compd* 22: 923–931
- Nemoto N, Takayama S, Gelboin HV (1978) Sulfate conjugation of benzo[a]pyrene metabolites and derivatives. *Chem Biol Interact* 23: 19–30
- Nerurkar PV, Okinaka L, Aoki C, Seifried A, Lum-Jones A, Wilkens LR, LeMarchand L (2000) CYP1A1, GSTM1, and GSTP1 genetic polymorphisms and urinary 1-hydroxypyrene excretion in non-occupationally exposed individuals. *Cancer Epidemiol Biomarkers Prev* 9: 1119–1122
- Nesnow S, Triplett LL, Slaga TJ (1985) Studies on the tumor initiating, tumor promoting and tumor co-initiating properties of respiratory carcinogens. In: Mass M, Kaufman D, Siegfried J, Steele V, Nesnow S (Eds) *Cancer of the Respiratory Tract Predisposing Factors*, Raven Press, New York, 257–278
- Nesnow S, Ross J, Beck S, Lasley J, Nelson G, Lamberg G, Platt KL, Agarwal SC (1994) Morphological transformation and DNA adduct formation by dibenz[a,h]anthracene and its metabolites in C3H10T1/2Cl8 cells. *Carcinogenesis* 15: 2225–2231
- Nesnow S, Davis C, Nelson G, Ross JA, Allison J, Adams L, King LC (1997) Comparison of the morphological transforming activities of dibenzo[a,l]pyrene and benzo[a]pyrene in C3H10T1/2Cl8 cells and characterization of the dibenzo[a,l]pyrene-DNA adducts. *Carcinogenesis* 18: 1973–1978
- Nesnow S, Mass MJ, Ross JA, Galati AJ, Lambert GR, Gennings C, Carter WH Jr., Stoner GD (1998) Lung tumorigenic interactions in strain A/J mice of five environmental polycyclic aromatic hydrocarbons. *Environ Health Perspect* 106, Suppl 6: 1337–1346
- Nesnow S, Davis S, Pimentel M, Mass MJ, Nelson GB, Ross JA (2000) Interaction analyses of binary mixtures of carcinogenic PAHs using morphological cell transformation of C3H10T1/2Cl8 mouse embryo fibroblasts in culture. *Polycyclic Aromat Compd* 21: 31–42
- Nesnow S, Davis C, Nelson GB, Lambert G, Padgett W, Pimentel M, Tennant AH, Kligerman AD, Ross JA (2002) Comparison of the genotoxic activities of the K-region dihydrodiol of benzo[a]pyrene with benzo[a]pyrene in mammalian cells: morphological cell transformation, DNA damage, and stable covalent DNA adducts. *Mutat Res* 521: 91–102
- Neumann HG (1996) Toxic equivalence factors, problems and limitations. *Food Chem Toxicol* 34: 1045–1051

- Ng KM, Chu I, Bronaugh RL, Franklin CA, Somers DA (1992) Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl)phthalate: Comparison of *in vitro* and *in vivo* results in the hairless guinea pig. *Toxicol Appl Pharmacol* 115: 216–223
- Nisbet C, LaGoy P (1992) Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Regul Toxicol Pharmacol* 16: 290–300
- Nishikawa T, Nakamura T, Fukushima A, Takagi Y (2005) Further evaluation of the skin nucleus test: results obtained using 10 polycyclic aromatic hydrocarbons. *Mutat Res* 588: 58–63
- NIST (2007) NIST Special publication 922. Polycyclic aromatic hydrocarbon structure index <http://ois.nist.gov/pah/>
- Noda N, Matsuzoe D, Konno T, Kawahara K, Yamashita Y, Shirakusa T (2004) Risk for K-ras gene mutations in smoking-induced lung cancer is associated with cytochrome P4501A1 and glutathione S-transferase micro 1 polymorphisms. *Oncol Rep* 12: 773–779
- Nordqvist M, Thakker DR, Vyas KP, Yagi H, Levin W, Ryan DE, Thomas PE, Conney AH, Jerina DM (1981) Metabolism of chrysene and phenanthrene to bay-region diol epoxides by rat liver enzymes. *Mol Pharmacol* 19: 168–178
- Norpoth K, Kemena A, Jacob J, Schümann C (1984) The influence of 18 environmentally relevant polycyclic aromatic hydrocarbons and Clophen A50, as liver monooxygenase inducers, on the mutagenic activity of benz[a]anthracene in the Ames test. *Carcinogenesis* 5: 747–752
- Norpoth K (1990) Systemische Kanzerogenese durch polycyclische aromatische Kohlenwasserstoffe (Systemic carcinogenicity induced by polycyclic aromatic hydrocarbons) (German). *Arbeitsmed Sozialmed Präventivmed* 25: 59–64
- Nousiainen U, Törrönen R, Hänninen O (1984) Differential induction of various carboxylesterases by certain polycyclic aromatic hydrocarbons in the rat. *Toxicology* 32: 243–251
- Noyes WF (1969) Carcinogen-induced neoplasia with metastasis in a South American primate, *Saguinus oedipus*. (33845). *Proc Soc Exp Biol Med* 131: 223–225
- NTP (National Toxicology Program) (1992) Toxicology and carcinogenesis studies of naphthalene (CAS No. 91-20-3) in B6C3F1 mice (inhalation studies). US Department of Health and Human Services, Research Triangle Park, NTP TR 410, NIH Publication No. 92-3141, North Carolina
- NTP (National Toxicology Program) (2000) Toxicology and carcinogenesis studies of naphthalene in F 344/N rats (inhalation studies), US Department of Health and Human Services, TR 500, Public Health Services, Bethesda, MD
- O'Brien KAF, Suverkropp C, Kaqnekal S, Plopper CG, Buckpitt AR (1989) Tolerance to multiple doses of the pulmonary toxicant, naphthalene. *Toxicol Appl Pharmacol* 99: 487–500
- OEHA (Office of Environmental Health Hazard Assessment) (1994) Part B. Health assessment, in Benzo[a]pyrene as a toxic air contaminant. Air Resources Board and Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Berkeley, CA
- Oesch F, Glatt H (1976) Evaluation of the importance of enzymes involved in the control of mutagenic metabolites. In: Montesano R, Bartsch H, Tomatis L (Eds) *Screening Tests in Chemical Carcinogenesis*, IARC, Lyon, France, 255–274
- Oesch F, Bückler M, Glatt H (1981) Activation of phenanthrene to mutagenic metabolites and evidence for at least two different activation pathways. *Mutat Res* 81: 1–10
- Okamoto H, Yoshida D (1981) The isolation and identification of mutation enhancing principles from cellulose pyrolysate. *Agric Biol Chem* 45: 1291–1294
- Old LJ, Benacerraf B, Carswell E (1963) Contact reactivity to carcinogenic polycyclic hydrocarbons. *Nature* 198: 1215–1216

- Oppermann UCT, Maser E (2000) Molecular and structural aspects of xenobiotic carbonyl metabolizing enzymes: role of reductases and dehydrogenases in xenobiotic phase I reactions. *Toxicology* 144: 71–81
- Oshiro Y, Balwierz PS, Soelter SG, Guzzie PJ, Rohrbacher E (1992) Evaluation of mouse peripheral blood micronucleus assay. *Environ Mol Mutagen* 19, Suppl 20: 47
- Oueslati R, Alexandrov K, Chouikha M, Chouroulinkov I (1992) Formation and persistence of DNA adducts in epidermal and dermal mouse skin exposed to benzo[a]pyrene *in vivo*. *In vivo* 6: 231–236
- vrebø S, Ryberg D, Haugen A, Leira HL (1998) Glutathione S-transferase M1 and P1 genotypes and urinary excretion of 1-hydroxypyrene in coke-oven workers. *Sci Total Environ* 220: 25–31
- Pahlman R, Pelkonen O (1987) Mutagenicity studies of different polycyclic aromatic hydrocarbons: The significance of enzymatic factors and molecular structure. *Carcinogenesis* 8: 773–778
- Paika IJ, Beauchesne MT, Randall M, Schreck RR, Latt SA (1981) *In vivo* SCE analysis of 20 coded compounds. In: De Serres FJ, Ashby J (Eds) Evaluation of Short-Term Tests of Carcinogens, Elsevier North-Holland, New York, 672–681
- Palackal NT, Lee SH, Harvey RG, Blair IA, Penning TM (2002) Activation of polycyclic aromatic hydrocarbon *trans*-dihydrodiol proximate carcinogens by human aldo-keto reductase (AKR1C) enzymes and their functional overexpression in human lung carcinoma (A549) cells. *J Biol Chem* 277: 24799–24808
- Pan G, Hanaoka T, Yamano Y, Hara K, Ichiba M, Wang Y, Zhang J, Feng Y, Shujuan Z, Guan D, Gao G, Liu N, Takahashi K (1998) A study of multiple biomarkers in coke oven workers – a cross-sectional study in China. *Carcinogenesis* 19: 1963–1968
- Pancirov RJ, Brown RA (1977) Polynuclear aromatic hydrocarbons in marine tissues. *Environ Sci Technol* 11: 989–992
- Pannett B, Coggon D, Acheson ED (1985) A job-exposure matrix for use in population based studies in England and Wales. *Br J Ind Med* 42: 777–783
- Partanen T, Heikkilä P, Hernberg S, Kauppinen T, Moneta G, Olejajärvi A (1991) Renal cell cancer and occupational exposure to chemical agents. *Scand J Work Environ Health* 17: 231–239
- Partanen T, Boffetta P (1994) Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies. *Am J Ind Med* 26: 721–740
- Pataki J, Huggins C (1969) Molecular site of substituents of benz[a]anthracene related to carcinogenicity. *Cancer Res* 29: 506–509
- Penning TM, Burczynski ME, Hung CF, McCoull KD, Palackal NT, Tsuruda LS (1999) Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active *o*-quinones. *Chem Res Toxicol* 12: 1–18
- Penning TM (2004) Aldo-keto reductases and formation of polycyclic aromatic hydrocarbon *o*-quinones. *Methods Enzymol* 378: 31–67
- Pesch B, Haerting J, Ranft U, Klimpel A, Oelschlägel B, Schill W (2000 a) Occupational risk factors for urothelial carcinoma: agent-specific results from a case-control study in Germany. MURC Study Group. Multicenter urothelial and renal cancer study. *Int J Epidemiol* 29: 238–247
- Pesch B, Haerting J, Ranft U, Klimpel A, Oelschlägel B, Schill W (2000 b) Occupational risk factors for renal cell carcinoma: agent-specific results from a case-control study in Germany. MURC Study Group. Multicenter urothelial and renal cancer study. *Int J Epidemiol* 29: 1014–1024



- Péter S, Palme GE, Röhrborn G (1979) Mutagenicity of polycyclic hydrocarbons. III. Monitoring genetic hazards of benz[*a*]anthracene. *Acta Morphol. Acad Sci Hung* 27: 199–204
- Petry T, Schmid P, Schlatter C (1996) The use of toxic equivalency factors in assessing occupational and environmental health risk associated with exposure to airborne mixtures of polycyclic aromatic hydrocarbons (PAHs). *Chemosphere* 32: 639–648
- Pfeiffer EH (1977) Oncogenic interaction of carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons in mice, in IARC (International Agency for Research on Cancer) Scientific Publications 16, Lyon, France, 69–77
- Phillips d h, Philip L, Sims P (1979) A quantitative determination of the covalent binding of a series of polycyclic hydrocarbons to DNA in mouse skin. *Int J Cancer* 23: 201–208
- Plasterer MR, Bradshaw WS, Booth GM, Carter MW, Schuler RL, Hardin BD (1985) Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: Naphthalene, p-nitrophenol, sodium selenite, dimethylphthalate, ethylenethiourea, and four glycol ether derivatives. *J Toxicol Environ Health* 15: 25–38
- Platt KL, Pfeiffer E, Petrovic P, Friesel H, Beermann D, Hecker E, Oesch F (1990) Comparative tumorigenicity of picene and dibenzo[*a,h*]anthracene in the mouse. *Carcinogenesis* 11: 1721–1726
- Plopper CG, Suverkrupp C, Morin D, Nishio S, Buckpitt A (1992) Relationship of cytochrome P-450 activity of Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. *J Pharmacol Exp Ther* 261: 353–363
- Poirier MC, Weston A, Schoket B, Shamkhani H, Pan CF, McDiarmid MA, Scott BG, Deeter DP, Heller JM, Jacobsen-Kram D, Rothman N (1998) Biomonitoring of United States army soldiers serving in Kuwait in 1991. *Cancer Epidemiol Biomarkers Prev* 7: 545–551
- Polia JA (1941) Investigation on the possible carcinogenic effect of anthracene and chrysene and some of their compounds. II. The effect of subcutaneous injection in rats. *J Ind Hyg Toxicol* 223: 449–451
- Pool BL, Klein P, Schmeizer P, Brendler SY, Schlehofer JR, Grimmer G (1989) Genetic toxicology of thiaarenes. *Environ Mol Mutagen* 15: 155–156, (Abstract)
- Pott F, Brockhaus A, Huth F (1973) Untersuchungen zur Kanzerogenität von polyzyklischen aromatischen Kohlenwasserstoffen im Tierexperiment (Investigations on the carcinogenicity of polycyclic aromatic hydrocarbons in animal studies) (German). *Zentralbl Bakteriol Hyg I Abt Orig B* 157: 34–43
- Pott F, Mohr U, Brockhaus A (1978) Untersuchungen zur Kombinationswirkung von Benzo[*a*]pyren und Dibenz[*a,h*]anthracen mit SO<sub>2</sub>- und NO<sub>2</sub>-Inhalation beim Goldhamster (Studies of the combined effect of benzo[*a*]pyrene and dibenz[*a,h*]anthracene with SO<sub>2</sub> and NO<sub>2</sub> inhalation in golden hamsters) (German). *Lufthyg Silikoseforschung* 11: 225–226
- Pott F, Ziem U, Reiffer FJ, Huth F, Ernst H, Mohr U (1987) Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. *Exp Pathol (Jena)* 32: 129–152
- Pott F, Heinrich U (1990) VI-2 Staub und Staubinhaltsstoffe/Polycyclische aromatische Kohlenwasserstoffe (PAH) (Dust and dust components/polycyclic aromatic hydrocarbons (PAH)) (German). In: Wichmann HE, Schlipkötter W, Füllgraf G (Eds) *Handbuch der Umweltmedizin*, Ecomed, Landsberg, 1–23
- Pott F (1975) Chirurgical observations relative to the cataract, the polypus of the nose, the cancer of the scrotum, the different kinds of ruptures, and the mortification of the toes and feet. Hawes, Clarke & Collins, London
- Prasana P, Jacobs MM, Yang SK (1987) Selenium inhibition of benzo[*a*]pyrene, 3-methylcholanthrene, and 3-methylcholanthrylene mutagenicity in *Salmonella typhimurium* strains TA98 and TA100. *Mutat Res* 190: 101–105

- Preuss R, Angerer J, Drexler H (2003 a) Naphthalene – an environmental and occupational toxicant. *Int Arch Occup Environ Health* 76: 556–576
- Preuss R, Roßbach B, Korinath G, Müller J, Drexler H, Angerer J (2003 b) Innere und äußere Belastung mit polycyclischen aromatischen Kohlenwasserstoffen (PAK) bei Beschäftigten eines Bitumen verarbeitenden Betriebes (Internal and external exposure to polycyclic aromatic hydrocarbons (PAH) among workers of a bitumen-processing plant) (German). *Gefahrstoffe Reinhalt Luft* 63: 461–467
- Preuss R, Roßbach B, Müller J, Angerer J (2003 c) Eine deutschlandweite Studie zur inneren und äußeren Exposition gegenüber polycyclischen aromatischen Kohlenwasserstoffen (PAK) am Arbeitsplatz (A study carried out throughout Germany on internal and external occupational exposure to polycyclic aromatic hydrocarbons (PAH)) (German). *Gefahrstoffe Reinhalt Luft* 63: 7–14
- Preuss R, Angerer J (2004 a) Simultaneous determination of 1- and 2-naphthol in human urine using on-line clean-up column-switching liquid chromatography-fluorescence detection. *J Chromatogr B* 801: 307–316
- Preuss R, Koch HM, Wilhelm M, Pischetsrieder M, Angerer J (2004 b) Pilot study on the naphthalene exposure of German adults and children by means of urinary 1- and 2-naphthol levels. *Int J Hyg Environ Health* 207: 441–445
- Preuss R, Drexler H, Böttcher M, Wilhelm M, Brüning T, Angerer J (2005) Current external and internal exposure to naphthalene of workers occupationally exposed to polycyclic aromatic hydrocarbons in different industries. *Int Arch Occup Environ Health* 78: 355–362
- Preuss R, Roßbach B, Wilhelm M, Brüning T, Angerer J (2006) External and internal exposure to polycyclic aromatic hydrocarbons (PAH) among workers in the production of fire-proof materials – proposal of a biological monitoring guidance value. *Int J Hyg Environ Health* 209: 575–580
- Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. *Environ Mutagen* 3: 11–32
- Purchase IFH, Longstaff E, Ashby J, Styles JA, Anderson D, Lefevre PA, Westwood FR (1976) Evaluation of six short term tests for detecting organic chemical carcinogens and recommendations for their use. *Nature* 264: 624–627
- Quarles JM, Sega MW, Schenley CK, Lijinsky W (1979) Transformation of hamster fetal cells by nitrosated pesticides in a transplacental assay. *Cancer Res* 39: 4525–4533
- Ramos J, Villa J, Ruiz A, Armstrong R, Matta J (2004) UV dose determines key characteristics of non-melanoma skin cancer. *Cancer Epidemiol Biomarkers Prev* 13: 2006–2011
- Randerath E, Mittal D, Randerath K (1988) Tissue distribution of covalent DNA damage in mice treated dermally with cigarette ‘tar’: Preference for lung and heart DNA. *Carcinogenesis* 9: 75–80
- Rappaport SM, Waidyanatha S, Serdar S (2004) Naphthalene and its biomarkers as measure of occupational exposure to polycyclic aromatic hydrocarbons. *J Environ Monit* 6: 413–416
- Rastetter WH, Nachbar RB, Russo-Rodriguez S, Wattlely RV, Thilly WG, Andon BM, Jorgensen WL, Ibrahim M (1982) Fluoranthene: Synthesis and mutagenicity of fluor diol epoxides. *J Org Chem* 47: 4873–4878
- Raulf-Heimsoth M, Angerer J, Pesch B, Marczynski B, Hahn JU, Spickenheuer A, Preuss R, Rühl R, Rode P, Brüning T (2007) Biological monitoring as a useful tool for the detection of a coal-tar contamination in bitumen-exposed workers. *J Toxicol Environ Health Part A* in press
- Raveh D, Slaga TJ, Huberman E (1982) Cell-mediated mutagenesis and tumor initiating activity of the ubiquitous polycyclic hydrocarbon, cyclopenta[cd]pyrene. *Carcinogenesis* 3: 763–766

- Reddy MV, Gupta RC, Randerath E, Randerath K (1984)  $^{32}\text{P}$ -Postlabelling test for covalent DNA binding of chemicals in vivo: Application of a variety of aromatic carcinogens and methylating agents. *Carcinogenesis* 5: 231–243
- Reddy MV, Olson JA, Stober GR, Daniel FB (1991) Induction of nuclear anomalies in the gastrointestinal tract by polycyclic aromatic hydrocarbons. *Cancer Lett* 56: 215–224
- Reed GA, Layton ME, Ryan MJ (1988) Metabolic activation of cyclopenteno[*cd*]pyrene by peroxy radicals. *Carcinogenesis* 9: 2291–2295
- Reichhart PA, Althoff J (1980) The effects of cyclophosphamide, nitrosomethylurea and benzo[*a*]pyrene on the rat incisor. *Exp Pathol* 18: 288–296
- Reif AE (1984) Synergism in carcinogenesis. *J Natl Cancer Inst* 73: 25–39
- Rice JE, Hosted T Jr., LaVoie EJ (1984) Fluoranthene and pyrene enhance benzo[*a*]pyrene-DNA adduct formation *in vivo* in mouse skin. *Cancer Lett* 24: 327–333
- Rice JE, Coleman DT, Hosted TJ, LaVoie EJ, McCaustland DJ, Wiley J (1985) Identification of mutagenic metabolites of indeno[1,2,3-*cd*]pyrene formed *in vitro* with rat liver enzymes. *Cancer Res* 45: 5421–5425
- Rice JE, Hosted T Jr., DeFloria MC, LaVoie EJ, Fischer DL, Wiley JC Jr. (1986) Tumor-initiating activity of major *in vivo* metabolites of indeno[1,2,3-*cd*]pyrene on mouse skin. *Carcinogenesis* 7: 1761–1764
- Rice JE, Rivenson A, Braley J, LaVoie EJ (1987) Methylated derivatives of pyrene and fluorene: evaluation of genotoxicity in the hepatocyte/DNA repair test and tumorigenic activity in newborn mice. *J Toxicol Environ Health* 21: 525–532
- Rice JE, Geddie NG, DeFloria MC, LaVoie EJ (1988 a) Structural requirements favoring mutagenic activity among methylated pyrenes in *S. typhimurium*. In: Cooke M, Dennis AJ (Eds) Polynuclear Aromatic Hydrocarbons. A Decade of Progress, Battelle Press, Columbus, OH, USA, 773–785
- Rice JE, Jordan K, Little P, Hussain N (1988 b) Comparative tumor-initiating activity of methylene-bridged and bay-region methylated derivatives of benz[*a*]anthracene and chrysene. *Carcinogenesis* 9: 2275–2278
- Rice JE, Weyand EH, Burrill C, LaVoie EJ (1990) Fluorine probes for investigating the mechanism of activation of indeno[1,2,3-*cd*]pyrene to a tumorigenic agent. *Carcinogenesis* 11: 1971–1974
- Riegel B, Wartman WB, Hill WT, Reeb BB, Shubik P, Stanger DW (1951) Delay of methylcholanthrene skin carcinogenesis in mice by 1,2,5,6-dibenzofluorene. *Cancer Res* 11: 301–306
- Rigdon RH, Neal J (1965) Effects of feeding benzo[*a*]pyrene on fertility, embryos, and young mice. *J Natl Cancer Inst* 34: 297–305
- Rigdon RH, Neal J (1966) Gastric carcinomas and pulmonary adenomas in mice fed benzo[*a*]pyrene. *Tex Rep Biol Med* 24: 195–207
- Rihs HP, Pesch B, Kappler M, Rabstein S, Roßbach B, Angerer J, Scherenberg M, Adams A, Wilhelm M, Seidel A, Brüning T (2005) Occupational exposure to polycyclic aromatic hydrocarbons in German industries: Association between exogenous exposure and urinary metabolites and its modulation by enzyme polymorphisms. *Toxicol Lett* 157: 241–255
- Rippe RM, Pott F (1989) Kanzerogenitätsuntersuchungen von Nitro-PAH (Nitroarenen) im Hinblick auf ihre Bedeutung für die krebserzeugende Wirkung von Dieselmotorabgas (Carcinogenicity studies of nitro-PAH (nitroarenes) with regard to their relevance for the carcinogenicity of diesel engine exhaust) (German). In *Umwelthygiene*, Vol 21: Stefan W Albers Verlag, Düsseldorf, 65–89

- Robinson DE, Mitchell AD (1981) Unscheduled DNA synthesis response of human fibroblasts, WI-38 cells, to 20 coded chemicals. In: De Serres FJ, Ashby J (Eds) Evaluation of Short-Term Tests for Carcinogens, Elsevier North Holland, New York, 517–527
- Rockette HE, Arena VC (1983) Mortality studies of aluminium reduction plant workers: pot-room and carbon department. *J Occup Med* 25: 549–557
- Roe F (1962) Effect of phenanthrene on tumour-initiation by 3,4-benzpyrene. *Br J Cancer* 16: 503–506
- Rogan EG, Cavalieri EL, Walker BA, Balasubramanian R, Wislocki PG, Roth RW, Saugier RK (1986) Mutagenicity of benzylic acetates, sulfates and bromides of polycyclic aromatic hydrocarbons. *Chem Biol Interact* 58: 253–275
- Roggeband R, van den Berg PTM, Steenwinkel MJST, van Delft JHM, van der Wulp CJM, Baan RA (1994 a) In situ detection of different PAH-DNA adducts by means of immunofluorescence microscopy, in Annual Report on Toxicology, 1993/1994, TNO Nutrition and Food Research Institute, Zeist, 79–80
- Roggeband R, Wolterbeek APM, Van den Berg PTM, Baan RA (1994 b) DNA adducts in hamster and rat tracheas exposed to benzo[a]pyrene *in vitro*. *Toxicol Lett* 72: 105–111
- Roller M, Kamino K, Rosenbruch M (1992) Carcinogenicity testing of bladder carcinogens and other organic compounds by the intraperitoneal and intravesical route. In: Seemayer NH, Hadnagy W (Eds) Environmental Hygiene. III, Springer Verlag, Berlin, 95–98
- Romanoff LC, Li Z, Young KJ, Blakely NC, Patterson DG Jr., Sandau CD (2006) Automated solid-phase extraction method for measuring urinary polycyclic aromatic hydrocarbon metabolites in human biomonitoring using isotope-dilution gas chromatography high-resolution mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 835: 47–54
- Romundstad P, Andersen A, Haldorsen T (2000) Cancer incidence among workers in six Norwegian aluminum plants. *Scand J Work Environ Health* 26: 461–469
- Rosenkranz HS, Poirier LA (1979) Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J Natl Cancer Inst* 62: 873–891
- Ross J, Nelson G, Erxson G, Kligerman A, Early K, Gupta RC, Nesnow S (1991) DNA adducts in rat lung, liver, and peripheral blood lymphocytes produced by ip administration of benzo[a]pyrene metabolites and derivatives. *Carcinogenesis* 12: 1953–1955
- Ross JA, Nelson GB, Holden KL, Kligerman AD, Erxson GL, Bryant MF, Early K, Beach AC, Gupta RC, Nesnow S (1992) DNA adducts and induction of sister chromatid exchanges in the rat following benzo[b]fluoranthene administration. *Carcinogenesis* 13: 1731–1734
- Ross JA, Nesnow S (1999) Polycyclic aromatic hydrocarbons: correlations between DNA adducts and ras oncogene mutations. *Mutat Res* 424: 155–166
- Roszbach B, Angerer J (2002) PAK-Belastung am Arbeitsplatz – Reduzierung hoher innerer Belastung durch betriebliche Maßnahmen anhand von Ambient und Biological Monitoring (PAH exposure at the workplace – reduction of high internal exposure by occupational measures via ambient and biological monitoring) (German). Report of the results for the period from January 1999 to September 2001, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine of University of Erlangen-Nuremberg
- Roszinsky-Köcher G, Basler A, Röhrborn G (1979) Mutagenicity of polycyclic hydrocarbons. V. Induction of sister-chromatid exchanges *in vivo*. *Mutat Res* 66: 65–67
- Roush GC, Kelly JA, Meigs JW, Flannewry JT (1964) Scrotal carcinoma in Connecticut metal-workers, sequel to a study of sinonasal cancer. *Am J Epidemiol* 116: 76–85
- Rugen PJ, Stern CD, Lamm SH (1989) Comparative carcinogenicity of the PAHs as a basis for acceptable exposure levels (AELs) in drinking water. *Regul Toxicol Pharmacol* 9: 273–283

- Rusch HP, Kline BE, Baumann CA (1942) The nonadditive effect of ultra-violet light and other carcinogenic procedures. *Cancer Res* 2: 183–188
- Russell LB (1977) Validation of the *in vivo* somatic mutation method in the mouse as a pre-screen for germinal point mutations. *Arch Toxicol* 38: 75–85
- Ruzgys A, Bouchard M, Viau C (2005) Development of a high-performance liquid chromatographic method for the simultaneous determination of pyrene-1,6- and 1, 8-dione in animal and human urine. *J Anal Toxicol* 29: 533–538
- Saffiotti U, Cefis F, Kolb LH (1968) A method for experimental induction of bronchogenic carcinoma. *Cancer Res* 28: 104–113
- Saffiotti U, Montesano R, Sellkumar AR, Kaufmann DG (1972) Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo[a]pyrene and ferric oxide. *J Natl Cancer Inst* 49: 1199–1204
- Sakai M, Yoshida D, Mizusaki S (1985) Mutagenicity of polycyclic hydrocarbons and quinones on *Salmonella typhimurium* TA97. *Mutat Res* 156: 61–67
- Sala M, Lasne C, Lu YP, Chouroulinkov I (1987) Morphological transformation in three mammalian cell systems following treatment with 6-nitrochrysene and 6-nitrobenzo[a]pyrene. *Carcinogenesis* 8: 503–507
- Salaman MH, Roe FJC (1956) Further tests for tumour-initiating activity: N,N-Di-(2-chloroethyl)-p-aminophenylbutyric acid (CB1348) as an initiator of skin tumour formation in the mouse. *Br J Cancer* 10: 363–378
- Salamone MF, Heddle JA, Katz M (1979 a) The use of the *Salmonella*/microsomal assay to determine mutagenicity in paired chemical mixtures. *Can J Genet Cytol* 21: 101–107
- Salamone MF, Heddle JA, Katz M (1979 b) The mutagenic activity of thirty polycyclic aromatic hydrocarbons (PAH) and oxides in urban airborne particulates. *Environ Int* 2: 37–43
- Salamone MF (1981) Toxicity of 41 carcinogens and noncarcinogenic analogs. In: De Serres FJ, Ashby J (Eds) Evaluation of short-term tests for carcinogens, Elsevier North-Holland, Amsterdam, 682–685
- Salamone MF, Heddle JA, Katz M (1981) Mutagenic activity of 41 compounds in the *in vivo* micronucleus assay. In: De Serres FJ, Ashby J (Eds) Evaluation of short-term tests for carcinogens, Elsevier North-Holland, Amsterdam, 686–697
- Sanders CL, Skinner D, Gelman RA (1986) Percutaneous absorption of 7,10 <sup>14</sup>C-benzo[a]pyrene and 7,12 <sup>14</sup>C-dimethylbenz[a]anthracene in mice. *J Environ Pathol Toxicol Oncol* 7: 25–34
- Sandmeyer EE (1981) Aromatic hydrocarbons. In: Clayton GD, Clayton FE (Eds) Patty's Industrial Hygiene and Toxicology, John Wiley and Sons, New York, 3333–3339
- Sardella DJ, Boger E, Ghoshal PK (1981) Active sites in hexacyclic carcinogens probed by the fluorine substitution methodology, Battelle Press, Columbus, OH, USA, 529–538
- Sartorelli P, Cenni A, Matteucci G, Montomoli L, Novelli MT, Palmi S (1999) Dermal exposure assessment of polycyclic aromatic hydrocarbons: *in vitro* percutaneous penetration from lubricating oil. *Int Arch Occup Environ Health* 72: 528–532
- Sax NI, Lewis JR Sr. (1984) Dangerous properties of industrial materials, 7th edn, Van Nostrand Reinhold Co, New York, 2451–2452
- Schimberg RW (1981) Industrial hygienic measurements of polycyclic aromatic hydrocarbons in foundries. In: Cooke M, Dennis AJ (Eds) Chemical analysis and biological fate: polynuclear aromatic hydrocarbons, Battelle Press, Columbus, OH, USA, 755–762
- Schmähl D (1955) Prüfung von Naphthalin und Anthracen auf cancerogene Wirkung an Ratten (Study of naphthalene and anthracene for carcinogenicity in rats) (German). *Z Krebsforsch* 60: 697–710

- Schmähl D, Schmidt KG, Habs M (1977) Syncarcinogenic action of polycyclic aromatic hydrocarbons in automobile exhaust gas condensates. In: Mohr U, Schmähl D, Tomatis L (Eds) *Air Pollution and Cancer in Man*, IARC (International Agency for Research on Cancer), Scientific Publication No 16, Lyon, FR, 53–59
- Schmeltz I, Tosk J, Hilfrich J, Hirota N, Hoffmann D, Wynder EL (1978) Bioassays of naphthalene and alkylnaphthalenes for co-carcinogenic activity. Relation to tobacco carcinogenesis. In: Jones PW, Freudenthal RI (Eds) *Carcinogenesis, Polynuclear Aromatic Hydrocarbons*, Vol 3, Raven Press, New York, 47–60
- Schmidt KG, Schmähl D, Misfeld J, Timm J (1976) Experimentelle Untersuchungen zur Syncarcinogenese. 7. Mitteilung: Syncarcinogene Wirkung von polycyclischen aromatischen Kohlenwasserstoffen (PAH) im Epicutantest an der Mäusehaut (Experimental studies into syncarcinogenesis. 7th Communication: Syncarcinogenic effects of polycyclic aromatic hydrocarbons (PAH) in epicutaneous tests in mice) (German). *Z Krebsforsch* 87: 93–100
- Schmoldt A, Jacob J, Grimmer G (1981) Dose-dependent induction of rat liver microsomal aryl hydrocarbon monooxygenase by benzo[k]fluoranthene. *Cancer Lett* 13: 249–257
- Schneider J, Xue WL, Warshawsky D (1994) Fluorescence spectroscopic studies on the identification and quantification of 7H-dibenzo[c,g]carbazole and dibenz[a,j]acridine metabolites. *Chem Biol Interact* 93: 139–153
- Schoket B, Hewer A, Grover PL, Phillips dh (1988) Covalent binding of components of coal-tar, creosote and bitumen to the DNA of the skin and lungs of mice following topical application. *Carcinogenesis* 9: 1253–1258
- Schoket B, Papp G, Lévy K, Mracková G, Kadlubar FF, Vincze I (2001) Impact of metabolic genotypes on levels of biomarkers of genotoxic exposure. *Mutat Res* 482: 57–69
- Schott K, Raulf-Heimsoth M, Angerer J, Hahn JU, Heger M, Preuss R, Rohde P, Rühl R, Zoubek G, Brüning T (2003) Auffälligkeiten bei einer Gussasphaltkolonie – Ursachenklärung einer erhöhten PAK-Belastung (Abnormalities in a mastic asphalt crew – clarification of the causes of increased PAH exposure) (German). *Arbeitsmed Sozialmed Umweltmed* 38: 594–597
- Schürch O, Winterstein A (1935) Über die krebserregende Wirkung aromatischer Kohlenwasserstoffe. [The cancer-inducing effect of aromatic hydrocarbons] (German). *Z Physiol Chem* 236: 79–91
- Schunk W (1979) Zur Frage der Beziehung zwischen der Exposition gegenüber polycyclischen Kohlenwasserstoffen und der Häufigkeit von Krebserkrankungen in zwei Chemiebetrieben Thüringens (Question of the relation between exposure to polycyclic hydrocarbons and the frequency of cancer in two chemical companies in Thuringia) (German). *Z Aerztl Fortbild* 73: 84–88
- Scicchitano DA (2005) Transcription past DNA adducts derived from polycyclic aromatic hydrocarbons. *Mutat Res* 577: 146–154
- Scribner JO (1973) Brief communication: Tumor initiation by apparently noncarcinogenic polycyclic aromatic hydrocarbons. *J Natl Cancer Inst* 50: 1717–1719
- Sega GA (1979) Unscheduled DNA synthesis (DNA repair) in the germ cells of male mice. Its role in the study of mammalian mutagenesis. *Genetics* 92: 49–58
- Seidel A, Soballa VJ, Raab G, Frank H, Grimmer G, Greim H, Jacob J, Doehmer J (1998 a) Regio- and stereoselectivity in the metabolism of benzo[c]phenanthrene mediated by genetically engineered V79 Chinese hamster cells expressing rat and human cytochromes P450. *Environ Toxicol Pharmacol* 5: 179–196
- Seidel A, Friedberg T, Löllmann B, Schwierzok A, Funk M, Frank H, Holler R, Oesch F, Glatt H (1998 b) Detoxification of optically active bay- and fjord-region polycyclic aromatic hydro-

- carbon dihydrodiol epoxides by human glutathione transferase P1-1 expressed in Chinese hamster V79 cells. *Carcinogenesis* 19: 1975–1981
- Seidel A, Dahmann D, Krekeler H, Jacob J (2002) Biomonitoring of polycyclic aromatic compounds in the urine of mining workers occupationally exposed to diesel-exhaust. *Int J Hyg Environ Health* 204: 333–338
- Seidel A, Frank H, Behnke A, Schneider D, Jacob J (2004) Determination of dibenzo[a,l]pyrene and other fjord-region PAH isomers with MW 302 in environmental samples. *Polycyclic Aromat Compd* 24: 759–771
- Seidel A, Jacob J (2005) Ergebnisbericht zum Forschungsvorhaben des HVBG (Hauptverband der Berufsgenossenschaften): PAH-Belastung am Arbeitsplatz – Reduzierung hoher innerer Belastung durch betriebliche Maßnahmen anhand von Ambient und Biological Monitoring. Teilprojekt B: Bestimmung mehrfach-hydroxylierter PAH-Metaboliten und der individuellen Suszeptibilität (Report of the results on the HVBG (German workmen's compensation insurance association) research project: PAH exposure at the workplace – reduction of high internal exposure by occupational measures via ambient and biological monitoring. Particular project B: Determination of poly-hydroxylated PAH metabolites and of individual susceptibility) (German). Forschungsbericht Hauptverband der Berufsgenossenschaften, St. Augustin
- Seidel A, Dettbarn G, John A, Jacob J (2005) Differences in the urinary excretion of PAH metabolites in nonsmoking cohorts of eastern and western Europe. *Toxicol Lett* 158, Suppl 1: 205
- Seidel A (Sep. 3 2007) Communication to the secretariat of the Commission
- Sellakumar A, Shubik P (1974) Carcinogenicity of different polycyclic hydrocarbons in the respiratory tract of hamsters. *J Natl Cancer Inst* 53: 1713–1719
- Serdar B, Waidyanatha S, Zheng Y, Rappaport SM (2003) Simultaneous determination of urinary 1- and 2-naphthols, 3- and 9-phenanthrols, and 1-pyrenol in coke oven workers. *Biomarkers* 8: 93–109
- Sharpe CR, Rochon J, Adam JM, Suissa S (1989) Case control study of hydrocarbon exposure in patients with renal cell carcinoma. *Can Med Assoc J* 140: 1309–1318
- Shear MJ, Leiter J (1941) Studies in carcinogenesis. XVI. Production of subcutaneous tumors in mice by miscellaneous polycyclic compounds. *J Natl Cancer Inst* 2: 241–259
- Shimada H, Suzuki H, Itoh S, Hattori C, Matsuura Y, Tada S, Watanabe C (1992) The micronucleus test of benzo[a]pyrene with mouse and rat peripheral blood reticulocytes. *Mutat Res* 278: 165–168
- Shimada H, Satake S, Itoh S, Hattori C, Hayashi M, Ishidate MJr (1990) Multiple dosing effects of benzo[a]pyrene in the mouse bone marrow micronucleus test. *Mutat Res* 234: 179–181
- Shimkin MB, Stoner GD (1975) Lung tumours in mice: Application to carcinogenesis bioassay. In: Klein G, Weinhouse S (Eds) *Advances in Cancer Research*, Vol 1, Raven Press, New York, 1–58
- Shopp GM, White KL Jr., Holsapple MP, Barnes DW, Duke SS, Anderson AC, Condie LW Jr., Hayes JR, Borzelleca JF (1984) Naphthalene toxicity in CD-1 mice: General toxicology and immunotoxicology. *Fundam Appl Toxicol* 4: 406–419
- Shou MG, Korzekwa KR, Crespi CL, Gonzalez FJ, Gelboin HV (1994) The role of 12 cDNA-expressed human, rodent, and rabbit cytochromes P450 in the metabolism of benzo[a]pyrene and benzo[a]pyrene *trans*-7,8-dihydrodiol. *Mol Carcinogen* 10: 159–168
- Shubik P, Della Porta G (1957) Carcinogenesis and acute intoxication with large doses of polycyclic hydrocarbons. *Am Med Assoc Arch Pathol* 64: 691–703
- Shubik P, Pietra G, Della Porta G (1960) Studies of skin carcinogenesis in the Syrian golden hamster. *Cancer Res* 20: 100–105
- Shum S, Jensen NM, Nebert DW (1979) The murine Ah locus: *in utero* toxicity and teratogenesis with genetic differences in benzo[a]pyrene metabolism. *Teratology* 20: 365–376

- Siemiatycki J (1991) Risk factors for cancer in the workplace, CRC Press, Boca Raton, FL
- Siemiatycki J, Dewar R, Nadon L, Gerin M, Richardson L, Wachholder S (1987) Associations between several sites of cancer and twelve petroleum derived liquids. Results from a case-referent study in Montreal. *Scand J Work Environ Health* 13: 493–504
- Siemiatycki J, Gerin M, Stewart P, Nadon L, Dewar R, Richardson L (1988) Associations between several sites of cancer and ten types of exhaust and combustion products. *Scand J Work Environ Health* 14: 79–90
- Silverstein M, Meizlish N, Park R, Mirer F (1985) Mortality among workers exposed to coal tar pitch volatiles and welding emissions: an exercise in epidemiologic triage. *Am J Public Health* 75: 1283–1287
- Simmon VF (1979) *In vitro* mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*. *J Natl Cancer Inst* 62: 893–899
- Simmon VF, Rosenkranz HS, Zeiger E, Poirier LA (1979) Mutagenic activity of chemical carcinogens and related compounds in the intraperitoneal host-mediated assay. *J Natl Cancer Inst* 62: 911–918
- Simon P, Lafontaine M, Delsaut P, Morel Y, Nicot T (2000) Trace determination of urinary 3-hydroxybenzo[a]pyrene by automated column-switching high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 748: 337–348
- Simpson CD, Wu MT, Christiani DC, Santella RM, Carmella SG, Hecht SS (2000) Determination of *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene in human urine by gas chromatography/negative ion chemical ionization/mass spectrometry. *Chem Res Toxicol* 13: 271–280
- Sims P (1962) Metabolism of polycyclic compounds. 19. The metabolism of phenanthrene in rabbits and rats: Phenols and sulphuric esters. *Biochem J* 84: 558–563
- Sims P, Grover PL, Swaisland A, Pal K, Hewer A (1974) Metabolic activation of benzo[a]pyrene proceeds by a diol-epoxide. *Nature* 252: 326–328
- Sirianni SR, Huang CC (1978) Sister chromatid exchange induced by promutagens/carcinogens in Chinese hamster cells cultured in diffusion chambers in mice. *Proc Soc Exp Biol Med* 158: 269–274
- Skopek TR, Thilly WG (1983) Rate of induced forward mutation at 3 genetic loci in *Salmonella typhimurium*. *Mutat Res* 108: 45–56
- Slaga TJ, Hubermann E, Selkirk JK, Harvey RG, Bracken WM (1978 a) Carcinogenicity and mutagenicity of benz[a]anthracene diols and diol-epoxides. *Cancer Res* 38: 1699–1704
- Slaga TJ, Bracken WM, Viaje A, Berry DL, Fischer SM, Miller DR, Levin W, Conney AH, Yagi H, Jerina DM (1978 b) Tumor initiating and promoting activities of various benzo[a]pyrene metabolites in mouse skin. *Carcinogenesis* 3: 371–382
- Slaga TJ, Gleason GL, Mills C, Ewald L, Fu PP, Lee HM, Harvey RG (1980) Comparison of the tumor-initiating activities of dihydrodiols and diol-epoxides of various polycyclic aromatic hydrocarbons. *Cancer Res* 40: 1981–1984
- Slaga TJ, Iyer RP, Lyga W, Secrist A, Daub GH, Harvey RG (1981) Comparison of the skin-tumor initiating activities of dihydrodiols, diol-epoxides, and methylated derivatives of various polycyclic aromatic hydrocarbons. In: Cooke M, Dennis AJ (Eds) Polynuclear aromatic hydrocarbons: chemical analysis and biological fate, Battelle Press, Columbus, OH, USA, 753–769
- Smith WE (1956) Sebaceous gland activity as a measure of the relative carcinogenicity of certain oils and tars. *New York Pathological Society* 32: 77–78



- Smith CJ, Grainger J, Patterson DG (1998) Separation of polycyclic aromatic hydrocarbon metabolites by  $\gamma$ -cyclodextrin-modified micellar electrokinetic chromatography with laser-induced fluorescence detection. *J Chromatogr A* 803: 241–245
- Smolarek TA, Baird WM, Fisher EP, DiGiovanni J (1987) Benzo[e]pyrene-induced alterations in the binding of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene to DNA in Sencar mouse epidermis. *Cancer Res* 47: 3701–3706
- Snell KC, Stewart HL (1962) Pulmonary adenomatosis induced in DBA/2 mice by oral administration of dibenz[a,h]anthracene. *J Natl Cancer Inst* 28: 1043–1049
- Solt D, Farber E (1976) New principle for the analysis of chemical carcinogenesis. *Nature* 263: 701–703
- Solt DB, Medline A, Farber E (1977) Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. *Am J Pathol* 88: 595–618
- Solt DB, Polverini PJ, Calderon L (1987) Carcinogenic response of hamster buccal pouch epithelium to 4 polycyclic aromatic hydrocarbons. *J Oral Pathol* 16: 294–302
- Sorahan T, Faux AM, Cooke MA (1994) Mortality among a cohort of United Kingdom steel foundry workers with special reference to cancers of the stomach and lung. 1946–1990. *Occup Environ Med* 51: 316–322
- Spornins VL, Mott AW, Baraney G, Wattenberg LW (1986) Effects of allyl methyl trisulfide on glutathione S-transferase activity and BP-induced neoplasia in the mouse. *Nutr Cancer* 8: 211–215
- Springer DL, Mann DB, Dankovic DA, Thomas BL, Wright CW, Mahlum DD (1989) Influences of complex organic mixtures on tumor-initiating activity, DNA binding and adducts of benzo[a]pyrene. *Carcinogenesis* 10: 131–137
- Stanton MF, Miller E, Wrench C, Blackwell R (1972) Experimental induction of epidermoid carcinoma in the lungs of rats by cigarette smoke condensate. *J Natl Cancer Inst* 49: 867–877
- Steineck G, Plato N, Norell SE, Hogstedt C (1990) Urothelial cancer and some industry-related chemicals: an evaluation of the epidemiologic literature. *Am J Ind Med* 17: 371–391
- Steiner PF, Falk HL (1951) Summation and inhibition effects of weak and strong carcinogenic hydrocarbons: 1,2-Benzanthracene, chrysene, 1:2:5:6-dibenzanthracene, and 20-methylcholanthrene. *Cancer Res* 11: 56–63
- Steiner PE, Edgcomb JH (1952) Carcinogenicity of 1,2-benzanthracene. *Cancer Res* 12: 657–659
- Steiner PF (1955) Carcinogenicity of multiple chemicals simultaneously administered. *Cancer Res* 15: 632–635
- Steinhoff D, Mohr U, Hahnemann S (1991) Carcinogenesis studies with iron oxides. *Exp Pathol* 43: 189–194
- Stevenson JL, von Haam E (1965) Carcinogenicity of benz[a]anthracene and benzo[c]phenanthrene derivatives. *Am Ind Hyg Assoc J* 26: 475–478
- Storer JS, DeLeon I, Millikan LE, Laseter JL, Griffing C (1984) Human absorption of crude coal tar products. *Arch Dermatol* 120: 874–877
- Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Coglian V (2005) WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of polycyclic aromatic hydrocarbons. *Lancet Oncol* 6: 931–932
- Struble CB, Larsen GL, Feil VJ, Bakke JE (1986) Metabolism of 9-hydroxy-9,10-dihydro-10-cysteinyl-phenanthrene in rats. In: Cooke M, Dennis AJ (Eds) Polynuclear Aromatic Hydrocarbons: Chemistry, Characterization and Carcinogenesis, Battelle Press, Columbus, OH, USA, 893–900

- Strunk P, Ortlepp K, Heinz H, Rossbach B, Angerer J (2002) Ambient and biological monitoring of coke plant workers – determination of exposure to polycyclic aromatic hydrocarbons. *Int Arch Occup Environ Health* 75: 354–358
- Sugiyama T (1973) Chromosomal aberrations and carcinogenesis by various benz[a]anthracene derivatives. *Gann* 64: 637–639
- Sundberg K, Widersten M, Seidel A, Mannervik B, Jernström B (1997) Glutathione conjugation of bay- and fjord-region diol epoxides of polycyclic aromatic hydrocarbons by glutathione transferases M1-1 and P1-1. *Chem Res Toxicol* 10: 1221–1227
- Sundberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, Mannervik B, Jernström B (1998 a) Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. *Carcinogenesis* 19: 433–436
- Sundberg K, Seidel A, Mannervik B, Jernström B (1998 b) Detoxication of carcinogenic fjord-region diol epoxides of polycyclic aromatic hydrocarbons by glutathione transferase P1-1 variants and glutathione. *FEBS Lett* 438: 206–210
- Suntzeff VA, Cowdry EV, Croninger A (1955) Microscopic visualization of the degeneration of sebaceous glands caused by carcinogens. *Cancer Res* 15: 637–640
- Suntzeff V, Croninger AB, Wynder EL, Cowdry EV, Graham EA (1957) Use of sebaceous-gland test of primary cigarette-tar fractions and of certain noncarcinogenic polycyclic hydrocarbons. *Cancer* 10: 250–254
- Surh YJ, Liem A, Miller EC, Miller JA (1990 a) The strong hepatocarcinogenicity of the electrophilic and mutagenic metabolite 6-sulfooxymethylbenzo[a]pyrene and its formation of benzylic DNA adducts in the livers of infant male B6C3F1 mice. *Biochem Biophys Res Commun* 172: 85–91
- Surh YJ, Blomquist JC, Liem A, Miller JA (1990 b) Metabolic activation of 9-hydroxymethyl-10-methylanthracene and 1-hydroxymethylpyrene to electrophilic, mutagenic and tumorigenic sulfuric acid esters by rat hepatic sulfotransferase activity. *Carcinogenesis* 11: 1451–1460
- Surh YJ, Liem A, Miller EC, Miller JA (1991) 7-Sulfooxymethyl-12-methylbenz[a]anthracene is an electrophilic mutagen, but does not appear to play a role in carcinogenesis by 7,12-dimethylbenz[a]anthracene or 7-hydroxymethyl-12-methylbenz[a]anthracene. *Carcinogenesis* 12: 339–347
- Surh YJ, Kwon HJ, Tannenbaum SR (1993) Sulfotransferase-mediated activation of 4-hydroxy- and 3,4-dihydroxy-3,4-dihydrocyclopenta[cd]pyrene, major metabolites of cyclopenta[cd]pyrene. *Cancer Res* 53: 1017–1022
- Swaen GMH, Slangen JJM, Volovics A, Hayes RB, Scheffers T, Sturmans F (1991) Mortality of coke plant workers in the Netherlands. *Br J Ind Med* 48: 130–135
- Swartz WJ, Mattison DR (1985) Benzo[a]pyrene inhibits ovulation in C57Bl/6N mice. *Anat Rec* 221: 268–276
- Tawfic HN (1965) Studies on ear duct tumors in rats. Part II: Inhibitory effects of methylcholanthrene and 1,2-benzanthracene on tumor formation by 4-dimethylaminostilbene. *Acta Pathol Jpn* 15: 255–260
- Teranishi K, Hamada K, Watanabe H (1975) Quantitative relationship between carcinogenicity and mutagenicity of polyaromatic hydrocarbons in *Salmonella typhimurium* mutants. *Mutat Res* 31: 97–102
- Thakker DR, Yagi H, Levin W, Wood AW, Conney AH, Jerina DM (1985) Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In: Anders MW (Ed) *Bioactivation of Foreign Compounds*, Academic Press, Inc., 177–242

- Thorslund T, Farrer D (1991) Development of relative potency estimates for PAHs and hydrocarbon combustion product fractions compared to benzo[a]pyrene and their use in carcinogenic risk assessments. US Environmental Protection Agency, EPA/600/R-92/134, Washington, DC
- Thyssen J, Althoff J, Kimmerle G, Mohr U (1980) Investigation of the carcinogenic burden of air pollution in man. XIX. Effect of inhaled benzo[a]pyrene in Syrian golden hamsters: Apilot study. *Zentralbl Bakteriol Hyg I Abt Orig B* 171: 441–444
- Thyssen J, Althoff J, Kimmerle G, Mohr U (1981) Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. *J Natl Cancer Inst* 66: 575–577
- Tobias S, Knecht U, Woitowitz HJ (1990) Verringerung der Krebsgefährdung durch Verwendung von Bitumen im Schwarzdeckenbau (Reduction of the cancer risk using bitumen in black top paving) (German). In: Meyer-Falke A, Jansen G (Eds) *Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin*, Gentner-Verlag, Stuttgart, 239
- Tokiwa H, Morita K, Takeyoshi H, Takahashi K, Ohnishi Y (1977) Detection of mutagenic activity in particulate air pollutants. *Mutat Res* 48: 237–248
- Tong C, Brat VS, Telang S, Laspia MF, Fazio M, Reiss B, Williams GM (1983) Effects of genotoxic polycyclic aromatic hydrocarbons in rat liver culture systems. In: Cooke M, Dennis AJ (Eds) *Polynuclear Aromatic Hydrocarbons: Formation, Metabolism, and Measurement*, Battelle Press, Columbus, OH, USA, 1189–1203
- Topham JC (1980) Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? *Mutat Res* 74: 379–387
- Topping DC, Martin d h, Nettesheim P (1981) Determination of cocarcinogenic activity of benzo[e]pyrene for respiratory tract mucosa. *Cancer Lett* 11: 315–321
- Troester MA, Lindstrom AB, Waidyanatha S, Kupper LL, Rappaport SM (2002) Stability of hemoglobin and albumin adducts of naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone. *Toxicol Sci* 68: 314–321
- Trush A, Esterline RL, Mallet WG, Mosebrook DR, Twerdok LE (1991) Further evidence for the role of myeloperoxidase in the activation of benzo[a]pyrene-7,8-dihydrodiol by polymorphonuclear leukocytes. *Adv Exp Med Biol* 283: 399–401
- Tsai PJ, Shieh HY, Lee WJ, Lai SO (2001) Health-risk assessment for workers exposed to polycyclic aromatic hydrocarbons (PAHs) in a carbon black manufacturing industry. *Sci Total Environ* 278: 137–150
- Tsuchimoto T, Matter BE (1981) Activity of coded compounds in the micronucleus test. In: de Serres FJ, Ashby J (Eds) *Evaluation of short-term tests for carcinogens. Report of the International Collaborative Program, Progress in mutation research, Vol 1*, Elsevier, Amsterdam, 705–711
- Tsuda H, Lee G, Farber E (1980) Induction of resistant hepatocytes as a new principle for a possible short-term *in vivo* test for carcinogens. *Cancer Res* 40: 1157–1164
- Tu MH, Perry D, Chen C (1979) Mutagenic effect of 7, 12-dimethylbenz[a]anthracene-epidioxide on *Salmonella typhimurium*. *Mol Pharmacol* 15: 189–191
- UBA (Umweltbundesamt) (1990) *Umweltsurvey 1990/92* (Environmental survey of 1990/92) (German). Data online under <http://www.uba.de/>
- UBA (Umweltbundesamt) (1998) *Umweltsurvey 1998* (Environmental survey of 1998) (German). Data online under <http://www.uba.de/>
- Unwin J, Cocker J, Scobbie E, Chambers H (2006) An assessment of occupational exposure to polycyclic aromatic hydrocarbons in the UK. *Ann Occup Hyg* 50: 395–403
- Upadhyaya P, Rao P, Hochalter JB, Li ZZ, Villalta PW, Hecht SS (2006) Quantitation of N-acetylS-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine in human urine: comparison with glutathione S-transferase genotypes in smokers. *Chem Res Toxicol* 19: 1234–1240

- Urso P, Gengozian N (1980) Depressed humoral immunity and increased tumor incidence in mice following *in utero* exposure to benzo[a]pyrene. *J Toxicol Environ Health* 6: 569–576
- USEPA (US Environmental Protection Agency) (1978) Ambient water quality criteria: Naphthalene. B/6 (PB-296 786), Washington, DC
- USEPA (US Environmental Protection Agency) (1984) Health effects assessment for polycyclic aromatic hydrocarbons (PAH). EPA 549/1-86-013, Cincinnati, OH
- USEPA (US Environmental Protection Agency) (1989) Mouse oral subchronic toxicity of pyrene. Study No. 042-010, Washington, DC
- Valaes T, Doxiadis SA, Fessas P (1963) Acute hemolysis due to naphthalene inhalation. *J Pediatr* 63: 904–915
- Valencia R, Houtchens K (1981) Mutagenic activity of 10 coded compounds in the *Drosophila melanogaster* sex-linked recessive lethal test. In: De Serres FJ, Ashby J (Eds) *Evaluation of Short-Term Tests for Carcinogens* Elsevier North-Holland, Amsterdam, 652–659
- Vanaanan V, Hameila M, Kalliokoski P, Nykyri E, Heikkilä P (2005) Dermal exposure to polycyclic aromatic hydrocarbons among road pavers. *Ann Occup Hyg* 49: 167–178
- Van Delft JHM, Steenwinkel MJST, van Asten JG, de Vogel N, Bruijntjes-Rozier TCDM, Schouten T, Cramers P, Maas L, van Herwijnen MH, van Schooten FJ, Hopmans PMJ (2001) Biological monitoring the exposure to polycyclic aromatic hydrocarbons of coke oven workers in relation to smoking and genetic polymorphisms for *GSTM1* and *GSTT1*. *Ann Occup Hyg* 45: 395–408
- Van der Bijl P, Gareis A, Lee H, van Eyk AD, Stander IA, Cilliers J (2002) Effects of two barrier creams on the diffusion of benzo[a]pyrene across human skin. *J South African Dental Assoc* 57: 49–52
- Van Dijk BA, Schouten LJ, Oosterwijk E, Hulsbergen-van de Kaa CA, Kiemeny LA, Goldbohm RA, Schalken JA, van den Brandt PA (2006) Cigarette smoking, von Hippel-Lindau gene mutations and sporadic renal cell carcinoma. *Br J Cancer* 95: 374–377
- Van Duuren BL, Sivak A, Degal A, Orris L, Langseth L (1966) The tumor-promoting agents of tobacco leaf and tobacco smoke condensate. *J Natl Cancer Inst* 37: 519–526
- Van Duuren BL, Sivak A, Langseth L, Goldschmidt BM, Segal A (1968) Initiators and promoters in tobacco carcinogenesis. *Natl Cancer Inst Monogr* 28: 173–180
- Van Duuren BL, Sivak A, Goldschmidt BM, Katz C, Melchionne S (1970) Initiating activity of aromatic hydrocarbons in two-stage carcinogenesis. *J Natl Cancer Inst* 44: 1167–1173
- Van Duuren BL, Goldschmidt BM (1976) Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J Natl Cancer Inst* 56: 1237–1242
- Van Rooij JG, Bodelier-Bade MM, De Looft AJ, Dijkmans AP, Jongeneelen FJ (1992) Dermal exposure to polycyclic aromatic hydrocarbons among primary aluminium workers. *Med Lav* 83: 519–529
- Van Rooij JGM, Bodelier-Bade M, Jongeneelen FJ (1993 a) Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. *Brit J Ind Med* 50: 623–632
- Van Rooij JGM, De Roos JHC, Bodelier-Bade MM, Jongeneelen FJ (1993 b) Absorption of polycyclic aromatic hydrocarbons through human skin: differences between anatomical sites and individuals. *Toxicol Environ Health* 38: 355–368
- Van Rooij JGM, Van Lieshout EMA, Bodelier-Bade MM, Jongeneelen FJ (1993 c) Effect of the reduction of skin contamination on the internal dose of creosote workers exposed to polycyclic aromatic hydrocarbons. *Scand J Work Environ Health* 19: 200–207

- Van Rooij JGM, Maassen LM, Bodelier-Bade MM, Jongeneelen FJ (1994) Determination of skin contamination with exposure pads among workers exposed to polycyclic aromatic hydrocarbons. *Appl Occup Environ Hyg* 9: 693–699
- Van Wendel de Joode B, van Hemmen JJ, Meijster T, Major V, London L, Kromhout H (2005 a) Reliability of a semi-quantitative method for dermal exposure assessment (DREAM). *J Expos Anal Environ Epidemiol* 15: 111–120
- Van Wendel de Joode B, Vermeulen R, van Hemmen JJ, Fransman W, Kromhout H (2005 b) Accuracy of a semiquantitative method for dermal exposure assessment (DREAM). *Occup Environ Med* 62: 623–632
- Veierod MB, Weiderpass E, Thorn M, Hansson J, Lund E, Armstrong B, Adami HO (2003) A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. *J Natl Cancer Inst* 95: 1530–1538
- Verma DK, Muir DCF, Cunliffe S, Julian JA, Vogt JH, Rosenfeld J, Chovli A (1982) Polycyclic aromatic hydrocarbons in Ontario foundry emissions. *Ann Occup Hyg* 25: 17–25
- Viau C, Carrier G, Vyskocil A, Dodd C (1995 a) Urinary excretion kinetics of 1-hydroxypyrene in volunteers exposed to pyrene by the oral and dermal route. *Sci Total Environ* 163: 179–186
- Viau C, Vyskocil A, Martel L (1995 b) Background urinary 1-hydroxypyrene levels in non-occupationally exposed individuals in the Province of Quebec, Canada, and comparison with its excretion in workers exposed to PAH mixtures. *Sci Total Environ* 163: 191–194
- Viau C, Bouchard M, Carrier G, Brunet R, Krishnan K (1999) The toxicokinetics of pyrene and its metabolites in rats. *Toxicol Lett* 108: 201–207
- Vogel EW, Zijlstra JA, Blijleven WGH (1983) Mutagenic activity of selected aromatic amines and polycyclic hydrocarbons in *Drosophila melanogaster*. *Mutat Res* 107: 53–77
- Vu-Duc TM, Lafontaine M (1996) 1-Hydroxypyrene in human urines as biomarker of exposure to PAH in work related processes in an aluminium production plant. *Polycyclic Aromatic Compd* 11: 1–9
- Waidyanatha S, Troester MA, Lindstrom AB, Rappaport SM (2002) Measurement of hemoglobin and albumin adducts of naphthalene-1,2-oxide, 1,2-naphthoquinone and 1,4-naphthoquinone after administration of naphthalene to F344 rats. *Chem Biol Interact* 141: 189–210
- Waldron HA, Waterhouse JAH, Tessema N (1984) Scrotal cancer in the West Midlands 1936–76. *Br J Ind Med* 41: 437–444
- Warren DL, Brown DR Jr., Buckpitt AR (1982) Evidence for cytochrome P450 mediated metabolism in the bronchiolar damage by naphthalene. *Chem Biol Interact* 40: 287–303
- Warshawsky D, Barkley W (1987) Comparative carcinogenic potencies of 7H-dibenzo[c,g]carbazole, dibenzo[a,j]acridine and benzo[a]pyrene in mouse skin. *Cancer Lett* 37: 337–344
- Watabe T, Ishizuka T, Isobe M, Ozawa N (1982) A 7-hydroxymethyl sulfate ester as an active metabolite of 7,12-dimethylbenz[a]anthracene. *Science* 215: 403–405
- Waterhouse JAH (1971) Cutting oils and cancer. *Ann Occup Hyg* 14: 161–170
- Wattenberg LW, Leong JL (1970) Inhibition of the carcinogenic action of benzo[a]pyrene by flavones. *Cancer Res* 30: 1922–1925
- Weisburger JH, Weisburger EK (1967) Tests for chemical carcinogenesis. In: Busch H (Ed) *Methods in Cancer Research*. Book 1, Academic Press, New York, 307–398
- Welch RM, Gommi B, Alvares AP, Conney AH (1972) Effect of enzyme-induction on the metabolism of benzo[a]pyrene and 3-methyl-4-monomethylaminoazobenzene in the pregnant and fetal rat. *Cancer Res* 32: 973–978
- Wenzel-Hartung R, Brune H, Grimmer G, Germann P, Timm J, Wosniok W (1990) Evaluation of the carcinogenic potency of four environmental polycyclic aromatic compounds following intrapulmonary application in rats. *Exp Pathol* 40: 221–227

- Wenzel-Hartung (1992) Teilvorhaben Kanzerogenitätsprüfung von umwelt- und arbeitsplatzrelevanten PAH nach intrapulmonaler Applikation bei Ratten. [Subproject: Carcinogenicity assessment of PAH of environmental and workplace relevance after intrapulmonary administration in rats]. *Government Reports Announcements & Index* 92: 172
- Wermuth B, Platt KL, Seidel A, Oesch F (1986) Carbonyl reductase provides the enzymatic basis of quinone detoxication in man. *Biochem Pharmacol* 35: 1277–1282
- West JAA, van Winkle LS, Morin D, Fleschner CA, Forman HJ, Plopper CG (2003) Repeated inhalation exposures to the bioactivated cytotoxicant naphthalene (NA) produce airway-specific Clara cell tolerance in mice. *Toxicological Sciences* 75: 161–168
- Wester RC, Maibach HI, Bucks DAW, Sedik L, Melendres J, Liao C, DiZio S (1990) Percutaneous absorption of [ $^{14}\text{C}$ ]DDT and [ $^{14}\text{C}$ ]benzo[a]pyrene from soil. *Fundam Appl Toxicol* 15: 510–516
- Weyand EH, Rice JE, Hussain N, LaVoie EJ (1987) Detection of DNA adducts of tumorigenic nonalternant polycyclic aromatic hydrocarbons by  $^{32}\text{P}$ -postlabelling. *Proc Am Assoc Cancer Res* 28: 102
- Weyand EH, LaVoie EJ (1988) Comparison of PAH DNA adduct formation and tumor initiating activity in newborn mice. *Proc Am Assoc Cancer Res* 29: 98
- Weyand EH, Patel S, LaVoie EJ, Cho B, Harvey RG (1990) Relative tumor initiating activity of benzo[a]fluoranthene, benzo[b]fluoranthene, naphtho[1,2-b]fluoranthene and naphtho[2,1-a]fluoranthene on mouse skin. *Cancer Lett* 52: 229–233
- Weyand EH, Bryla P, Wu Y, He ZM, LaVoie EJ (1993) Detection of the major DNA adducts of benzo[j]fluoranthene in mouse skin: Nonclassical dihydrodiol epoxides. *Chem Res Toxicol* 6: 117–124
- White J, White A (1939) Inhibition of growth of the rat by oral administration of methylcholanthrene, benzpyrene, or pyrene and the effect of various dietary supplements. *J Biol Chem* 131: 149–161
- White PA (2002) The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. *Mutat Res* 515: 85–98
- WHO (World Health Organization) (1998) Selected non-heterocyclic polycyclic aromatic hydrocarbons. IPCS Environmental Health Criteria 202, WHO, Geneva, Switzerland
- Whong WZ, Stewart JD, Ong T (1992) Comparative study of DNA adducts formation and cytogenic effects of two constituents in coke oven emissions with an *in vivo* rat lung cell system. *Environ Mol Mutagen* 19, Suppl 20: 70
- Whong WZ, Stewart DJ, Cutler D, Ong T (1994) Induction of *in vivo* DNA adducts by 4 industrial by-products in the rat lung-cell system. *Mutat Res* 312: 165–172
- Wielgosz SM, Brauze D, Pawlak AL (1991) Ah locus-associated differences in induction of sister chromatid exchanges and in DNA adducts by benzo[a]pyrene in mice. *Mutat Res* 246: 129–137
- Willems MI, Roggeband R, Baan RA, Wilmer JWGM, De Raat WK, Lohmann PHM (1991) Monitoring the exposure of rats to benzo[a]pyrene by the determination of mutagenic activity in excreta, chromosome aberrations and sister chromatid exchanges in peripheral blood cells, and DNA adducts in peripheral blood lymphocytes and liver. *Mutagenesis* 6: 151–158
- Williams TM, Harris RL, Arp EW, Symons MJ, van Ert MD (1980) Worker exposure to chemical agents in the manufacture of rubber tires and tubes. *Am Ind Hyg Assoc J* 41: 204–211
- Williams GM, Laspia MF, Dunkel VC (1982) Reliability of the hepatocyte primary culture/DNA repair test in testing of coded carcinogens and noncarcinogens. *Mutat Res* 97: 359–370
- Wilson NK, Chuang JC, Lyu C, Menton R, Morgan MK (2003) Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. *J Expos Anal Environ Epidemiol* 13: 187–202

- Winker N, Weniger P, Klein W, Ott E, Kocsis F, Schoket B, Korpert K (1995) Detection of polycyclic aromatic hydrocarbon exposure damage using different methods in laboratory animals. *J Appl Toxicol* 15: 59–62
- Wislocki PG, Wood AW, Chang RL, Levin W, Yagi H, Hernandez O, Dansette PM, Jerina DM, Conney AH (1976) Mutagenicity and cytotoxicity of benzo[a]pyrene arene oxides, phenols, quinones, and dihydrodiols in bacterial and mammalian cells. *Cancer Res* 36: 3350–3357
- Wislocki PG, Buening MK, Levin W, Lehr RE, Thakkes DR, Jerina DM, Conney AH (1979) Tumorigenicity of the diastereomeric benz[a]anthracene 3,4-diol-1,2-epoxides and the (+) and (–)-enantiomers of benz[a]anthracene in newborn mice. *J Natl Cancer Inst* 63: 201–204
- Wolfe JM, Bryan WR (1939) Effects induced in pregnant rats by injection of chemically pure carcinogenic agents. *Am J Cancer* 36: 359–368
- Wolff RK, Griffith WC, Henderson RF, Hahn FF, Harkema JR, Rebar AH, Eidson AF, McClellan RO (1989) Effects of repeated inhalation exposures to 1-nitropyrene, benzo[a]pyrene, Ga<sub>2</sub>O<sub>3</sub> particles, and SO<sub>2</sub> alone and in combination of particle clearance, bronchoalveolar lavage fluid composition, and histopathology. *J Toxicol Environ Health* 27: 123–138
- Wood AW, Levin W, Ryan D, Thomas PE, Yagi H, Mah HD, Thakker DR, Jerina DM, Conney AH (1977 a) High mutagenicity of metabolically activated chrysene 1,2-dihydrodiol: Evidence for bay region activation of chrysene. *Biochem Biophys Res Commun* 78: 847–854
- Wood AW, Levin W, Lu AY, Ryan D, West SB, Yagi H, Mah HD, Jerina DM, Conney AH (1977 b) Structural requirements for the metabolic activation of benzo[a]pyrene to mutagenic products: effects of modifications in the 4,5-, 7,8-, and 9,10-positions. *Mol Pharmacol* 13: 1116–1125
- Wood AW, Levin W, Thomas PE, Ryan D, Karle JM, Yagi H, Jerina DM, Conney AH (1978) Metabolic activation of dibenzo[a,h]anthracene and its dihydrodiols to bacterial mutagens. *Cancer Res* 38: 1967–1973
- Wood AW, Chang RL, Levin W, Ryan DE, Thomas PE, Mah HD, Karle JM, Yagi H, Jerina DM, Conney AH (1979) Mutagenicity and tumorigenicity of phenanthrene and chrysene epoxides and diol epoxides. *Cancer Res* 39: 4069–4077
- Wood AW, Levin W, Chang RL, Huang MT, Ryan DE, Thomas PE, Lehr RE, Kumar S, Koreeda M, Akagi H (1980) Mutagenicity and tumor-initiating activity of cyclopenta[cd]pyrene and structurally related compounds. *Cancer Res* 40: 642–649
- Wood AW, Chang RL, Levin W, Ryan DE, Thomas PE, Lehr RE, Kumar S, Sardella DJ, Boger E, Yagi H, Sayer JM, Jerina DM, Conney AH (1981) Mutagenicity of the bay region diol-epoxides and other benzo-ring derivatives of dibenzo[a,h]pyrene and dibenzo[a,i]pyrene. *Cancer Res* 41: 2589–2597
- Wu MT, Huang SL, Ho CK, Yeh YF, Christiani DC (1998) Cytochrome P450 1A1 *MspI* polymorphism and urinary 1-hydroxypyrene concentrations in coke-oven workers. *Cancer Epidemiol Biomarkers Prev* 7: 823–829
- Wu MT, Simpson CD, Christiani DC, Hecht SS (2002) Relationship of exposure to coke-oven emissions and urinary metabolites of benzo[a]pyrene and pyrene in coke-oven workers. *Cancer Epidemiol Biomarkers Prev* 11: 311–314
- Wynder EL, Hoffmann D (1959 a) The carcinogenicity of benzofluoranthenes. *Cancer* 15: 1194–1199
- Wynder EL, Hoffmann D (1959 b) A study of tobacco carcinogenesis. VII. The role of higher polycyclic hydrocarbons. *Cancer* 12: 1079–1086
- Wynder EL, Hoffmann D (1961) Carcinogenicity of dibenzo[a,i]pyrene. *Nature* 192: 1092–1093
- Wynder E, Hoffmann D (1970) The epidermis and the respiratory tract as bioassay systems in experimental carcinogenesis. *Br J Cancer* 24: 574–582

- Xu X, Kelsey KT, Wiencke JK, Wain JC, Christiani DC (1996) Cytochrome P 450 CYP 1A1 MspI polymorphism and lung cancer susceptibility. *Cancer Epidemiol Biomarkers Prev* 5: 687–692
- Yamagiwa K, Ichikawa K (1915) Über die künstliche Erzeugung von Papilloma (Artificial generation of papilloma) (German). *Verh Jap Path Ges* 5: 142–148
- Yang SK, Roller PP, Fu PP, Harvey RG, Gelboin HV (1977) Evidence for a 2,3-epoxide as an intermediate in the microsomal metabolism of benzo[a]pyrene. *Biochem Biophys Res Commun* 77: 1176–1182
- Yang Y, Griffiths WJ, Midtvedt T, Sjøvall J, Rafter J, Gustafsson JA (1999) Characterization of conjugated metabolites of benzo[a]pyrene in germ-free rat urine by liquid chromatography/electrospray tandem mass spectrometry. *Chem Res Toxicol* 12: 1182–1189
- Yang M, Jang JY, Kim S, Lee SM, Chang SS, Cheong HK, Le E, Kang D, Kim H, Kawamoto T, Dhin HD (2003) Genetic effects on urinary 1-hydroxypyrene in a Korean population. *Carcinogenesis* 24: 1085–1089
- Ye YR, Duke CC, Holder GM (1995) Identification of hepatic metabolites of two highly carcinogenic polycyclic aza-aromatic compounds, 7,9-dimethylbenz[c]acridine and 7,10-dimethylbenz[c]acridine. *Chem Res Toxicol* 8: 188–202
- Zeegers MP, Swaen GM, Kant I, Goldbohm RA, van den Brandt PA (2001) Occupational risk factors for male bladder cancer: results from a population based case cohort study in the Netherlands. *Occup Environ Med* 58: 590–596
- Zhang J, Ichiba M, Zhang S, Hanaoka T, Pan G, Yamano Y, Takahashi K, Tomokuni K (2001) Urinary 1-hydroxypyrene in coke oven workers relative to exposure, alcohol consumption, and metabolic enzymes. *Occup Environ Med* 58: 716–721
- Zhao ZH, Quan WY, Tian DH (1990) Urinary 1-hydroxypyrene as an indicator of human exposure to ambient polycyclic aromatic hydrocarbons in coal-burning environment. *Sci Total Environ* 92: 145–154
- Zhong BZ, Gu ZW, Stewart J, Ong T (1995) Micronucleus formation induced by three polycyclic aromatic hydrocarbons in rat bone marrow and spleen erythrocytes following intratracheal instillation. *Mutat Res* 326: 147–153
- Zijlstra JA, Vogel EW (1984) Mutagenicity of 7,12-dimethylbenz[a]anthracene and some other aromatic mutagens in *Drosophila melanogaster*. *Mutat Res* 125: 243–261
- Zinkham WH, Childs B (1958) A defect of glutathione metabolism in erythrocytes from patients with a naphthalene-induced hemolytic anemia. *Pediatrics* 22: 461–471
- Zuelzer WW, Apt L (1949) Acute hemolytic anemia due to naphthalene poisoning. *J Am Med Assoc* 141: 185–191.

completed 27.06.2007