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ANNEX

Background document to the opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food (expressed on 4 December 2002)

Polycyclic Aromatic Hydrocarbons – Occurrence in foods, dietary exposure and health effects

Summary of background information on polycyclic aromatic hydrocarbons with emphasis on occurrence in foods, dietary exposure and health effects. The summary is based on available background monographs prepared in other frameworks and contributions of members of the SCF Task Force on PAH.

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1 EXPOSURE ASSESSMENT

1.1 Occurrence of PAH in foods

Raw foods should usually not contain high levels of PAH. In areas remote from urban or industrial activities, the levels of PAH found in unprocessed foods reflect the background contamination, which originates from long distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires. In the neighbourhood of industrial areas or along highways, the contamination of vegetation can be ten-fold higher than in rural areas (Hancock *et al.*, 1970, personal communication from Lambré, C., 2002; Larsson and Sahlberg, 1982).

Processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting, frying) are major sources generating PAH (Guillen *et al.*, 1997; Phillips, 1999). Levels as high as 200 µg/kg food have been found for individual PAH in smoked fish and meat. In barbecued meat, 130 µg/kg has been reported whereas the average background values are usually in the range of 0.01-1 µg/kg in uncooked foods. Contamination of vegetable oils (including olive residue oils) with PAH usually occurs during technological processes like direct fire drying, where combustion products may come into contact with the oil seeds or oil (Speer *et al.*, 1990; Standing Committee on Foodstuffs, 2001).

Examples of PAH levels in a number of foods can be found in table 1.1.1.

1.1.1 Sources of environmental PAH contamination

Foods can be contaminated by PAH that are present in air (by deposition), soil (by transfer) or water (deposition and transfer). The sources, natural and mostly anthropogenic, of PAH in the environment are numerous and include (IPCS, 1998):

- ✓ Stubble burning (Ramdahl and Moller, 1983) and spreading of contaminated sewage sludge on agricultural fields (Hembrock-Heger and Konig, 1990; cited by IPCS, 1998).
- ✓ Exhausts from mobile sources (motor vehicles and aircrafts). Close to an emission source such as a motorway, very high concentrations of PAH were detected in the surface layer, but soil at a depth of 4-8 cm was two times less contaminated (Butler *et al.*, 1984; cited by IPCS, 1998). Close to highways, concentrations of PAH in the soil in the range of 2-5 mg/kg can be found whereas in unpolluted areas, the levels are in the range of 5-100 µg/kg. The distribution and concentration of PAH in soil, leaf litter, and soil fauna depend broadly on the distance from the roadside.

- ✓ Industrial plants (e.g. aluminum foundries, incinerators).
- ✓ Wood preservation, use of tar coated wood. Oysters and mussels grown in beds with tar or creosote coated wood posts may be contaminated with PAH.
- ✓ Domestic heating with open fireplaces. Levels of PAH in the atmosphere appear to be higher in the winter than in the summer period.
- ✓ Burning of coal for thermal and electric energy. The quantity emitted varies widely depending on the quality of coal and on the combustiton process.
- ✓ Burning of automobile tires or of creosote treated wood releases considerable amounts of PAH.
- ✓ PAH present in tobacco smoke contaminate both the atmosphere of the kitchen and the foods during preparation and cooking.
- ✓ Oil pollution of surface waters and soils.
- ✓ Forest fires and volcanic eruptions (Hites *et al.*, 1980; cited in IPCS, 1998).

1.1.2 Physical and chemical properties of PAH influencing their occurrence in foods

The occurrence of PAH in foods is influenced by the same physicochemical characteristics that determine their absorption and distribution in man. These are their relative solubility in water and organic solvents, which determine their capacity for transport and distribution between different environmental compartments and their uptake and accumulation by living organisms. The transportation of PAH in the atmosphere is influenced by their volatility. The chemical reactivity of PAH influences adsorption to organic material or degradation in the environment. All these factors determine the persistence and capacity of PAH to bioaccumulate in the food chain.

PAH are lipophilic and generally have a very poor aqueous solubility. PAH accumulate in lipid tissue of plants and animals. PAH will not tend to accumulate in plant tissues with a high water content and limited transfer from the soil to root vegetables will occur. The rate of transfer varies widely and is also influenced by soil characteristics, the plant and the presence of co-pollutants. PAH adsorb strongly to the organic fraction of soils and do not penetrate deeply into most soils, therefore limiting both leaching to groundwater and availability for uptake by plants.

Some PAH are semi volatile but most of them tend to adsorb on organic particulate matter. Heavier PAH preferentially associate with particulate matter so atmospheric fall out is a principal route of contamination (Edwards, 1983; Nielsen *et al.*, 1996). PAH with 5 or more aromatic rings are found predominantly on particulates, (usually on small (< 2.5 μm) particles such as fly ash and soot). PAH with 2 or 3 rings are almost entirely in the vapor phase, those with 4 rings being in an intermediate position.

Consequently, vegetables with large leafs, grazing cattle and poultry which may ingest particulate matter from soil are susceptible to contamination by PAH adsorbed to particles. The waxy surface of vegetables and fruits can concentrate low molecular mass PAH mainly through surface adsorption. PAH concentrations are generally greater on plant surface (peel, outer leaves) than on internal tissue. Careful washing may remove up to 50% of the total PAH. Particle bound PAH are easily washed off the surface whereas those in the waxy layer are less efficiently removed, washing may alter the apparent high to low molecular mass PAH profile.

When particulates fall out into surface water, they are transported in suspension and surface adsorbed PAH finally end up in fresh water or marine sediments. PAH are strongly bound to these sediments which constitute a potential pollution reservoir for PAH release under specific conditions. Sediment-dwelling and filtering organisms are most susceptible to contamination. Most organisms have a high bio-transformation potential for PAH resulting in no significant bio-magnification in the aquatic food chain. However filter-feeding bivalves (e.g. mussels and oysters) filter large volumes of water and have a low metabolic capacity for PAH, they may accumulate PAH. The water-soluble low molecular mass PAH are rapidly degraded in water but sustained release of PAH in waste water can result in elevated concentrations in bivalves grown close to industrialized areas. The accumulation of sediment-adsorbed PAH depends on the contaminant (Baumard *et al.*, 1998, personal communication from Lambré, C., 2002).

Degradation

PAH are chemically stable and very poorly degraded by hydrolysis (Howard *et al.*, 1991; cited in IPCS, 1998) but are susceptible to oxidation and photo-degradation in light. PAH half lives in air range from a few hours to days and estimated PAH half lives in soils vary from several months to several years. Abiotic degradation may remove 2-20% of two and three ring PAH in contaminated soils (Park *et al.*, 1990; cited in IPCS, 1998). PAH with 4 or more aromatic rings persist in the environment but they are often strongly adsorbed to organic matter. Following degradation, oxidized reaction products may be formed which tend to react with biological components. Reaction with nitrogen oxides and nitric acid in the atmosphere can form nitro derivatives, which could contaminate foods. Thus although parent compounds cannot always be detected in PAH contaminated foods, degradation products or derivatives, some of which have significant toxicity, may be present. The half-lives in soil and air depends on various parameters (e.g. type of adsorption onto particles, molecular weight) and range from hours to days for air and months to years for soil.

Biodegradation

The most significant information on biodegradation is summarized below (IPCS, 1998):

- ✓ The biotransformation potential of aquatic organisms depends on the activity of their cytochrome P450-dependent mixed-function oxidases (James, 1989; cited in IPCS, 1998).
- ✓ Biotransformation mainly takes place in liver, lung, kidney, placenta, intestinal tract, and skin (Cerniglia, 1984; cited in IPCS, 1998).
- ✓ The initial transformation step in invertebrates is usually slower than in vertebrates.
- ✓ Invertebrates excrete PAH metabolites inefficiently.
- ✓ There are marked differences in biotransformation of PAH between different species of crustaceans.
- ✓ There are marked differences in biotransformation for different PAH within each species of crustaceans.
- ✓ Limited information prevents conclusions on biotransformation by algae, plants and fungi.

1.1.3 Contamination of food with PAH during processing and smoking

Processing procedures, such as smoking and drying, and cooking of food is commonly thought to be the major source of contamination by PAH. Depending on a number of parameters: time, fuel used, distance from the heat source and drainage of fat, type (grilling, frying, roasting), cooking results in the production in the food of a number of compounds including PAH. Although not precisely known, it is likely that there are several mechanisms of formation of PAH such as melted fat that undergoes pyrolysis when dripping onto the heat and pyrolysis of the meat due to the high temperature (Lijinsky and Shubik, 1965 a, b). A comparison of PAH levels in duck breast steaks, undergoing various processing and cooking treatments for 0.5 hour to 1.5 hours, showed that charcoal grilled samples without skin contained the highest amount of total PAH (320 µg/kg), followed by charcoal grilling with skin (300 µg/kg), smoking (210 µg/kg), roasting (130 µg/kg), steaming (8.6 µg/kg) and liquid smoke flavouring (0.3 µg/kg). For PAH that are classified as carcinogenic (IARC class 1 or 2 A and B), the trend was the same with the exception that smoked samples contained the highest amount (35 µg/kg). In addition, the highest amounts of total and carcinogenic PAH were observed after smoking of duck breast samples for 3 hours (53 µg/kg) (Chen and Lin, 1997).

Contamination of water may lead to intake of PAH through drinking water and cooked foods. The levels are usually below 1 ng/L in drinking water but can be higher when asphalt or coal tar coating of storage tanks and water distribution pipes are used.

1.1.4 Analytical Methods

Following homogenization of the foodstuff, PAH are extracted using different techniques prior to clean up and purification. PAH are most often identified and quantified using either gas chromatography (GC) with flame ionization detection (FID) or coupled to mass spectrometry (MS) or high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection or coupled to MS.

In addition to potential losses of PAH during homogenization, extraction and clean up, there are a number of other factors that may lead to erroneous results. During sample collection and storage it is important that the sample not be exposed to tobacco smoke, light and high temperatures (leading to volatilization and/or chemical conversion). Also storage for a prolonged time before analysis may result in the reaction of some PAH with components of the food matrix.

Attention should also be paid to the possible co-elution of some PAH. For example, under the gas chromatographic conditions generally used, chrysene + triphenylene, the benzo[*b*+*j*+*k*]fluoranthenes, and the dibenzo[*a,h*+*a,c*]anthracenes may co-elute and give rise to only a single peak. When HPLC is used the separation of benzo[*b*]fluoranthene + perylene, benzo[*k*]fluoranthene + dibenz[*a,c*]anthracene, and benzo[*j*]fluoranthene + benzo[*e*]pyrene may be critical.

1.1.5 Examples of data for various contaminated foods

Examples of PAH levels in foods can be found in table 1.1.1. The criteria used to select the pertinent studies from the literature are described in chapter 1.2 "Profile of PAH occurring in food".

Barbecue
PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, the time of cooking and the temperature. For example a heavily barbecued lamb sausage contained 14 µg/kg of carcinogenic PAH (Mottier *et al.*, 2000).

The presence of PAH was studied in several samples of meat and fish that were grilled on two geometrically different gas barbecues. In contrast to a horizontal barbecue, the vertical

Table 1.1.1 PAH concentrations measured in individual foods (µg/kg)

Food group	meat and meat products		fish and marine foods					mussels			vegetables					
Food	meat	grilled meat [b]	fish	smoked herring	smoked fish [h]	smoked fish [e]	smoked fish [d]	canned	"control" site [i]	from shellfish farm [m]	kale	lettuce [a]	lettuce	lettuce [c]	chicory [f]	olives
Reference	[1]	[12]	[1]	[12]	[6]	[6]	[27]	[10]	[16]	[16]	[3]	[4]	[11]	[11]	[8]	[8]
PAH																
Acenaphthene																
Fluorene									0.4	0.3						
Anthracene					6.3	21.0	14.0	0.5	< 0.2	< 0.2			< 0.06	< 0.05	10.0	32.0
Phenanthrene					32.0	65.3	81	6.1	1.5	1.6			4.3	1.6	383	802
Fluoranthene	0.5	297	0.8	107	9.1	26.0	16.3	11.2	< 1.4	4.1	117	28.0	9.3	5.5	146	176
Pyrene	0.6	354	0.8	111	5.3	20.5	10.2	7.4	1.2	3.2	70		6.4	6.6	69.0	119
Benzo[a]fluorene													0.9	0.9	11.0	20.7
Benzo[b]fluorene													0.7	1.6	5.0	14.7
Benzofluorene, sum																
Benzo[ghi]fluoranthene																
Benz[a]anthracene	0.1	108	0.1	26.7	0.6	2.5	1.7	3.7	0.8	2.7	15				7.0	15.3
Benzo[c]phenanthrene									0.3	0.8	9.2					
Chrysene	0.2		0.7		0.6	2.5										
Chrysene+triphenylene							2.7	8.7	1.6	5.2	62				20.0	47.7
Benzo[b]fluoranthene	0.04		0.1		0.1	1.2	0.2		2.7	15.4		6.1				
Benzo[k]fluoranthene	0.01		0.04		0.1	0.5	0.4[n]		1.5	8.0		3.7				
Benzo[b+j+k]fluoranthene		197		16.5				7.9			28.7		< 2.3	3.1	11.0	29.3
Benzo[a]pyrene	0.1	157	0.1	8.4	0.1	1.2	0.8	1.3	0.9	5.9	4.2	5.6	0.5	0.6	3.0	11.3
Benzo[e]pyrene	0.03	60.9	0.1	4.8			0.3	4.8	3.9	17.6	7.9		0.7	0.7	4.0	11.3
Perylene					0.02	0.3	< 0.1	1.8	0.5	1.7			< 0.2	0.1	1.0	2.7
Anthanthrene													< 0.05			
Benzo[ghi]perylene	0.1	114	0.1	3.0	0.03	0.7	< 0.1	1.6	1.6	9.2	7.7	10.0	< 0.6	0.4	3.0	6.0
Indeno[1,2,3-cd]pyrene						1.1	< 0.1	1.1	< 0.8	4.5	7.9	2.4	< 0.4		3.0	8.7
Dibenz[a,h]anthracene	0.01		0.03		< 0.02	< 0.1			< 0.2	1.6	1.0					
Dibenz[a,h+ac]anthracene		< 6.1		< 1.0				0.4					< 0.1	0.1		
Dibenzo[a,h]pyrene											0.7					
Dibenzo[a,i]pyrene											0.3					

Notes, see end of table.

Table 1.1.1 (Cont'd)

Food group	fruits and confectionary				cereals					fatty foods					
Food	grapes [f]	bisquits	pudding, biscuits and cakes	sugar and sweets	bran	breakfast cereals	milled wheat	bread	white bread	sunflower oil	sunflower oil	sunflower oil	soya oil	olive oil	corn oil
Reference	[8]	[5]	[2]	[5]	[7]	[2]	[7]	[5]	[2]	[13]	[14]	[15]	[14]	[15]	[15]
PAH															
Acenaphthene					1.6		0.7								
Fluorene					5.9		1.7								
Anthracene	2.5	< 0.3		< 0.3	9.4			< 0.3		0.9	< 3.0	0.3	< 0.4	2.5	< 0.1
Phenanthrene	29.5	< 2.0		< 2.0	94		10.0	< 2.0				3.8		43.3	1.0
Fluoranthene	26.5	1.0	1.4	0.7	130	0.4	1.8	1.0	0.7	2.7	3.4	3.1	< 1.5	12.5	2.5
Pyrene	11.5		2.0		47	0.6	1.6		0.5	2.7	1.0	2.6	< 2.4	9.2	2.6
Benzo[a]fluorene	< 1.5														
Benzo[b]fluorene	< 1.0														
Benzofluorene, sum					29		0.6								
Benzo[ghi]fluoranthene		< 0.7		< 0.7				< 0.7							
Benz[a]anthracene	2.0	< 0.1	1.3	0.2	11	0.1	< 0.1	0.2	0.1	1.0		0.9		1.4	2.6
Benzo[c]phenanthrene		< 0.4		< 0.4				< 0.4							
Chrysene		< 0.4	0.7	0.7		0.1		< 0.4	0.2	1.5					
Chrysene+triphenylene	9.0				9.7		0.3					1.5		5	3.9
Benzo[b]fluoranthene		0.2	0.3	0.2		0.03		0.2	0.1	1.6	1.3		1.2		
Benzo[k]fluoranthene		0.1	0.3	0.1		0.04		0.1	0.1	0.6	0.7		0.6		
Benzo[b+j+k]fluoranthene	3.5				16		0.6					1.5		2.3	2.9
Benzo[a]pyrene	1.5	0.1	0.4	0.15	5.4	0.04	0.2	0.1	0.1	1.0	1.3	0.7	0.9	0.6	1.2
Benzo[e]pyrene	1.5		0.7		5.2	0.1	0.2		0.1			0.7		0.7	1.3
Perylene	< 1.0				0.7		< 0.1					0.2		< 0.2	0.3
Anthanthrene											0.1		< 0.2		
Benzo[ghi]perylene	< 1.0	< 0.1	0.6	< 0.1		0.1		0.2	0.1	1.2	1.4	0.5	1.3	< 0.4	1.0
Indeno[1,2,3-cd]pyrene	< 1.0	< 0.2	0.7	< 0.2		0.1		< 0.2	0.2	0.9	1.2	0.4	0.8	0.4	0.8
Dibenz[a,h]anthracene			0.1			< 0.01			< 0.01	0.3	< 0.5		< 0.2		
Dibenz[a,h+ac]anthracene												< 0.1		< 0.1	< 0.1
Dibenzo[a,h]pyrene															
Dibenzo[a,i]pyrene															

Notes, see end of table.

Table 1.1.1 (Cont'd)

Food group									others			
Food	corn oil	corn oil	vegetable oils	lard and dripping	margarine	cheese	butter	chocolate	beer	skimmed milk powder	dried fruit and pulses	desiccated coconut
Reference	[13]	[14]	[2]	[2]	[2]	[2]	[2]	[2]	[9]	[2]	[2]	[2]
PAH												
Acenaphthene												
Fluorene												
Anthracene	5.5	1.3										
Phenanthrene												
Fluoranthene	22.7	6.3	3.2	2.4	1.8	0.1	0.6	2.5		1.2	0.7	29.4
Pyrene	19.1	4.7	3.5	2.3	2.1	0.8	1.2	3.6		0.8	1.3	24.5
Benzo[a]fluorene												
Benzo[b]fluorene												
Benzofluorene, sum												
Benzo[ghi]fluoranthene												
Benz[a]anthracene	10.7		1.9	0.8	1.5	0.1	0.1	0.5		0.2	0.2	3.8
Benzo[c]phenanthrene												
Chrysene	11.7		2.1	0.7	1.9	0.1	0.1	0.7		0.2	0.3	4.8
Chrysene+triphenylene												
Benzo[b]fluoranthene	10.5	0.9	0.7	0.2	0.8	0.04	0.03	0.1	0.1	0.1	0.1	2.8
Benzo[k]fluoranthene	5.1	0.5	1.0	0.4	1.1	0.1	0.03	0.2	0.1	0.1	0.1	1.3
Benzo[b+j+k]fluoranthene												
Benzo[a]pyrene	10.7	0.9	1.3	0.4	1.7	0.04	0.1	0.2	0.04	0.1	0.1	3.4
Benzo[e]pyrene			1.8	0.7	2.3	0.1	0.1	0.2		0.2	0.2	2.5
Perylene												
Anthanthrene		< 0.1										
Benzo[ghi]perylene	6.1	< 0.4	1.5	0.5	1.8	0.1	0.2	0.6	0.1	0.2	0.1	4.5
Indeno[1,2,3-cd]pyrene	7.3	0.7	2.3	0.8	2.9	0.04	0.2	0.3	0.04	0.2	0.2	1.9
Dibenz[a,h]anthracene	2.3	< 0.2	0.2	0.1	0.3	< 0.01	< 0.01	0.03	< 0.01	0.01	0.01	0.1
Dibenz[a,h+ac]anthracene												
Dibenzo[a,h]pyrene												
Dibenzo[a,i]pyrene												

Notes, see end of table.

Notes table 1.1.1

- References: [1] Dennis *et al.*, 1983, [2] Dennis *et al.*, 1991, [3] Vaessen *et al.*, 1984, [4] Germany, Ministry of Environment, 1994, [5] de Vos *et al.*, 1990 [6] Karl and Leinemann, 1996, [7] Tuominen *et al.*, 1988 , [8] Corradetti *et al.*, 1990, [9] Moret *et al.*, 1995, [10] Speer *et al.*, 1990, [11] Wickström *et al.*, 1986, [12] Dennis *et al.*, 1984, [13] Welling and Kaandorp, 1986, [14] Stijve and Hischenhuber, 1987, [15] Speer and Montag, 1988, [16] D. Gott, Personal communication, [27] Larsson, 1982
- For the criteria used to select the studies, see chapter 1.2.
- [a] From an industrial area. [b] Sausage and pork chops. [c] Urban site with some industrial sources. [d] Median PAH levels in the edible part of more than eight species, from both traditional kilns and kilns with external smoke generation. [e] Mean PAH levels in the edible part of five species, from traditional smoking kilns. [f] Contaminated by industrial sources. [g] Benzo[a]anthracene+chrysene. [h] Mean PAH levels in the edible part of eight species, from kiln with external smoke generator. [i] 3-month mean at a control site for the mussels reported in the next column (see note m). [m] 5-month mean of two sites downstream an aluminium smelter. [n] Benzo[k]fluoranthene+benzo[j]fluoranthene.

barbecue prevented fat from dripping onto the heat source, and the PAH level were very low and 10-30 times lower than with the horizontal system (Saint-Aubert *et al.*, 1992).

Smoked foods

On a quantitative basis, the data reported in the literature are highly variable. Such variations can be attributed in part to the different procedures used to evaluate the presence of PAH, but the main reason for such discrepancies is the difference in procedures used for smoking. Such variables include: the type and composition of wood, type of generator, oxygen accessibility, temperature of smoke generation, and smoking time. PAH content in smoked fishery products from modern smoking kilns with external smoke generation and products from traditional smoking kilns have been compared. The average benzo[*a*]pyrene concentration determined for the traditional kilns was 1.2 µg/kg with a sum of carcinogenic compounds of 9 µg/kg, and 0.1 µg/kg and 4.5 µg/kg respectively, for the modern kilns (Karl and Leinemann, 1996). In Annex II of Directive 88/388/EEC (EEC, 1988), a maximum level of 0.03 µg benzo[*a*]pyrene per kg as a result of the use of (smoke) flavourings has been set for foodstuffs or beverages as consumed.

Regarding the generation of liquid smoke flavorings, it has been showed that poplar wood generated the highest number and concentration of both total and carcinogenic PAH, while oak, cherry tree, beech samples were similarly less effective. Hardwoods instead of softwoods have also been recommended, indeed, dry woods generate more PAH because of their higher smoke generation temperature (Guillen *et al.*, 2000).

Vegetable oils

Vegetable oils and fats are a significant source of PAH in the diet, either directly, as in the case of vegetable oils used for seasoning and margarine used for cooking, or indirectly by their incorporation into other foods such as the cereal-based products, biscuits and cakes (Dennis *et al.*, 1991).

The occurrence of PAH in vegetable oils (including olive residue oils) is mostly related to the drying processes of the seeds where combustion gases may come into contact with the seeds (Speer *et al.*, 1990; Standing Committee on Foodstuffs, 2001). The levels of PAH in crude edible oils vary widely and refining (based on the deodorization step) reduces the concentration of a number of the lower molecular weight compounds such as fluoranthene, while no corresponding effect is observed for the higher molecular weight PAH. The level of the latter may be reduced by treatment with activated charcoal (Larsson *et al.*, 1987); this refining method has been reported to be widely used (Dennis *et al.*, 1991).

Coffee, tea

Roasting and drying of coffee beans and tealeaves increase the PAH content (Stall and Eisenbrand, 1988). However, the PAH concentrations found in the beverages were lower than in the beans/leaves (Lintas *et al.*, 1979; Maier, 1991). A Finnish study showed that roasted ground coffee and dried tealeaves contained high levels of PAH namely 100-200 µg/kg and 480-1400 µg/kg, respectively. However, PAH could not be detected in tea and coffee beverages (Hietaniemi *et al.*, 1999).

A Spanish study on coffee brew samples showed that their total PAH (8 compounds) content depends mainly on the sample elaboration. For instance, coffee brew made from de-caffeinated and green coffee beans were found to contain mean concentrations of 2.0 and 1.7 ng PAH/L coffee brew, respectively. The highest concentration (2.9 ng PAH/L of coffee brew) was found in coffee brew prepared from torrefied (roasted in the presence of sugar) beans (Kayali-Sayadi *et al.*, 1999). All the samples contained benzo[*a*]pyrene and dibenzo[*a,h*]anthracene. These results are in good agreement with available data for benzo[*a*]pyrene in coffee brew, which are at the level of 1 ng/L (De Kruijf, 1987, personal communication from Lambré, C., 2002).

Human milk

In a study conducted in the Federal Republic of Germany in 1984 a number of individual PAH compounds were found at concentrations ranging from 5 to 15 ng/kg human milk. Benzo[*a*]pyrene was detected at a concentration of 6.5 ng/kg (number of samples not available; Deutsche Forschungsgemeinschaft, 1984, cited in IPCS, 1998). In an Austrian study (Lechner *et al.,* 1991), benzo[a]pyrene was not detected in any of 41 samples of human milk from the region of Tyrol (limit of detection: 0.1-1 µg/kg).

In another German study, PAH levels ranging from 3 to 30 ng/kg human milk were found (Heeschen, 1985; cited in IPCS, 1998).

A Scientific Co-operation task (EC, 2001a) aimed at the collection of occurrence data of PAH in food in EU member states is currently ongoing. A report will become available in 2003.

1.1.6 Measures to reduce PAH contamination of foods

The amount of PAH formed during cooking or processing of food depends markedly on the conditions used. Simple practices are known to result in a significantly reduced contamination of foods by PAH (Lijinsky and Ross, 1967; Lijinsky, 1991; Knize *et al.*, 1999) as well as by other undesirable contaminants. This may include selecting

preferentially lean meat and fishes, avoiding contact of foods with flames for barbecuing, using less fat for grilling, and, in general, cooking at lower temperature for a longer time. Broiling (heat source above) instead of grilling can significantly reduce the levels of PAH. Actually the fat should not drip down onto an open flame sending up a column of smoke that coats the food with PAH. The use of medium to low heat, and placement of the meat further from the heat source, can greatly reduce formation of PAH. The intensity of flavour is not necessarily associated with the depth of the brown color of grilled foods. It is therefore needless to overcook the food to get the flavour. However, cooking must always remains effective as regards inactivation of any possible contaminating bacteria or endogenous toxins.

In some countries (e.g. Norway) the food authorities have banned the sale of shellfish and mussels caught in areas contaminated with PAH. The public is advised not to catch and consume PAH-contaminated shellfish and mussels. Depuration of contaminated mussels in clean water will not reduce their level of PAH significantly.

The waxy surface of vegetables and fruits can concentrate low molecular mass PAH mainly through surface adsorption. The concentrations of PAH are generally higher on plant surface (peel, outer leaves) than in internal tissue. Consequently, careful washing may remove, on average, up to 50% of the total PAH. Particle bound high molecular mass PAH which remain on the surface are easily washed off whereas low molecular mass compounds which are in the vapor phase can penetrate the waxy layer of fruits and vegetables and, therefore, are less efficiently removed by washing.

1.2 Profiles of PAH occurring in food

The Committee noted that there were large differences in the choice of the PAH determined by various authors making comparisons between results from analysis of food by different authors almost impossible. The Committee therefore examined the possibility of using benzo[*a*]pyrene (which almost always is included in any analysis) as a marker for PAH in foods. The patterns of PAH distributions (profiles) relative to benzo[*a*]pyrene in various foods were examined. In particular, the Committee considered the uncertainties in using benzo[*a*]pyrene as a marker for the class of PAH compounds or for a defined set of PAH (e.g. the carcinogenic PAH).

The PAH profiles were calculated from studies selected from the literature according to the following criteria:

- ✓ The concentrations of both benzo[*a*]pyrene and several other PAH are quantified; i.e. they are not reported as 'not detected' or 'less than'.
- ✓ The concentration of benzo[*a*]pyrene is not equal to (nor a little above) its limit of detection: at this concentration level the accuracy is poor and hence any profile is poorly reliable.
- ✓ The concentrations in the individual sample(s) or the mean concentration(s) are reported, and not only the ranges.
- ✓ The study was performed in the European Union.
- ✓ The study was published as from 1980.
- ✓ The study does not provide clearly inconsistent results.
- ✓ The selected foods are of some relevance due to their higher consumption or their representativeness (e.g., high number of samples or consumption in different geographical areas).

Table 1.2.1 shows PAH profiles relative to benzo[*a*]pyrene in different foods. For any food/ investigation, the profile is reported as a set of ratios between the available concentrations of any PAH and benzo[*a*]pyrene ([PAH]/[BaP]). For data completeness, the PAH concentrations as measured in the individual foods from which the profiles were calculated, are given in table 1.1.1 in chapter 1.1[1].

[1] The [PAH]/[BaP] ratio calculated from these concentrations may not be exactly the same as the one reported in table 1.2.1. This is because, for some foods, the profile in table 1.2.1 is the mean of the profiles in the individual samples, and not the profile calculated from the mean concentrations.

Table 1.2.1 PAH profiles relative to BaP in different foods ([PAH]/[BaP])

Food group	meat and meat products		fish and marine foods					mussels		
Food	meat	grilled meat [b]	fish	smoked herring	smoked fish [h]	smoked fish [e]	smoked fish [s]	canned	'control' site [i]	from shellfish farm [m]
Number of samples	5	3	5	1	35	27	58	3	3	12
Analysis	HPLC	GC/FID	HPLC	GC/FID	HPLC and GC/MS	HPLC and GC/MS	GC/FID	GC/MS	GC/MS (?)	GC/MS (?)
Reference [r]	[1]	[12]	[1]	[12]	[6]	[6]	[27]	[10]	[16]	[16]
PAH [d]	**BaP profile ([PAH]/[BaP])**									
Acenaphthene										
Fluorene									0.6	0.2
Anthracene					63.0	17.5	20.6	0.3	< 0.2	< 0.1
Phenanthrene					320	54.4	91	4.3	2.1	0.6
Fluoranthene	9.6	9.4	6.2	12.7	91.0	21.7	20.7	8.1	< 2.0	1.4
Pyrene	11.0	10.6	6.1	13.2	53.0	17.1	16.0	5.4	1.4	1.0
Benzo[a]fluorene										
Benzo[b]fluorene										
Benzofluorene, sum										
Benzo[ghi]fluoranthene										
Benz[a]anthracene	1.0	1.3	1.1	3.2	6.0	2.1	2.8	2.7	0.9	0.8
Benzo[c]phenanthrene									0.4	0.3
Chrysene	3.0		5.0		6.0	2.1				
Chrysene+triphenylene							3.6	6.5	< 1.5	1.4
Benzo[b]fluoranthene	0.8		1.0		1.0	1.0	0.5		2.6	3.0
Benzo[k]fluoranthene	0.2		0.3		0.7	0.4	[t] 0.7		1.4	1.6
Benzo[b+j+k]fluoranthene		1.7		2.0				5.9		
Benzo[e]pyrene	0.6	0.9	0.9	0.6			0.4	3.6	4.2	4.5
Perylene					0.2	0.3	0.4	1.2	0.6	0.4
Anthanthrene										
Benzo[ghi]perylene	1.0	1.3	0.9	0.4	0.3	0.6	< 0.2	1.3	1.6	2.2
Indeno[1,2,3-cd]pyrene						0.9	< 0.2	0.9	< 0.8	0.9
Dibenz[a,h]anthracene	0.2		0.2		< 0.2	< 0.1			< 0.2	0.3
Dibenz[a,h+ac]anthracene		< 0.1		< 0.1				0.3		
Dibenzo[a,h]pyrene										
Dibenzo[a,i]pyrene										

Notes: see end of table.

Table 1.2.1 (Cont'd)

Food group	vegetables				fruits and confectionary					
Food	kale	lettuce[a]	lettuce	lettuce [c]	chicory [f]	olives [f]	grapes [f]	bisquits	puddings, biscuits and cakes	sugar and sweets
Number of samples	16	?	20	1	1	3	2	10	6	10
Analysis	GC/FID	?	GC/MS	GC/MS	GC/FID	GC/FID	GC/FID	HPLC	HPLC	HPLC
Reference [r]	[3]	[4]	[11]	[11]	[8]	[8]	[8]	[5]	[2]	[5]
PAH [d]										
Acenaphthene										
Fluorene										
Anthracene			< 0.1	< 0.1	3.3	3.5	2.0	< 3.0		< 2.0
Phenanthrene			7.2	2.7	128	79.4	23.3	< 20.0		< 13.3
Fluoranthene	27.9	5.0	15.2	9.3	48.7	18.0	21.0	10.0	3.1	4.7
Pyrene	16.7		13.1	11.2	23.0	12.3	9.3		4.6	
Benzo[a]fluorene			1.4	1.5	3.7	2.0	< 1.0			
Benzo[b]fluorene			1.0	2.7	1.7	1.4	< 0.8			
Benzofluorene, sum										
Benzo[ghi]fluoranthene								< 7.0		< 4.7
Benz[a]anthracene	3.6				2.3	1.5	1.3	< 1.0	3.0	1.3
Benzo[c]phenanthrene	2.2							< 4.0		< 2.7
Chrysene								< 4.0	1.5	4.7
Chrysene+triphenylene	14.8				6.7	4.7	6.8			
Benzo[b]fluoranthene		1.1						2.0	0.6	1.3
Benzo[k]fluoranthene		0.7						1.0	0.7	0.7
Benzo[b+j+k]fluoranthene	6.8		3.3	5.3	3.7	2.6	2.5			
Benzo[e]pyrene	1.9		1.4	1.3	1.3	1.0	1.0		1.6	
Perylene			0.2	0.2	0.3	0.3	< 0.8			
Anthanthrene			< 0.1							
Benzo[ghi]perylene	1.8	1.8	0.8	0.6	1.0	0.5	< 0.8	< 1.0	1.3	< 0.7
Indeno[1,2,3-cd]pyrene	1.9	0.4	0.7		1.0	0.8	< 0.8	< 2.0	1.7	< 1.3
Dibenz[a,h]anthracene									0.1	
Dibenz[a,h+ac]anthracene	0.2		0.1	0.2						
Dibenzo[a,h]pyrene	0.2									
Dibenzo[a,i]pyrene	0.1									

Notes: see end of table.

Table 1.2.1 (Cont'd)

Food group	cereals					fatty foods							
Food	bran	breakfast cereals	milled wheat	bread	white bread	sunflower oil			soya oil	olive oil	corn oil		
Number of samples	1	3	19	10	10	4	1	4	3	7	5	3	2
Analysis	GC/MS	HPLC	GC/MS	HPLC	HPLC	HPLC	HPLC	GC/MS	HPLC	GC/MS	GC/MS	HPLC	HPLC
Reference [r]	[7]	[2]	[7]	[5]	[2]	[13]	[14]	[15]	[14]	[15]	[15]	[13]	[14]
PAH [d]													
Acenaphthene	0.3		3.5										
Fluorene	1.1		8.5										
Anthracene	1.7			< 3.0		1.1	< 2.3	0.5	< 0.4	5.5	0.1	0.7	1.8
Phenanthrene	17.4		50.0	< 20.0				6.3		89.4	0.7		
Fluoranthene	24.1	8.8	9.0	10.0	7.1	3.9	2.6	4.8	2.2	23.0	2.4	3.2	5.1
Pyrene	8.7	14.5	8.0		5.3	3.6	0.8	3.8	2.0	19.4	2.2	2.6	3.8
Benzo[a]fluorene													
Benzo[b]fluorene													
Benzofluorene, sum	5.4		3.0										
Benzo[ghi]fluoranthene				< 7.0									
Benz[a]anthracene	2.0	2.3	< 0.5	2.0	1.2	1.0	<[g] 2.0	1.5	<[g] 2.8	2.6	1.6	1.2	<[g] 2.1
Benzo[c]phenanthrene				< 4.0									
Chrysene		2.8		< 4.0	1.5	1.7						1.5	
Chrysene+triphenylene	1.8		1.5					2.4		9.9	3.2		
Benzo[b]fluoranthene		0.8		2.0	0.5	1.7	1.0		1.2			1.2	1.0
Benzo[k]fluoranthene		1.0		1.0	0.8	0.7	0.5		0.6			0.5	0.5
Benzo[b+j+k]fluoranthene	3.0		3.0					2.4		4.3	2.4		
Benzo[e]pyrene	1.0	2.3	1.0		1.1			1.0		1.1	1.0		
Perylene	0.1		< 0.5					0.3		0.3	0.2		
Anthanthrene							0.08		0.2				< 0.2
Benzo[ghi]perylene		1.8		2.0	1.2	1.0	1.1	0.7	1.4	0.5	0.7	1.4	< 0.7
Indeno[1,2,3-cd]pyrene		2.8		< 2.0	2.0	0.8	0.9	0.6	0.9	0.5	0.6	0.7	0.7
Dibenz[a,h]anthracene		< 0.3			< 0.1	0.3	< 0.4		0.2			0.2	< 0.3
Dibenz[a,h+ac]anthracene								< 0.2		< 0.2	< 0.1		
Dibenzo[a,h]pyrene													
Dibenzo[a,i]pyrene													

Notes: see end of table.

Table 1.2.1 (Cont'd)

Food group							others			
Food	vegetable oils	lard and dripping	margarine	cheese	butter	chocolate	beer	skimmed milk powder	dried fruit and pulses	desiccated coconut
Number of samples	18	7	19	4	4	6	6	5.0	7	7
Analysis	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC
Reference [r]	[2]	[2]	[2]	[2]	[2]	[2]	[9]	[2]	[2]	[2]
PAH [d]										
Acenaphthene										
Fluorene										
Anthracene										
Phenanthrene										
Fluoranthene	2.4	6.7	1.1	3.0	10.7	12.1		11.0	8.8	8.7
Pyrene	2.7	6.4	1.3	19.5	20.0	17.2		7.1	16.1	7.3
Benzo[a]fluorene										
Benzo[b]fluorene										
Benzofluorene, sum										
Benzo[ghi]fluoranthene										
Benz[a]anthracene	1.5	2.1	0.9	3.5	1.5	2.2		1.6	2.3	1.1
Benzo[c]phenanthrene										
Chrysene	1.7	1.8	1.2	3.3	2.3	3.4		1.9	3.5	1.4
Chrysene+triphenylene										
Benzo[b]fluoranthene	0.6	0.6	0.5	1.0	0.5	0.7	1.8	0.9	0.6	0.8
Benzo[k]fluoranthene	0.8	1.0	0.6	1.5	0.5	0.9	1.5	1.0	1.0	0.4
Benzo[b+j+k]fluoranthene										
Benzo[e]pyrene	1.4	1.9	1.4	2.3	1.7	0.9		2.0	2.4	0.7
Perylene										
Anthanthrene										
Benzo[ghi]perylene	1.1	1.3	1.1	2.8	3.8	2.7	3.3	2.0	1.6	1.3
Indeno[1,2,3-cd]pyrene	1.8	2.3	1.7	1.0	2.5	1.4	1.0	1.6	2.5	0.6
Dibenz[a,h]anthracene	0.2	0.4	0.2	< 0.3	< 0.2	0.1	< 0.1	0.1	0.1	0.04
Dibenz[a,h+ac]anthracene										
Dibenzo[a,h]pyrene										
Dibenzo[a,i]pyrene										

Notes: see end of table.

Table 1.2.1 (Cont'd)

	BaP profile ([PAH]/[BaP]), overall results							
						Max/Min		
							≥ 90% of data[v]	
	Mean	Median	Min	Max	n	All data		n excluded
Acenaphthene	1.9	1.9	0.3	3.5	2	12	12	0
Fluorene	2.6	0.8	0.2	8.5	4	43	43	0
Anthracene	5.8	1.8	< 0.1	63	23	> 788	> 219	2
Phenanthrene	48.9	< 20.0	0.6	320	19	533	213	1
Fluoranthene	12.3	8.8	1.1	91	42	83	14	4
Pyrene	10.5	8.4	0.8	53	38	69	18	3
Benzo[a]fluorene	1.9	1.5	< 1.0	3.7	5	> 3.7	> 3.7	0
Benzo[b]fluorene	1.5	1.4	< 0.8	2.7	5	> 3.4	> 3.4	0
Benzofluorene, sum	4.2	4.2	3.0	5.4	2	1.8	1.8	0
Benzo[ghi]fluoranthene	< 6.2	< 7.0	< 4.7	< 7.0	3	-	-	0
Benz[a]anthracene	1.9	1.6	< 0.5	6.0	39	> **12**	**4.1**	4
Benzo[c]phenanthrene	< 2.3	< 2.4	0.3	< 4.0	6	< 13	< 13	0
Chrysene	2.8	2.3	1.2	6.0	21	5.2	3.5	2
Chrysene+triphenylene	5.0	3.6	1.4	14.8	13	11	7.1	1
Benzo[b]fluoranthene	1.1	1.0	0.5	3.0	29	**6.1**	**4.1**	3
Benzo[k]fluoranthene	0.8	0.7	0.2	1.6	29	**8.0**	**3.9**	3
Benzo[b+j+k]fluoranthene	3.5	3.0	1.7	6.8	14	**4.0**	**3.5**	1
Benzo[e]pyrene	1.5	1.3	0.4	4.5	31	11	6.3	3
Perylene	0.4	0.3	0.1	1.2	16	9.3	6.0	1
Anthanthrene	< 0.1	< 0.1	0.08	0.2	4	2.6	2.6	0
Benzo[ghi]perylene	1.3	1.1	< 0.2	3.8	41	> 19	7.7	4
Indeno[1,2,3-cd]pyrene	1.2	0.9	< 0.3	2.8	35	> **9.2**	**5.0**	3
Dibenz[a,h]anthracene	0.2	0.2	0.04	0.4	24	**10**	> **3.8**	2
Dibenz[a,h+ac]anthracene	0.2	0.2	< 0.1	0.3	9	> **2.6**	> **2.5**	0
Dibenzo[a,h]pyrene	0.2	-	-	-	1	-	-	0
Dibenzo[a,i]pyrene	0.1	-	-	-	1	-	-	0

Notes: see end of table.

Table 1.2.1 (Cont'd)

	BaP profile ([PAH]/[BaP]) in							
	coke-oven fumes [n]			urban air [o]			coal tar [u]	
	n = 6		fumes median/	n = 4		urban air median/	n = 2	coal tar median/
	Range	Median	foods median [z]	Range	Median	foods median [z]	Median	foods median [z]
Acenaphthene							0.8	0.4
Fluorene							1.9	2.2
Anthracene							1.2	0.7
Phenanthrene							3.9	> 0.2
Fluoranthene							2.5	0.3
Pyrene							2.7	0.3
Benzo[a]fluorene								
Benzo[b]fluorene								
Benzofluorene, sum								
Benzo[ghi]fluoranthene								
Benz[a]anthracene	0.9-2.8	**1.7**	**1.0**	0.5-1.9	**0.9**	**0.6**	**1.3**	**0.8**
Benzo[c]phenanthrene								
Chrysene								
Chrysene+triphenylene							1.2	0.3
Benzo[b]fluoranthene							**1.1**	**1.1**
Benzo[k]fluoranthene							**0.4**	**0.5**
Benzo[b+j+k]fluoranthene	0.6 - ≥ 2.3	**2.2**	**0.7**	2.1-4.4	**> 2.4**	**> 0.8**	**≥ 1.5**	**≥ 0.5**
Benzo[e]pyrene								
Perylene								
Anthanthrene								
Benzo[ghi]perylene							0.8	0.7
Indeno[1,2,3-cd]pyrene	0.4-0.7	**0.6**	**0.7**	1.0-1.6	**1.5**	**1.6**	**0.7**	**0.8**
Dibenz[a,h]anthracene	0.04-0.2	**≤ 0.2**	**≤ 1.1**	0.1-0.3	**0.2**	**1.1**		
Dibenz[a,h+ac]anthracene							**0.1**	**0.9**
Dibenzo[a,h]pyrene								
Dibenzo[a,i]pyrene								

Notes: see end of table.

Notes table 1.2.1

- References: [1] Dennis *et al.*, 1983, [2] Dennis *et al.*, 1991, [3] Vaessen *et al.*, 1984, [4] Germany, Ministry of Environment, 1994, [5] de Vos *et al.*, 1990 [6] Karl and Leinemann, 1996, [7] Tuominen *et al.*, 1988 , [8] Corradetti *et al.*, 1990, [9] Moret *et al.*, 1995, [10] Speer *et al.*, 1990, [11] Wickström *et al.*, 1986, [12] Dennis *et al.*, 1984, [13] Welling and Kaandorp, 1986, [14] Stijve and Hischenhuber, 1987, [15] Speer and Montag, 1988, [16] D. Gott, Personal communication, [17] Bjørseth *et al.*, 1978, [18] Andersson *et al.*, 1983, [19] Haugen *et al.*, 1986, [20] Jongeneelen *et al.*, 1990, [21] Buchet *et al.*, 1992, [22] Grimmer *et al.*, 1993, [23] Brown *et al.*, 1996, [24] Nielsen *et al.*, 1996, [25] Dörr *et al.*,1996, [26] Menichini *et al.*, 1999,[27] Larsson, 1982, [28] Culp *et al.*, 1998, [29] Koganti *et al.*, 2000
- Unlisted PAH were not determined in any study. PAH in boldface are classified as 2A or 2B by IARC.
- Values are generally rounded to the nearest 0.1 but calculations were performed with all figures originally available. Barred values: see column "Max/Min, ≥ 90% of data" and note v.
- [a] From an industrial area. [b] Sausage and pork chops. [c] Urban site with some industrial sources. [d] Ranked according to increasing molecular mass and, for isomers, in alphabetical order. [e] Traditional smoking kilns (five species). [f] Contaminated by industrial emissions.[g] Benz[a]anthracene + chrysene. [h] External smoke generator (eight species). [i] 3-month mean at a control site for the mussels reported in the next column (see note m). [m] 6-month mean of two sites downstream an aluminium smelter.

  [n] Cumulative data elaborated from refs. 17-22. [o] Four European cities (London, Augsburg (D) and Rome: annual means; Copenhagen: winter mean), years '90, cumulative data from refs. 23-26. [r] Data re-elaborated; for the references, see notes at end of table 1.1.1. [s] More than eight species, from both traditional kilns and kilns with external smoke generation. [t] Benzo[k]fluoranthene+benzo[j]fluoranthene. [u] Median values from two coal tar mixtures showing very similar profiles (from refs. 28-29). [v] See text. The number of data excluded is reported in the rightside column. The values excluded are those barred with an oblique line. [z] Ratio between the values in the pertinent column "Median" and the column "overall results - Median".

For any individual PAH, the ratio between the maximum and the minimum [PAH]/[BaP] is taken as an indicator of the maximum observed variability of [PAH]/[BaP] among different foods.

The ratio for each PAH was also re-calculated excluding (at most and when applicable) 10% of the pertinent studies giving the results most distant from the overall mean/median value (i.e., as many studies were used in the re-calculation as corresponded to a minimum of 90% of all the studies concerning that PAH; hence this procedure was applied to PAH determined in at least 10 studies). This exercise allows evaluating the overall profile variability not affected by extreme values, which possibly derive from specific individual foods/investigations or from any poorly accurate determination (which is not infrequent in the analysis of PAH complex mixtures, especially due to co-eluting problems).

For the sake of comparison with the use of benzo[*a*]pyrene as an indicator of carcinogenicity by inhalation, the profiles are also reported for coke-oven fumes, urban air and coal tar (profiles are limited to carcinogenic PAH for the first two matrices). This is because: [a] WHO established a carcinogenic unit risk for benzo[*a*]pyrene as indicator air constituent for PAH, based on epidemiological data from studies in coke-oven workers (WHO 2001); [b] according to the EC (2001b) Position Paper on 'Ambient air pollution on PAH', benzo[*a*]pyrene can be used as a marker of the carcinogenic risk of airborne PAH compounds; [c] some experimental studies concerning PAH carcinogenicity by ingestion (and considered in this report, see chapter 2.4) included comparison with coal tar administration in the diet (Culp *et al.*, 1998; Koganti *et al.*, 2000).

Finally, to examine whether it is possible to distinguish the PAH profiles on the basis of different sources, two subsets of foods were selected and compared: those which are expected to be contaminated only by atmospheric contamination and those where PAH are formed during processing (tables 1.2.3-1.2.5).

The following conclusions may be drawn on the variability of [PAH]/[BaP] in different foods:

- ✓ The lower-molecular 3- and 4-ring PAH (fluorene, anthracene, phenanthrene, fluoranthene and pyrene) show variability markedly higher than higher-molecular mass PAH.
- ✓ The variability for almost all the higher-molecular mass PAH - from benzofluorenes upwards - results to be within a factor of ten. The variability markedly decreases after excluding (at most) 10% of data, resulting within one order of magnitude for all these PAH. This holds especially for carcinogenic PAH (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*j*]fluoranthene, indeno[1,2,3-

cd]pyrene, dibenz[*a,h*]anthracene), whose profile relative to benzo[*a*]pyrene results to be constant in different foods within a factor of five.

- ✓ It appears that benzo[*a*]pyrene may be used as an indicator of occurrence and concentrations of PAH from benzofluorenes upwards, at least in terms of orders of magnitude. Benzo[*a*]pyrene may however not be used as an indicator of lower-molecular PAH.
- ✓ The median profile of carcinogenic PAH is very similar to those in coke-oven fumes and in urban air, within an approximate factor of two.
- ✓ The PAH median profile is also similar to that one of coal tar, well within one order of magnitude. In particular, for carcinogenic PAH, the ratios between the values of [PAH]/[BaP] in coal tar and in foods (median values) are in the range 0.5-1.1.

In evaluating the variability of profiles resulting from table 1.2.1, it should be taken into account that a significant part of variability is likely to be consequent to: [a] the different analytical procedures adopted in different investigations, [b] any poor accuracy possibly present in some investigations and/or some PAH (see above). So, when the comparison of profiles in different foods is based on analyses performed in the same laboratory with the same procedure, as is the case of the investigation of Dennis *et al.* (1991), the variability for the higher-molecular PAH (from benz[*a*]anthracened upwards) results to be at most within a 5-fold factor, without excluding any food (table 1.2.2); a higher [PAH]/[BaP] ratio of 8.7 is only observed for dibenz[*ah*]anthracene. The accuracy of its determination however is affected by the measured concentration levels laying around the detection limit.

In comparison with foods contaminated by deposition and with all foods overall considered, processed foods show markedly different profiles only for anthracene and phenanthrene, these PAH being more abundant in processed foods. As to the other PAH, and especially for the carcinogenic ones, the profiles appear to be similar, any difference resulting to be within an approximate factor of two (both between them and in comparison with ‘all foods’).

Table 1.2.2 PAH profiles relative to BaP ([PAH]/[BaP]) in different foods, as calculated from the investigation of Dennis et al. (1991)

Food	White bread	Puddings, biscuits and cakes	Breakfast cereals	Lard and dripping	Vegetable oils	Margarine
Number of samples	10	6	3	7	18	19
PAH	**[PAH]/[BaP]**					
Fluoranthene	7.1	3.1	8.8	6.7	2.4	1.1
Pyrene	5.3	4.5	14.5	6.4	2.7	1.3
Benz[a]anthracene	**1.2**	**3.0**	**2.3**	**2.1**	**1.5**	**0.9**
Chrysene	1.5	1.5	2.8	1.8	1.7	1.1
Benzo[b]fluoranthene	**0.5**	**0.6**	**0.8**	**0.6**	**0.6**	**0.5**
Benzo[k]fluoranthene	**0.8**	**0.7**	**1.0**	**1.0**	**0.8**	**0.6**
Benzo[e]pyrene	1.1	1.5	2.3	1.9	1.4	1.4
Benzo[ghi]perylene	1.2	1.3	1.8	1.3	1.1	1.1
Indeno[1,2,3-cd]pyrene	**2.0**	**1.7**	**2.8**	**2.3**	**1.8**	**1.7**
Dibenz[a,h]anthracene	**< 0.1**	**0.1**	**< 0.3**	**0.4**	**0.2**	**0.1**

Food	Cheese	Butter	Chocolate	Skimmed milk powder	Dried fruit and pulses	Desiccated coconut	**[PAH]/[BaP], overall results** (12 foods, 96 samples)				
Number of samples	4	4	6	5	7	7					
PAH	**[PAH]/[BaP]**						Mean	Median	Min	Max	Max/Min
Fluoranthene	3.0	10.7	12.1	11.0	8.8	8.7	7.0	7.9	1.1	12.1	11.1
Pyrene	19.5	20.0	17.2	7.1	16.1	7.3	10.2	7.2	1.3	20.0	15.9
Benz[a]anthracene	**3.5**	**1.5**	**2.2**	**1.5**	**2.3**	**1.1**	**1.9**	**1.8**	**0.9**	**3.5**	**4.1**
Chrysene	3.3	2.3	3.4	1.9	3.5	1.4	2.2	1.9	1.1	3.5	3.0
Benzo[b]fluoranthene	**1.0**	**0.5**	**0.7**	**0.9**	**0.6**	**0.8**	**0.7**	**0.6**	**0.5**	**1.0**	**2.0**
Benzo[k]fluoranthene	**1.5**	**0.5**	**0.9**	**1.0**	**1.0**	**0.4**	**0.8**	**0.8**	**0.4**	**1.5**	**3.9**
Benzo[e]pyrene	2.3	1.7	0.9	2.0	2.4	0.7	1.6	1.6	0.7	2.4	3.2
Benzo[ghi]perylene	2.8	3.8	2.7	2.0	1.6	1.3	1.8	1.5	1.1	3.8	3.5
Indeno[1,2,3-cd]pyrene	**1.0**	**2.5**	**1.4**	**1.5**	**2.5**	**0.6**	**1.8**	**1.8**	**0.6**	**2.8**	**5.0**
Dibenz[a,h]anthracene	**< 0.3**	**< 0.2**	**0.1**	**0.1**	**0.1**	**0.04**	**< 0.2**	**0.1**	**0.04**	**0.36**	**8.7**

Unlisted PAHs were not determined in the investigation. PAHs in boldface are classified as 2A or 2B by IARC.
Analysis by HPLC

Table 1.2.3 PAH profiles in foods expected to be contaminated by atmospheric deposition

Food	kale	lettuce	lettuce	chicory	olives	lettuce	grapes						
Number of samples	16	?	20	1	3	1	2						
Reference [a]	[3]	[4]	[11]	[8]	[8]	[11]	[8]						
								[PAH]/[BaP]					
PAH								Mean	Median	Min	Max	n	Max/Min
Acenaphthene												0	
Fluorene												0	
Anthracene			< 0.1	3.3	3.5	< 0.1	2.0	1.8	2.0	< 0.1	4	5	> 41
Phenanthrene			7.2	128	79.4	2.7	23.3	48.1	23.3	2.7	128	5	47
Fluoranthene	27.9	5.0	15.2	48.7	18.0	9.3	21.0	20.7	18.0	5.0	49	7	10
Pyrene	16.7		13.1	23.0	12.3	11.2	9.3	14.3	12.7	9.3	23	6	2
Benzo[a]fluorene			1.4	3.7	2.0	1.5	< 1.0	1.9	1.5	< 1.0	3.7	5	> 3.7
Benzo[b]fluorene			1.0	1.7	1.4	2.7	< 0.8	1.5	1.4	< 0.8	2.7	5	> 3.4
Benzofluorene, sum												0	
Benzo[ghi]fluoranthene												0	
Benz[a]anthracene	3.6			2.3	1.5		1.3	2.2	1.9	1.3	3.6	4	3
Benzo[c]phenanthrene	2.2							2.2	-	-	-	1	-
Chrysene												0	
Chrysene+triphenylene	14.8			6.7	4.7		6.8	8.2	6.8	4.7	14.8	4	3
Benzo[b]fluoranthene		1.1						1.1	-	-	-	1	-
Benzo[k]fluoranthene		0.7						0.7	-	-	-	1	-
Benzo[b+j+k]fluoranthene	6.8		3.3	3.7	2.6	5.3	2.5	4.0	3.5	2.5	6.8	6	2.7
Benzo[e]pyrene	1.9		1.4	1.3	1.0	1.3	1.0	1.3	1.3	1.0	1.9	6	2
Perylene			0.2	0.3	0.3	0.2	< 0.8	0.4	0.3	0.2	< 0.8	5	< 3.9
Anthanthrene			< 0.1					0.1	-	-	-	1	-
Benzo[ghi]perylene	1.8	1.8	0.8	1.0	0.5	0.6	< 0.8	1.0	0.8	0.5	1.8	7	4
Indeno[1,2,3-cd]pyrene	1.9	0.4	0.7	1.0	0.8		< 0.8	0.9	0.8	0.4	1.9	6	4.4
Dibenz[a,h]anthracene												0	
Dibenz[a,h+ac]anthracene	0.2		0.1			0.2		0.2	0.2	0.1	0.2	3	1.9
Dibenzo[a,h]pyrene	0.2							0.2	-	-	-	1	-
Dibenzo[a,i]pyrene	0.1							0.1	-	-	-	1	-

[a] For references, see table 1.1.1

Table 1.2.4 PAH profiles in processed foods

Food	grilled meat	smoked herring	smoked fish	smoked fish	smoked fish	dried fruit and pulses	desiccated coconut						
Number of samples	3	1	35	27	58	7	7						
Reference [a]	[12]	[12]	[6]	[6]	[27]	[2]	[2]						
								[PAH]/[BaP]					
PAH								Mean	Median	Min	Max	n	Max/Min
Acenaphthene												0	
Fluorene												0	
Anthracene			63.0	17.5	20.6			33.7	20.6	17.5	63	3	3.6
Phenanthrene			320	54.4	91			155.1	91.0	54.4	320	3	5.9
Fluoranthene	9.4	12.7	91.0	21.7	20.7	8.8	8.7	24.7	12.7	8.7	91	7	10
Pyrene	10.6	13.2	53.0	17.1	16.0	16.1	7.3	19.1	16.0	7.3	53	7	7.3
Benzo[a]fluorene												0	
Benzo[b]fluorene												0	
Benzofluorene, sum												0	
Benzo[ghi]fluoranthene												0	
Benz[a]anthracene	1.3	3.2	6.0	2.1	2.8	2.3	1.1	2.7	2.3	1.1	6.0	7	5.4
Benzo[c]phenanthrene												0	
Chrysene			6.0	2.1		3.5	1.4	3.3	2.8	1.4	6.0	4	4.2
Chrysene+triphenylene					3.6			3.6	-	-	-	1	-
Benzo[b]fluoranthene			1.0	1.0	0.5	0.6	0.8	0.8	0.8	0.5	1.0	5	2.0
Benzo[k]fluoranthene			0.7	0.4	0.7	1.0	0.4	0.6	0.7	0.4	1.0	5	2.6
Benzo[b+j+k]fluoranthene	1.7	2.0						1.8	1.8	1.7	2.0	2	1.1
Benzo[e]pyrene	0.9	0.6			0.4	2.4	0.7	1.0	0.7	0.4	2.4	5	6.0
Perylene			0.2	0.3	0.4			0.3	0.3	0.2	0.4	3	2.0
Anthanthrene												0	
Benzo[ghi]perylene	1.3	0.4	0.3	0.6	< 0.2	1.6	1.3	0.8	0.6	0.2	1.6	7	8.2
Indeno[1,2,3-cd]pyrene				0.9	< 0.3	2.5	0.6	1.1	0.7	0.3	2.5	4	8.3
Dibenz[a,h]anthracene			< 0.2	< 0.1		0.1	0.04	0.1	0.1	0.04	0.2	4	5.0
Dibenz[a,h+ac]anthracene	< 0.1	< 0.1						0.1	0.1	0.1	0.1	2	1.0
Dibenzo[a,h]pyrene												0	
Dibenzo[a,i]pyrene												0	

[a] For references, see table 1.1.1

Table 1.2.5 Comparison of PAH profiles [PAH]/[BaP] from different sources [a]

	[PAH]/[BaP]													
	Foods expected to be contaminated by atmospheric deposition (from table 1.2.3)						Processed foods (from table 1.2.4)						All foods (from table 1.2.1)	
	Mean	**Median**	Min	Max	n	Max/Min	Mean	**Median**	Min	Max	n	Max/Min	Mean	**Median**
Anthracene	1.8	**2.0**	< 0.1	4	5	> 41	33.7	**20.6**	17.5	63	3	3.6	5.8	**1.8**
Phenanthrene	48.1	**23.3**	2.7	128	5	47	155.1	**91.0**	54.4	320	3	5.9	48.9	**< 20.0**
Fluoranthene	20.7	**18.0**	5.0	49	7	10	24.7	**12.7**	8.7	91	7	10	12.3	**8.8**
Pyrene	14.3	**12.7**	9.3	23	6	2	19.1	**16.0**	7.3	53	7	7.3	10.5	**8.4**
Benz[a]anthracene	2.2	**1.9**	1.3	3.6	4	3	2.7	**2.3**	1.1	6.0	7	5.4	1.9	**1.6**
Chrysene+triphenylene	8.2	**6.8**	4.7	14.8	4	3	3.6	**3.6**	-	-	1	-	5.0	**3.6**
Benzo[b]fluoranthene	1.1	**1.1**	-	-	1	-	0.8	**0.8**	0.5	1.0	5	2.0	1.1	**1.0**
Benzo[k]fluoranthene	0.7	**0.7**	-	-	1	-	0.6	**0.7**	0.4	1.0	5	2.6	0.8	**0.7**
Benzo[b+j+k]fluoranthene	4.0	**3.5**	2.5	6.8	6	2.7	1.8	**1.8**	1.7	2.0	2	1.1	3.5	**3.0**
Benzo[e]pyrene	1.3	**1.3**	1.0	1.9	6	2	1.0	**0.7**	0.4	2.4	5	6.0	1.5	**1.3**
Perylene	0.4	**0.3**	0.2	< 0.8	5	< 3.9	0.3	**0.3**	0.2	0.4	3	2.0	0.4	**0.3**
Benzo[ghi]perylene	1.0	**0.8**	0.5	1.8	7	4	0.8	**0.6**	0.2	1.6	7	8.2	1.3	**1.1**
Indeno[1,2,3-cd]pyrene	0.9	**0.8**	0.4	1.9	6	4.4	1.1	**0.7**	0.3	2.5	4	8.3	1.2	**0.9**
Dibenz[a,h+ac]anthracene	0.2	**0.2**	0.1	0.2	3	1.9	0.1	**0.1**	0.1	0.1	2	1.0	0.2	**0.2**

[a] Summary of statistics from tables 1.2.1, 1.2.3 and 1.2.4

1.3 Intake estimates

1.3.1 Dietary intakes

This section presents the estimates of the dietary intake of PAH as derived from the national studies published so far. It is not possible to generate other intake estimates for the European population because: (i) food consumption surveys were carried out only in a very few countries and were not based on common criteria, and (ii) the available information on PAH occurrence data in national diets is scarce and generally limited to a few foodstuffs.

Comparative intake data for individual PAH were collated from five total diet studies (TDS) conducted in the United Kingdom (two studies: Dennis *et al.*, 1983; COT, 2002), Italy (Turrio-Baldassarri *et al.*, 1996), The Netherlands (De Vos *et al.*, 1990) and Austria (Pfannhauser, 1991). Moreover, benzo[*a*]pyrene intakes are also available for Sweden (Beckman Sundh *et al.*, 1998), Germany (State Committee for Air Pollution Control, 1992; cited in IPCS, 1998) and the USA (Butler *et al.*, 1993; Kazerouni *et al.*, 2001). The US intake, which is not of direct concern in this document, is considered here for the sake of comparison.

The protocols adopted in these surveys are reported here below as they are known from the original literature; other details are given with the intake results in table 1.3.1. The protocols are not homogeneous; in particular, there may be some gaps in the representativity of some food classes in some total-diet samples. The comparability is also affected by the 'not detected' concentration data (as to the variability of their occurrence and of the limit of detection (LOD), and their treatment in the calculations). In particular, 'not detected' data were assumed equal to zero or to the LOD, resulting, respectively, in underestimated or overestimated concentrations/intakes. Consequently, the intakes estimated from different studies should not be directly compared.

UK/1. Total-diet samples were prepared at five colleges in geographically diverse areas. They were based on a national average diet (Buss and Lindsay, 1978). The reported results are the means of the five samples; no bias, however, was apparent for samples from different geographical locations.

UK/2. Total-diet samples were collected throughout one year (2000) at 2 weekly intervals from 24 randomly selected locations representative of the UK as a whole. A total of 115 retail food samples were purchased, prepared/cooked for consumption and combined to form 20 food groups. Using the mean PAH concentrations in each food group, mean and high-level (97.5^{th} percentile) dietary intakes were estimated for adults and children of different age ranges. Consumption data were from the National Diet and Nutrition Surveys.

Italy. Food intakes were determined from a 10,000 household survey (Turrini *et al.*, 1991) for four geographical areas (north-eastern, west-eastern, southern, whole nation).The reference diet samples were built accordingly. A 5-day diet sample (about 8.5 kg) was set-up for each diet, including any local food item; food and beverages were prepared according to the prevalent regional habits. The market basket composition is reported in table 1.3.2.

The Netherlands. A food consumption survey was carried out among about 200 18-yr-old men, with an emphasis on food consumption in the previous 14 days. Correspondingly, 10 samplings (market baskets) were performed during a period of 2.5 years (one every three months) in a town in the centre of The Netherlands. The market basket composition is reported in table 1.3.2.

Austria. A duplicate diet study was performed by analysing 10 samples constituted by the weekly composites of portions from each meal, as consumed by 10 persons.

Sweden. The benzo[*a*]pyrene intake was calculated on the basis of national food consumption data and the available analytical data in Swedish foods.

USA/1. The survey was conducted during three phases, each one for a period of two weeks, in a town of New Jersey. A total of 9-10 homes were monitored in each phase. Portions of each meal consumed by one participant per household were collected and composited into weekly samples.

USA/2. The consumption of different food items was estimated by a Food Frequency Questionnaire based on the Second National Health and Nutrition Examination Survey. The questionnaire was completed by 228 male/female subjects in Washington and included details on frequency of consumption and portion sizes. Benzo[*a*]pyrene concentrations were measured in the most common food items consumed in the American diet. Food items were collected in a town of Maryland and included both house and national brands. They were bought in stores. Cooked meats were purchased from fast food stores and restaurants.

The dietary intakes, as estimated from these national surveys, are shown in table 1.3.1. The results allow to draw the following conclusions.

- ✓ Notwithstanding the different protocols and the different dietary habits, the PAH intakes result to be fairly uniform in the four EU countries where a total diet study is available for a set of PAH. In fact, for each PAH, the estimated intakes are generally within one order of magnitude, differences being relatively small.

- ✓ In particular, the PAH intakes calculated in the Dutch and Italian studies, as well as in the older UK study (Dennis *et al.*, 1983), are quite similar. Lower mean intakes were calculated in the Austrian study and in the more recent UK study (COT, 2002).

- ✓ According to surveys conducted in six EU countries, the mean or national-averaged dietary intake of benzo[*a*]pyrene for an adult person was estimated in the range 0.05 to 0.29 µg/day. Higher intakes were calculated at regional level (0.32 µg/day in southern Italy) or for individual diets (up to 0.36 µg/day in Austria) or in the worst-case scenario obtained after assuming ingestion of foods each one at the highest found contamination (0.42 µg/day in The Netherlands).

- ✓ The mean or national-averaged benzo[*a*]pyrene intakes are consistent with the mean benzo[*a*]pyrene intakes estimated in the two US surveys (0.05-0.14 µg/day). Quite higher individual intakes were however calculated in one survey (Butler *et al.*, 1993): up to 0.57 µg/day in the two winter phases and up to 1.15 µg/day in the third one (in late summer). According to the authors, the food diaries did not support a contribution of seasonal differences in cooking style (e.g., increased barbecuing during summer) to seasonal trends.

- ✓ From the findings of the five TDSs, the personal intakes of other PAH may span three orders of magnitude, up to some µg/day. The highest intakes seem to be found among the lower-molecular (3- and 4-ring) PAH. The scarcity of data and their poor comparability (both for food consumption and PAH occurrence) do not allow, however, to draw more precise conclusions on a ranking of PAH intakes.

- ✓ Based on the mentioned limitations of this data collation, the intakes so far estimated should be used in terms of orders of magnitude. In fact, there is indication of an important variability (spatial or temporal or inter-individual) possibly associated with the mean calculated values:

    - The differences in PAH intake among northern and southern Italy resulted to be within a low factor of two.
    - The range of the German benzo[*a*]pyrene intake was reported within a factor of seven (but no information is available on how this range was calculated).
    - The range of the Austrian intake for 10 individuals during the same week spans one to two orders of magnitude, pending on the PAH.
    - An even higher inter-individual variability was reported for the benzo[*a*]pyrene intake in a US survey (Butler *et al.*, 1993). A considerable variability was also

evident in the daily estimated intakes for a given individual from one week to the next (no similar study is available for European countries).

Finally, the Committee noted that:

- ✓ only one national intake estimate is available based on recent surveys (COT, 2002): all the other intakes are based on a food consumption survey or on food collection performed in the 80s (in the 70s in one case);
- ✓ no estimate is available on the intake by specific risk groups.

Table 1.3.1 Dietary intake of PAH as estimated by national surveys (μg/day per person)

Country	Italy		The Netherlands			UK /1	UK /2 [a]			
Reference	[1]		[2]			[3]	[9]			
Years of consumption survey	1980-84		1982-83			1976	1986-87			
Years of food collection	1993-95		1984-86			1979	2000			
Treatment of nd data			nd=0	nd=LOD	nd=LOD	nd=0	nd=0	nd=LOD	nd=0	nd=LOD
PAH	National diet	Regional maximum [b]	Lower-bound mean	Upper-bound mean	Maximum [c]	Lower-bound mean	Lower-bound mean	Upper-bound mean	Lower-bound high level [d]	Upper-bound high level [d]
Acenaphthylene							0.13	0.14	0.23	0.25
Acenaphthene							0.98	0.98	1.61	1.61
Fluorene							0.59	0.56	0.98	0.98
Anthracene			0.03	0.64	0.70		0.07	0.08	0.13	0.14
Phenanthrene			0.87	4.51	5.13		1.54	1.54	2.73	2.73
Fluoranthene			0.99	1.66	2.11	0.99	0.35	0.35	0.60	0.60
Pyrene						1.09	0.35	0.35	0.60	0.60
3,6-Dimethylphenanthrene			0.11	0.31	0.49					
Benzo[ghi]fluoranthene			0.02	1.46	1.48					
Cyclopenta[cd]pyrene							0.01	0.03	0.03	0.06
Benz[a]anthracene	0.41	0.36	0.20	0.36	0.65	0.22	0.05	0.06	0.08	0.10
Benzo[c]phenanthrene			0.11	0.91	1.01					
Chrysene	1.46 [e]	1.70	0.86	1.53	3.90	0.50	0.11	0.11	0.18	0.19
6-Methylchrysene [f]			0.58	0.73	2.58					
Benzo[b]fluoranthene			0.31	0.36	0.59	0.18	0.04	0.11	0.07	0.18
Benzo[j]fluoranthene										
Benzo[k]fluoranthene			0.10	0.14	0.24	0.06	0.01	0.09	0.03	0.15
Benzo[b+j+k]fluoranthene	1.10	0.73	>0.41	>0.50	>0.83	>0.24	>0.05	>0.20	>0.10	>0.33
Benzo[a]pyrene	0.17	0.32	0.12	0.29	0.42	0.25	0.04	0.11	0.07	0.19
Benzo[e]pyrene						0.17	0.04	0.06	0.08	0.10
Perylene										
Anthanthrene							0	0.02	0	0.04
Benzo[ghi]perylene			0.20	0.36	1.03	0.21	0.05	0.06	0.09	0.11
Indeno[1,2,3-cd]pyrene	0.16	0.20	0.08	0.46	0.55	<0.02	0.03	0.10	0.06	0.17
Dibenz[a,h]anthracene	0.08	0.17				0.03	0	0.04	0	0.06
Dibenz[a,j]anthracene [f]			0.54	1.03	2.66					
Dibenzo[a,e]pyrene [f]			0.01	0.63	0.64					

Table 1.3.1 (Cont'd)

Country	Austria		Sweden	Germany	USA /1		USA /2		Overall EU data	
Reference	[4]		[5]	[6]	[7]		[8]			
Years of consumption survey	1989		1985		1987-88		1994-96			
Years of food collection										
Treatment of nd data										
PAH	Median	Range		Range	Mean	Max	Median	Max	Range of mean values [g]	Maximum [h]
Acenaphthylene									0.14	0.25
Acenaphthene									0.98	1.61
Fluorene									0.6	0.98
Anthracene	<0.04	<0.03-5.6							<0.04 - 0.64	5.6
Phenanthrene	<0.33	<0.33-2.0							<0.33 - 4.51	5.13
Fluoranthene	0.60	<0.04-4.30							0.35 - 1.66	4.30
Pyrene	0.60	<0.02-3.97							0.35 - 1.09	3.97
3,6-Dimethylphenanthrene									0.31	
Benzo[ghi]fluoranthene									1.46	
Cyclopenta[cd]pyrene									0.03	0.06
Benz[a]anthracene	<0.02	<0.02-0.14							<0.02 - 0.41	0.65
Benzo[c]phenanthrene									0.91	1.01
Chrysene	0.20	<0.03-0.90							0.11 - 1.53	3.90
6-Methylchrysene [f]									0.73	2.58
Benzo[b]fluoranthene	0.005	<0.05-1.02							0.005 - 0.36	1.02
Benzo[j]fluoranthene	<0.03	<0.03-0.90							<0.03	0.90
Benzo[k]fluoranthene	0.04	<0.02-0.30							0.04 - 0.14	0.30
Benzo[b+j+k]fluoranthene	<0.08	<0.10-2.22							<0.08 - 1.10	2.22
Benzo[a]pyrene	0.05	<0.01-0.36	0.08	0.02-0.14	0.14	1.15	*ca.* 0.05[i]	*ca.* 0.15[i]	0.05 - 0.29	0.42
Benzo[e]pyrene									0.06	0.10
Perylene	0.008	<0.004-0.20							0.01	0.20
Anthanthrene	<0.002	<0.001-0.3							<0.002	0.3
Benzo[ghi]perylene	0.12	<0.01-7.6							0.06 - 0.36	7.6
Indeno[1,2,3-cd]pyrene	<0.02	<0.02-0.31							<0.02 - 0.46	0.55
Dibenz[a,h]anthracene	<0.02	<0.01-0.10							<0.02 - 0.08	0.17
Dibenz[a,j]anthracene [f]									1.03	2.66
Dibenzo[a,e]pyrene [f]									0.63	0.64

Notes table 1.3.1:

- Unlisted PAH were not determined in any study. nd: not detected; LOD: limit of detection. Lower-bound values: calculated by assuming 'nd' values to be zero. Upper-bound values: calculated by assuming 'nd' values to be equal to LOD.

- [a] Dietary exposures of adults. Original data in ng/kg bw were converted by considering a body weight of 70 kg.
  [b] Maximum of three regional diets (noth-western, north-eastern and southern Italy).
  [c] Ingestion of foods with the maximum PAH concentration and taking values < LOD to be equal to LOD (worst-case scenario).
  [d] 97.5th percentile of dietary intakes.
  [e] CHR + TRI.
  [f] Value surprisingly high with respect to literature data on the occurrence of this PAH relative to other PAH.
  [g] The range includes the Italian 'National diet', the Austrian median, the German mean value (0.08), the UK/1 and Swedish values, the Dutch and UK/2 upper-bound means.
  [h] For the UK/2 study, the 'upper-bound high level ' values were included.
  [i] 0.04<median<0.06; estimated from a figure.
  [j] 0.14<maximum<0.16; estimated from a figure.

- References: [1] Turrio-Baldassarri *et al.*, 1996. [2] De Vos *et al.*, 1990. [3] Dennis *et al.*, 1983. [4] Pfannhauser, 1991. [5] Larsson, 1986; cited in Beckman Sundh *et al.*, 1998. [6] State Committee for Air Pollution Control, 1992; cited in IPCS, 1998; re-elaborated data. [7] Butler *et al.*, 1993. [8] Kazerouni *et al.*, 2001. [9] COT, 2002.

Table 1.3.2 Composition (g/person x day) of the market basket constituting the Italian and Dutch total diet studies.

Food group	Italy [c]	The Netherlands [e]
Cereals	248-320	329
Potatoes and potato products		260
Meat/fish	164-188	117 (108/9) [d]
Milk/cheese	227-300	602
Eggs	22-24	33 [a]
Legumes	21-29	18
Oils/fats	48-57	64
Nuts		11
Fruits/vegetables	451-515	482 (256/226) [d]
Sugar/sweets	59-87	78
Beverages [b]	90-168	
Wine/beer/spirits	159-212	
Drinks and drinking-water		1617
Various/ready	8-15	20

[a] Poultry and eggs.

[b] Alcohol free, including mineral water.

[c] From: Turrio-Baldassarri *et al.*, 1996. The range refers to the intakes in the different areas (national, north-western, north-eastern, southern).

[d] The two values in brackets refer to the two sub-foodgroups.

[e] From: De Vos *et al.*, 1990.

1.3.2 Intake by children

The PAH intake by schoolchildren and toddlers of seven age groups (between 1.5 and 18 years) was estimated in the UK survey of COT (2002). The same methodology adopted for the adult population (see 'UK/2' study in the previous section) was used for children. The intakes (per kg body weight) are presented in table 1.3.3 together with those of adults for comparison purposes (the years of consumption surveys are given in the table, note b).

The daily intake of benzo[*a*]pyrene , as well as of all PAH, progressively decreases with increasing age. This holds true for both the mean exposure and the high level (97.5^{th} percentile) exposure. The youngest age group (1.5-2.5 years) is the highest exposed one, with an intake (of both benzo[*a*]pyrene and all PAH) about 2.4-fold higher than for adults.

The available information on levels of PAH in human milk was inadequate to assess the intake by infants during periods of breastfeeding.

1.3.3 Temporal trends in intake

An evaluation of temporal trends in dietary intakes is possible only for the United Kingdom: two TDS are available (Dennis *et al.*, 1983; COT, 2002) with foods collected at an interval of 21 years (1979 and 2000). A direct comparison, however, is limited by some factors: the food groupings and the sets of PAH determined are different, and the 1979 data were calculated only as 'lower-bound' values (i.e. 'not detected' data were treated as 'zero', see also table 1.3.1).

On the basis of the available data and after re-elaborating the 1979 figures, the COT made a comparison of 1979 and 2000 data and focused on the three PAH classified by IARC as probably carcinogenic: benzo[*a*]pyrene , benz[*a*]anthracene and dibenz[*a,h*]anthracene. The following results were obtained:

- the estimated lower-bound intake of benzo[*a*]pyrene and benz[*a*]anthracene is 4- to 5-fold lower for 2000 than for 1979, both as mean exposure and as high level exposure (97.5^{th} percentile);
- also the upper-bound intakes for 2000 are lower than the lower-bound intakes for 1979: about 1.5-fold and 3-fold for benzo[*a*]pyrene and benz[*a*]anthracene , respectively;
- Dibenz[*a,h*]anthracene intake is also likely to have decreased but the extent of the decrease cannot be estimated as dibenz[*a,h*]anthracene was not detected in any food group in 2000.

A decrease in benzo[*a*]pyrene intake was also observed by COT (2002) for children. A comparison between the 1979 and 2000 surveys is shown in table 1.3.4. The upper-bound intakes in 2000 are about 65% of the lower-bound intakes in 1979, both as mean and as high level estimates.

Table 1.3.3 Dietary intakes of PAH for consumers of different age in UK (ng/kg body weight/day). [a,b]

	Average intake									High level (97.5th percentile) intake								
		Schoolchildren				Toddlers					Schoolchildren				Toddlers			
		Age range (years)							1.5-2.5/		Age range (years)							1.5-2.5/
PAH	Adults	15-18	11-14	7-10	4-6	3.5-4.5	2.5-3.5	1.5-2.5	adults [d]	Adults	15-18	11-14	7-10	4-6	3.5-4.5	2.5-3.5	1.5-2-5	adults [d]
Acenaphthylene	2.0	1.7	2.1	2.9	3.8	3.8	4.0	4.4	2.2	3.5	3.0	3.9	5.1	6.3	6.4	6.9	8.2	2.3
Acenaphthene	14	12	15	22	27	27	28	31	2.2	23	21	25	33	40	42	46	52	2.3
Fluorene	8	7.5	9.3	13	17	17	18	20	2.5	14	12	16	20	25	26	29	33	2.4
Anthracene	1.2	1.1	1.4	1.9	2.4	2.4	2.5	2.6	2.2	2.0	1.8	2.4	2.9	3.6	3.6	3.9	4.5	2.3
Phenanthrene	22	21	27	39	49	50	53	56	2.5	39	36	46	59	74	76	83	94	2.4
Fluoranthene	5.0	4.5	5.9	8.4	11	11	12	12	2.4	8.5	7.8	10	13	16	17	18	21	2.5
Pyrene	5.0	4.7	6.1	8.8	11	11	12	13	2.6	8.6	8.0	10	14	16	17	19	21	2.4
Cyclopenta[cd]pyrene	0.4	0.4	0.5	0.8	1.0	0.9	1.0	1.1	2.8	0.8	0.8	0.9	1.2	1.4	1.4	1.6	1.8	2.3
Benz[a]anthracene	0.8	0.8	1.0	1.4	1.7	1.7	1.8	1.8	2.3	1.4	1.4	1.6	2.2	2.6	2.5	2.8	3.1	2.2
Chrysene	1.6	1.4	1.8	2.6	3.3	3.2	3.3	3.5	2.2	2.7	2.6	3.0	3.9	4.8	4.8	5.2	5.9	2.2
Benzo[b]fluoranthene	1.5	1.4	1.7	2.5	3.2	3.0	3.3	3.6	2.4	2.6	2.4	2.9	3.8	4.8	4.6	5.2	6.0	2.3
Benzo[k]fluoranthene	1.3	1.2	1.4	2.1	2.7	2.6	2.8	3.2	2.5	2.2	2.0	2.5	3.3	4.3	3.9	4.7	5.4	2.5
Benzo[a]pyrene	1.6	1.4	1.8	2.6	3.3	3.1	3.4	3.8	2.4	2.7	2.5	3.0	4.0	5.0	4.8	5.4	6.2	2.3
Benzo[e]pyrene	0.8	0.7	0.9	1.3	1.7	1.6	1.7	1.8	2.3	1.4	1.3	1.6	2.1	2.5	2.5	2.7	3.0	2.1
Anthanthrene	0.3	0.3	0.3	0.5	0.6	0.6	0.7	0.8	2.7	0.5	0.5	0.6	0.8	1.0	0.9	1.1	1.3	2.6
Benzo[ghi]perylene	0.9	0.8	1.1	1.5	1.9	1.8	1.9	2.0	2.2	1.5	1.5	1.7	2.4	2.8	2.8	3.2	3.6	2.4
Indeno[1,2,3-cd]pyrene	1.4	1.3	1.6	2.2	2.9	2.8	3.0	3.4	2.4	2.4	2.2	2.6	3.6	4.5	4.3	4.9	5.8	2.4
Dibenz[a,h]anthracene	0.5	0.5	0.6	0.9	1.1	1.0	1.1	1.2	2.4	0.9	0.9	1.0	1.4	1.8	1.6	1.8	2.1	2.3
Sum of 19 PAHs [c]	69	63	80	115	145	146	155	166	2.4	116	106	131	172	208	217	236	276	2.4
mean									2.4									2.3

From: COT, 2002.

[a] Upper-bound estimates (i.e., by assuming 'not detected' values to be equal to the limit of detection).

[b] Years of consumption surveys: adults, 1986-87; schoolchildren, 1998; toddlers, 1992-93.

[c] PAHs are those listed in this table + benzo[b]naphto[2,1-d]tiophene.

[d] Ratio of the intake of age range 1.5-2.5 to that of adults.

Table 1.3.4 Comparison of the mean and high level intakes of benzo[*a*]pyrene for children (in ng benzo[*a*]pyrene/kg bw/day), as estimated in the UK in the 1979 and 2000 surveys.

	1979 (lower bound estimate)		2000 (upper bound estimate)	
	Mean	High	Mean	High
Schoolchildren (age: 4-18 years)	2.3-5.0	4.2-7.8	1.4-3.3	2.5-5.0
Toddlers (age: 2.5-4.5 years)	4.9-5.3	7.8-9.6	3.1-3.8	4.8-6.2

From: COT, 2002.

High level intake: 97.5th percentile.

1.3.4 The contribution from individual food groups to PAH intake

The contribution from individual food groups to the intake of each PAH was calculated in the UK surveys (Dennis *et al.*, 1983; COT, 2002) and in the Dutch one (De Vos *et al.*, 1990). Two sets of results (Dennis *et al.*, 1983, and De Vos *et al.,* 1990) are sufficiently comparable as to the food groupings and are reported in table 1.3.5.

In the former UK study (Dennis *et al.*, 1983), the major contributors were the 'oils and fats'group and the 'cereals' groups. They contributed, respectively, 50% and 30% to benzo[*a*]pyrene intake; their median contribution to the intake of 11 PAH was, respectively, 34% and 31%, respectively. The 'oils and fats' group had the highest individual PAH levels, but the 'cereals' group, although never showing high individual PAH levels, was a major contributor due to its relative weight to the total diet. The third major contributor was 'vegetables' (8% and 12%, respectively), likely due to the atmospheric fall-out of particle-bound PAH. Smoked meat and smoked fish made a very small contribution to the pertinent food groups ('meat' and 'fish') and these in turn were not major components of the diet. Consequently, barbecued food provided a very small part of the dietary intake of PAH.

These results were substantially confirmed in the subsequent study of the Dutch diet (De Vos *et al.*, 1990). The major contributors to the daily benzo[*a*]pyrene intake were 'oils and fats' (47%) and 'cereal products' (36%), followed by 'sugar and sweets' (14%). The group 'cereal products' was found to contribute most (about 27%) to the intake of the sum of 17 PAH (listed in table 1.3.1) - again attributed to the high consumption share - , followed by 'sugar and sweets' (about 18%) and 'oils and fats' (about 14%). It should be noted, however, this Dutch ranking may be affected by the high occurrence of 'not detected'

values for most PAH and most foodstuffs, as well as by the surprisingly high concentrations of some compounds (namely, dibenzo[*a,e*]pyrene, dibenz[*a,j*]anthracene, 6-methylchrysene and 3-methylcholanthrene). The authors could not explain the surprisingly large share of the 'sugar and sweets' group (sugar, chocolate products, jellies, licorice). The relatively high contribution of the 'oils and fats' group was, at least partly, attributed to the well known elevated PAH concentrations possibly present in vegetable oils.

The UK estimated contributions were also confirmed in a Swedish study (Larsson, 1986; cited in IPCS, 1998 and in Beckman Sundh *et al.*, 1998). When considering the sum of 11 PAH (list not available), cereals were found to be the highest contributors (about 34%), followed by vegetables (about 18%) and by oils and fats (about 16%). Significant intakes were also found for 'fruit and sugar' group and for smoked meat products.

Smoked fish and grilled foods showed the highest PAH levels in the Swedish study. They made a modest contribution to the intake, however, since they are minor components of the usual diet. This confirms the above-mentioned conclusion of the UK study (Dennis *et al.*, 1983) that barbecued food provides a very small part of the PAH intake. It holds at least in areas where barbecuing is an infrequent activity. This cooking method, however, may significantly contribute if it is part of the usual diet, especially with long cooking times and high levels of doneness (very well-done rather than medium-done). Consistent with the Swedish study, in the USA (Kazerouni *et al.*, 2001) the highest benzo[*a*]pyrene levels (up to about 4 ng/g of cooked meat) were found in grilled/barbecued very well-done steaks and hamburgers, and in grilled/barbecued well-done chicken with skin. Differently from the available European surveys, however, grilled/barbecued meat contributed a high 21% to the mean daily intake of benzo[*a*]pyrene (resulting in the second contributing food group, after 'bread, cereal and grain' with 29%).

Overall, the results of these studies were consistent in the finding that the food groups contributing most to the total intake appeared to be cereals, oils and fats, and vegetables. This inter-country homogeneity is also consistent with the substantial similarity in the composition of market baskets constituting the Italian and Dutch total diets (table 1.3.2; these two surveys are the only available): for the food groups considered in both the studies, the variation in the daily intake is within an approximate factor of two.

This consistency was partially confirmed in the most recent total diet study, performed in the UK (COT, 2002). In that survey, cereals and vegetables are still major contributors but the contribution from 'oils and fats' has fallen since the previous survey (Dennis *et al.*, 1983); 'beverages', 'milk and diary products' and 'fruit and sugar' now make significant contributions. The temporal trend in intake of benzo[*a*]pyrene , as well as of benz[*a*]anthracene and dibenz[*a,h*]anthracene, since 1979 in the UK (already mentioned in

the section 1.3.3 ‘Temporal trends in intake’) appears particularly noticeable for oils and fats and for cereals. On the contrary, intakes from beverages, milk and dairy products appear to have risen: this finding, however, may be affected by the high number of ‘not detected’ concentrations in 1979 resulting in lower-bound intakes supposed to be considerably underestimated.

A comparison of the most contributing food groups in different surveys is shown in table 1.3.6.

A more detailed evaluation of contributions from different food groups is not feasible due to a number of factors possibly involved: the sampling strategy applied (e.g., differences in the coverage of products collected to represent a whole food group), the criteria adopted in reporting results, the large variations in concentrations in some of the foodstuffs and food groups, the low number of available complete studies (three: the UK and Dutch studies).

Table 1.3.5 Mean personal intake of PAH: percentage contribution from different food groups in the UK[a] and Dutch[b] diets.

	Cereals		Meat		Fish		Oils/fats		Nuts		Fruit		Sugar & sweets		Vegetables		Potato products		Legumes		Beverages		Milk		Miscellaneous		Percentage total	
PAH	UK	NL	UK	NL	UK	NL	UK	NL	UK [c]	NL	UK [d]	NL	UK [c]	NL	UK	NL	UK [c]	NL	UK [c]	NL	UK	NL	UK	NL	UK [c]	NL	UK	NL
PHE	-	0	-	0	-	0	-	0	100		-	0	0		-	0	0		0		-	0	-	0		0	-	100
FA	32	34	7	9	2	0.6	14	8	2		16	17	6		22	22	0		0		1	0	6	0		2	100	101
PY	39	-	8	-	1	-	20	-	-		13	-	-		16	-	-		-		0	-	1	-		-	98	-
BaA	38	54	4	0	1	0	34	28	1		9	0	17		12	0	0		0		0	0	2	0		0	100	100
CHR	36	7	5	0	3	0	19	20	23		8	0	29		29	13	0		0		0	0	2	0		7	102	99
BbFA	30	37	3	12	2	1	42	22	1		6	4	9		15	12	2		0		0	0	2	0		2	100	101
BkFA	29	41	3	12	2	1	41	26	0		5	0	10		19	8	0		0		0	0	2	0		3	101	99
BaP	30	36	3	0	1	0	50	47	0		5	0	14		8	0	0		0		0	0	2	0		2	99	99
BeP	31	-	3	-	1	-	53	-	-		3	-	-		9	-	-		-		0	-	0	-		-	100	-
BghiP	30	53	4	0	1	0	48	41	0		5	0	0		9	0	6		0		0	0	2	0		0	99	100
IP	0	0	0	0	0	0	0	84	0		0	0	0		0	16	0		0		0	0	0	0		0	0	100
DBahA	56	-	8	-	4	-	20	-	-		8	-	-		10	-	-		-		0	-	0	-		-	106 [i]	-
mean	32	29	4	4	2	0.3	31	31	14		7	2	9		14	8	1		0		0		2			2		
median	31	36	4	0	1	0	34	26	1		6	0	9		12	8	0		0		0		2			2		
Sum of 17 PAHs [e]		27		9 [h]		1		14 [f]	[f]			10	18			7	3		0			0		0 [g]		2		98

-: not determined;

[a] UK data are from: Dennis et al., 1983.

[b] Dutch data are re-elaborated from: De Vos et al., 1990. Median PAH concentrations were used to calculate the intake; 'not detected' values were entered as zero.

[c] Not analysed in the UK diet.

[d] Sugar is included in the UK 'Fruit' food group.

[e] Approximate data estimated from a figure (see also text). The PAHs are those listed in table 1.3.1 plus 3-methylcholanthrene.

[f] Nuts are included in the value of the 'Oils/fats' food group.

[g] Diary products are also included in this food group. A contribution of ca. 7% is reported in the original figure but it appears inconsistent with the zero values given for the individual PAHs.

[h] Poultry and eggs are also included in this value.

[i] This percentage total is the actual sum of the individual percentages reported in the original paper: they are supposed to contain an error.

Table 1.3.6 Food groups most contributing to PAH intake: summary of rankings from national studies.

	United Kingdom [1]		United Kingdom [4] [f]		The Netherlands [2]		Sweden [3]
Ranking	BaP	11 PAHs [a]	BaP	Sum of 19 PAHs [e]	BaP [d]	Sum of 17 PAHs [b]	Sum of 11 PAHs [c]
1	Oils & fats (50%)	Oils & fats (34%)	Beverages (28%)	Cereals [l] (35%)	Oils & fats (47%)	Cereals [h] (27%)	Cereals (34%)
2	Cereals (30%)	Cereals (31%)	Cereals [l] (24%)	Vegetables [m] (13%)	Cereals [h] (36%)	Sugar & sweets (18%)	Vegetables (18%)
3	Vegetables [i] (8%)	Vegetables [i] (12%)	Vegetables [m] (12%)	Fruit & sugar [n] (13%)	Sugar & sweets (14%)	Oils & fats (14%)	Oils & fats (16%)
4	Fruit & sugar (5%)	Fruit & sugar (6%)	Milk & diary products (12%)	Meat [g] (13%)	Miscellaneous (2%)	Fruits (10%)	

Notes table 1.3.6

- Percent contributions should not be directly compared other than in general terms, because food groups are not necessarily formed by the same foods.
- [a] Median value of 11 PAH (listed in Table 1.3.5).
  [b] Approximate data estimated from a figure (see also text). The PAH are those listed in Table 1.3.1 plus 3-methylcholanthrene.
  [c] List of PAHs: not available.
  [d] Data re-elaborated.
  [e] PAH are those listed in Table 1.3.1 (columns 'UK/2 intakes') plus benzo[b]naphto[2,1-d]thiophene.
  [f] Population mean intakes based on upper-bound concentrations (see Table 1.3.1).
  [g] Group including "carcass meat, offal, meat products and poultry".
  [h] Group including "bread, bisquits, rice, macaroni, etc.".
  [i] Group including "root and other vegetables".
  [l] Group including "bread and miscellaneous cereals".
  [m] Group including "green vegetables, potatoes, other vegetables, canned vegetables".
  [n] Group including "fresh fruit, fruit products, sugars and preserves".
- References: [1] Dennis et al., 1983. [2] De Vos et al., 1990. [3] Larsson, 1986; cited in IPCS , 1998. [4] COT, 2002; and UK Food Standard Agency, personal communication.

Table 1.3.6 Food groups most contributing to PAH intake: summary of rankings from national studies.

	United Kingdom [1]		United Kingdom [4] [d,f]		The Netherlands [2]		Sweden [3]
Ranking	BaP	11 PAHs [a]	BaP	Sum of 19 PAHs [e]	BaP [d]	Sum of 17 PAHs [b]	Sum of 11 PAHs [c]
1	Oils & fats (50%)	Oils & fats (34%)	Cereals (25%)	Cereals (36%)	Oils & fats (47%)	Cereals (27%)	Cereals (34%)
2	Cereals (30%)	Cereals (31%)	Beverages (25%)	Meat products[g] & eggs (16%)	Cereals (36%)	Sugar & sweets (18%)	Vegetables (18%)
3	Vegetables (8%)	Vegetables (12%)	Milk & diary products (15%)	Vegetables (10%)	Sugar & sweets (14%)	Oils & fats (14%)	Oils & fats (16%)
4	Fruit & sugar (5%)	Fruit & sugar (6%)	Meat products[g] & eggs (9%)	Milk & diary products (9%)	Miscellaneous (2%)	Fruit (10%)	

[a] Median value of 11 PAH (listed in table 1.3.5).

[b] Approximate data estimated from a figure (see also text). The PAH are those listed in table 1.3.1 plus 3-methylcholanthrene.

[c] List of PAHs: not available.

[d] Data re-elaborated.

[e] PAH are those listed in table 1.3.1 (columns 'UK/2') plus benzo[b]naphto[2,1-d]tiophene.

[f] Mean intakes based on upper-bound concentrations (see table 1.3.1).

[g] It includes carcass meat, offals and poultry.

References:

[1] Dennis et al., 1983. [2] De Vos et al., 1990. [3] Larsson, 1986; cited in IPCS, 1998. [4] COT, 2002.

Table 1.3.7 PAH concentrations in drinking water and urban air, European countries.

	Drinking water (ng/L), published since 1990						Urban air, since 1990, annual mean concentration (ng/m^3)							
			Italy		Switzerland			UK				Germany	Italy	Overall
	NL [e]	Norway	Naples [f]	Udine	Lausanne	Range [d]	Europe 90s [g]	London 1991-92	London 1997	Manchester 1995	Middlesbrough 1995	Augsburg 1992-93	Rome 1993-98	order of magnitude
Reference	[1]	[2]	[3]	[4]	[5]		[6]	[7]	[8]	[9]	[9]	[10]	[11]	
PAH														
Anthracene	<12						0.2-2.6	2.2	1.4	0.8	5.1	0.6		1
Phenanthrene	<80							23.7	20	11	71	14		20
Fluoranthene	<20	<0.6-24	7.7-21		3.3	1-10		16.1	4.8	2.6 [a]	8.0	4.3		5
Pyrene		<0.3-15	7.6-17			0.1-10	0.2-15	16.4	4.0	2.2	4.7	2.6		5
Benz[a]anthracene	<4	0.1-5.5	<5			0.1-5	0.2-4.2	3.6	0.5	0.3	0.6	0.4	0.8	1
Benzo[c]phenanthrene	<16							11.1						
Chrysene	<16						0.3-2.2	5.7		0.5	1.0			1
Chrysene+triphenylene			7.7-13			10						1.3		1
Benzo[b]fluoranthene	<2	0.05-4.0		<5	0.4-0.6	0.05-1		2.1	0.6	0.6	1.2			1
Benzo[j]fluoranthene		0.03-0.14				0.01-0.1								
Benzo[k]fluoranthene	<1.2	0.02-0.10		<5	0.1-0.9	0.01-1	0.2-1	2.0	0.5	0.3 [b]	0.9			1
Benzo[b+j+k]fluoranthene			<5									2.3	3.0	3
Benzo[a]pyrene	<4	<0.04-2.0	<5	<5	0.1-1	0.1-1	0.4-3.1	2.0	0.3	0.4	0.5	0.5	1.4	1
Benzo[e]pyrene			<5				0.2-3.7	5.1				0.6	1.5	1
Perylene												0.08		0.1
Anthanthrene								0.8						1
Benzo[ghi]perylene	<4	0.4-1.1	<5	<5	nd	0.1-1	0.5-4.7	3.1	0.9	0.3	0.5	0.7		1
Indeno[1,2,3-cd]pyrene	<8	0.4-1.2	<5	<5	tr	0.1-1	0.3-2.6	2.6	0.5		0.5	0.8	1.5	1
Dibenz[a,h]anthracene		1.2	<5	nd			0.06-0.4	0.1		0.8	0.1 [c]	0.2	0.2	0.1

[a] FA + methylphenanthrene. [b] BkFA + DBacA. [c] DBahA + DBacA. [d] Range of the orders of magnitude. [e] Approximate values. [f] n = 3.
[g] Urban data including traffic-oriented measurements.

References:

[1] De Vos et al., 1990. [2] Berglind, 1982; Kveseth et al., 1982; both cited in IPCS EHC 202. [3] S. Di Rosa, pers. comm. [4] Moret et al., 1995.
[5] Vu Duc and Huynh, 1981. [6] PAH Position Paper, Annexes, 2001. [7] Brown et al., 1996. [8] Coleman et al., 1998, cited in Coleman et al., 1999. [9] Branson et al., 1997.
[10] Dorr et al., 1996. [11] Menichini et al., 1999.

1.3.5 Comparison with the contribution to the PAH intake from other sources

The contribution by food ingestion to the total intake of PAH was compared with the intake from drinking water and inhalation of air. The available PAH concentrations in drinking water and in urban atmosphere, as measured in the 1990s in European countries, are reported in table 1.3.7.

As regards drinking water, the available data are few. This is likely due to the very low expected levels, commonly below the analytical limit of detection. The measurements are not easily comparable because of differences in the analytical procedure (particularly regarding the filtration of the sample before extraction) and the coating of the distribution pipes. So, the overall results were approximated to the nearest order of magnitude.

The atmospheric measurements reported in table 1.3.7 were limited to the investigations performed during the whole year due to the well-known seasonal variability of PAH concentrations. As regards the measurement of 3- and 4-ring PAH, the table includes only the investigations where vapour phase was also collected, besides particulate phase. Air concentrations generally show a good consistency among different investigations and the typical level of concentration is given as an order of magnitude.

Based on these concentration data and on the estimated dietary intake (table 1.3.1), table 1.3.8 shows the estimated contribution to the total personal PAH intake from the three routes of exposure.

Benzo[*a*]pyrene is the compound showing the highest number of measurements, by any route of exposure, and the results are also quite consistent relatively to the other PAH. This makes it possible to compare the individual mean benzo[*a*]pyrene intakes (table 1.3.8). Due to the overall uncertainties associated to the original concentration data and mainly to the mean intakes adopted, the results of this comparison are to be considered as approximate estimates, acceptable as indicative of the actual exposure.

As regards the mean benzo[*a*]pyrene intake by food ingestion, the average value of the 'range of mean values' (table 1.3.1) is used in the calculation. The total mean daily intake of benzo[*a*]pyrene results to be about 0.2 µg/person. The dietary intake accounts for about 90% of it. Drinking-water contributes by less than 1%, thus resulting to be a relatively insignificant route of exposure. The remaining is attributable to air inhalation. The latter contribution was calculated on the basis of a 'typical' benzo[*a*]pyrene concentration in urban air (1 ng/m^3), commonly measured at road level close to vehicular emissions: the actual personal exposure, however, is expected to be lower, at least for most of the

population. Hence, the mean intake by food ingestion is likely to contribute by more than 90%.

Table 1.3.8 Estimate of the mean daily intake by different routes for an adult non-smoker (ng/person)

PAH	Food [a]	Drinking water [b]	Air [c]
Anthracene	<30-640		20
Phenanthrene	<330-4510		400
Fluoranthene	600-1660	2-20	100
Pyrene	600-1090	0.2-20	100
Benz[a]anthracene	<20-410	0.2-10	20
Chrysene	200-1530	20 [d]	20
Benzo[b]fluoranthene	5-360	0.1-2	20
Benzo[j]fluoranthene	<30	0.02-0.2	
Benzo[k]fluoranthene	40-140	0.02-2	20
Benzo[b+j+k]fluoranthene	<70-1100		60
Benzo[a]pyrene	50-290	0.2-2	20
Benzo[e]pyrene	200		20
Benzo[ghi]perylene	120-360	0.2-2	20
Indeno[1,2,3-cd]pyrene	<20-460	0.2-2	20
Dibenz[a,h]anthracene	<10-80		2

[a] Range of mean values (from table 1.3.1).

[b] Range from the orders of magnitude of concentration (see table 1.3.7), assuming ingestion of 2 L/day.

[c] From the order of magnitude of concentration (see table 1.3.7), assuming a ventilation rate of 20 m^3/day. Occupational exposure is excluded.

[d] CHR + TRI.

These benzo[*a*]pyrene intake figures are consistent with those calculated in an US study: 12 and 140 ng/day as the mean intakes by, respectively, inhalation (based on personal air samples) and ingestion of food (based on prepared food samples) (Butler *et al.*, 1993).

An evaluation of the relative intakes of other PAH is made difficult by the wide ranges of the estimated ingestion by both food and water, as well as by the low number of available data. Taken overall, the data indicate that food contributes by far most of the intake also for the other PAH, including the lower-molecular ones.

1.3.6 Contribution from tobacco smoking to the benzo[*a*]pyrene intake

The contribution from the inhalation route may increase markedly for smokers and persons exposed to passive smoking.

The mainstream smoke of commercially available filter cigarettes was analysed for benzo[*a*]pyrene in Spain (Kayali and Rubio-Barroso, 1995), UK (Evans *et al.*, 1993) and USA (Tomkins *et al.*, 1985): the benzo[*a*]pyrene content was found in the range 2-20 ng/cigarette.

In the UK investigation, the sales-weighted mean was found to be 13 ng benzo[*a*]pyrene per cigarette for 20 brands representing 58% of UK sales. Assuming this concentration as a mean delivery and that 80% of inhaled particle-bound benzo[*a*]pyrene from mainstream smoke is deposited in the respiratory tract (IARC,1986, p.311), the additional benzo[*a*]pyrene intake for a person smoking 20 cigarettes/day is 210 ng, which is in the same order of magnitude of the mean intake by ingestion of food.

Personal exposure to passive smoking is also of high concern for PAH intake. Sidestream smoke was found to contain 10 times more PAH than mainstream smoke (Grimmer *et al.*, 1987; cited in IPCS, 1998); in particular, benzo[*a*]pyrene content per cigarette in sidestream smoke was 100 ng vs. 11 ng in mainstream smoke.

Persons living in rooms with many smokers may be exposed to benzo[*a*]pyrene concentrations in an approximate range of 5-20 ng/m^3 (Grimmer *et al.*, 1977, cited in: Grimmer, 1983; Valerio *et al.*, 1996). By assuming an exposure to 10 ng/m^3 for 5 hours/day, the additional benzo[*a*]pyrene intake by passive smoking is about 40 ng/day.

2 HAZARD IDENTIFICATION / CHARACTERISATION

Unless otherwise stated, the information used in this assessment is taken from the recent IPCS Environmental Health Criteria document on PAH (IPCS, 1998).

2.1 Absorption, Distribution, Metabolism and Excretion

2.1.1 Absorption

The two major determinants of gastrointestinal absorption are aqueous solubility and lipophilicity, since absorption requires compounds to go into solution in the lumen of the intestine, pass through the cell walls of intestinal cells and be removed to the circulation.

PAH are lipophilic compounds with low aqueous solubility. Those considered in this opinion have log K_{ow} values ranging from 3.4 to 7.3 and aqueous solubilities from 0.17 to 31740 µg/L at 25 ˚C. Although aqueous solubility generally decreases as the log K_{ow} value increases, there is considerable variability amongst compounds with similar log K_{ow} values reflecting the influence of molecular structure on aqueous solubility.

There are three main routes of absorption in humans, lung and respiratory tract following inhalation of aerosols or particulates containing PAH, dermal following skin contact and gastrointestinal tract following ingestion in water or food. For the purposes of this evaluation only the last route will be considered in detail.

Rees and colleagues in 1972 showed rapid absorption of benzo[*a*]pyrene following intragastric administration in rats with highest levels seen in the thoracic lymph nodes after 3-4 hours. Based on results in whole animals and intestinal sacs, these workers suggested that absorption of benzo[*a*]pyrene involved two phases, uptake by the mucosa followed by diffusion through the intestinal wall (Rees *et al.,*1972 as cited in IPCS, 1998).

Laurent and colleagues (2001) described studies on the absorption of two PAH (benzo[*a*]pyrene (log K_{ow} 6.5, aqueous solubility 3.8 µg/L) and phenanthrene (log K_{ow} 4.6, aqueous solubility 1290 µg/L)) following oral administration to pigs in a lipophilic milieu. Two castrated Large White pigs were catheterised in the portal vein and brachiocephalic artery. Fourteen days post-surgery they were fed 1 litre of milk containing 50 µCi of [7,10-^{14}C]-benzo[*a*]pyrene or 15 µCi of [9-^{14}C]-phenanthrene and 10 ml arterial and portal blood samples were collected before administration and then hourly for 6 hours at 9 and 24 hours.

Radioactivity was detectable within 1 hour and peaked at 5-6 hours and reached background by 24 hours. The peak radioactivity was higher for phenanthrene despite the 3-fold lower dose. Values in the portal vein were slightly higher than those in the brachiocephalic artery. The peak time corresponds with that observed for milk fat and is much longer than glucose (45 minutes) or protein (30 minutes). No areas under the curves (AUCs) or other pharmacokinetic parameters are reported.

Rahman and colleagues (1986) showed the presence of bile increased the intestinal absorption of PAH in Sprague-Dawley rats, absorption of benzo[*a*]pyrene (log K_{ow} 6.50, solubility 3.8 µg/L) (and 7,12-dimethylbenz[*a*]anthracene) being affected more than that of anthracene (log K_{ow} 4.5, solubility 78 µg/L) or pyrene (log K_{ow} 5.18, solubility 135 µg/L) (Rahman *et al.,*1986 as cited in IPCS, 1998). Kawamura and co-workers (1988) demonstrated that the composition of the diet influenced the absorption of co-administered ^{14}C-benzo[*a*]pyrene in Wistar rats. The radio-labelled benzo[*a*]pyrene was orally administered in a solution, emulsion or suspension of 200 mg of food or food component and blood samples collected over the first 6 hours and at 24 hours post-administration. The foods and components studied were triolein, soya bean oil, cellulose, bread, rice flake, lignin, water, starch, katsuobushi (dried bonito), ovalbumin, potato flake and spinach. The AUCs for administration in lipophilic foods (triolein and soya bean oil) were 50% and 42% of that following intravenous administration in saline. The AUCs for the other foods tested were 20-25% of that following intravenous administration in saline except for cellulose (around 30%), all were significantly lower than the lipophilic foods. These data suggest that the bioavailability of PAH from food will be in the range of 20-50% and that it increases with increasing content of lipophilic components in the food.

2.1.2 Distribution

Distribution of PAH has been studied in rodents and levels in tissues are influenced by several factors; the PAH, route of administration, vehicle, time of tissue sampling after treatment and presence or absence of inducers or inhibitors of hydrocarbon metabolism. However three common traits are observed; there are detectable levels of PAH (probably more accurately PAH-derived material) in almost all organs, those organs rich in adipose tissue act as depots from which material is slowly released and high levels are found in the gastrointestinal tract irrespective of the route of administration.

Following intravenous administration benzo[*a*]pyrene was rapidly removed from the bloodstream with a distribution half-life of less than 1 minute. The rate of clearance of radioactivity after administration of radio-labelled benzo[*a*]pyrene was increased after pre-treatment with inducers of metabolism either benzo[*a*]pyrene or phenobarbital.

Whole body autoradiography was used to study distribution in mice and their fetuses following intravenous administration of ^{14}C-labelled 3-methylcholanthrene to pregnant dams. Radioactivity was widely distributed in maternal tissues and was detected in fetuses showing that it crossed the placenta (Takahashi and Yasuhira 1973, Takahashi 1978 as cited in IPCS, 1998). Similar results have been obtained following inhalation, intragastric or intravenous administration of benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene to rats and mice (Shendrikova *et al.,*1973; 1974; Shendrikova and Aleksandrov 1974; Neubert and Tapken 1988; Withey *et al.,*1992, as cited in IPCS, 1998). In a small study in humans, samples of milk, placenta, maternal and umbilical cord blood were taken from 24 women and analysed for selected PAH. The highest levels of benzo[*a*]pyrene, dibenz[*a,c*]anthracene and chrysene were observed in milk and umbilical cord blood but levels were only above the detection limit in half of the samples. Nevertheless the authors concluded that both fetuses and infants were exposed to PAH which were presumed to be from the maternal diet (Madhavan and Naidu, 1995 as cited in IPCS, 1998).

Table 2.1.1 Mean levels of PAH in cancer free liver (N=6) and fat (N=10) biopsy samples obtained at autopsy from humans

PAH	Liver concentration µg/kg wet weight	Fat concentration µg/kg wet weight
benz[*a*]anthracene	<0.005	<0.005
dibenz[*a,h*]anthracene	<0.005	<0.005
pyrene	0.38	1.1
anthracene	0.17	0.26
benzo[*b*]fluoranthene	0.07	0.16
benzo[*ghi*]perylene	0.04	0.05
benzo[*k*]fluoranthene	0.01	0.03
benzo[*a*]pyrene	0.02	0.02
benzo[*e*]pyrene	<0.005	0.08

From: Obana *et al.* (1981)

Limited data are available describing measurement of tissue levels of PAH in autopsy or biopsy samples from humans. Graf and colleagues reported average benzo[*a*]pyrene levels of 0.32 µg/100 g dry tissue in liver, spleen, kidney, heart and skeletal muscle and 0.2 µg/100 g dry tissue in lung based on autopsy samples from normal humans with a wide age range (Graf *et al.,*1975 as cited in IPCS, 1998). Obana and colleagues (1981) measured the levels of nine PAH in cancer free liver and fat biopsies and the results are shown in table

2.1.1. Pyrene was the most abundant PAH but the range for all the PAH levels varied markedly (up to 40-fold). The variation in individual PAH profiles was consistent.

2.1.3 Elimination and excretion

The majority of PAH metabolites are excreted in urine, bile and faeces. Urinary excretion has been studied more extensively than faecal excretion, although research on faecal metabolism has increased following recognition of the importance of enterohepatic cycling of PAH metabolites.

The effect of interactions between different PAH on their elimination is complex and compound specific. Bartosek and colleagues (1984) administered three PAH to rats at two dose levels of total PAH, 11.4 mg or 22.8 mg in olive oil. Chrysene, benz[*a*]anthracene and triphenylene were administered singly and benz[*a*]anthracene was also administered as equimolar mixtures (11.4 mg of each) with chrysene or triphenylene. Increasing the benz[*a*]anthracene dose from 11.4 mg to 22.8 mg had a greater effect (roughly three-fold in blood and liver) on the relative availability of non-transformed benz[*a*]anthracene as determined by the AUC in blood and tissues. The authors postulated that this was due to saturation of elimination processes. In contrast the availability of chrysene was lower and appeared to decrease as the dose increased, attributed to its limited intestinal absorption. The availability of triphenylene altered in a dose related manner. All these data were supported by the results of perfusion experiments in isolated livers. Following co-administration of chrysene and benz[*a*]anthracene the faecal elimination of chrysene was unaffected (remaining at 25% of dose) whereas that of benz[*a*]anthracene doubled from 6 to 13%. Similarly benz[*a*]anthracene co-administration doubled the faecal elimination of triphenylene from 3 to 6%. Benz[*a*]anthracene also resulted in increased relative availability of chrysene in other tissues (blood, liver, brain, adipose tissue and mammary tissue) measured by the AUC in these tissues. In perfused livers chrysene clearance fell in the presence of benz[*a*]anthracene from 6.85 ml/min to 4.85 ml/min. The effect was more pronounced in the lipid rich adipose and mammary tissue. In contrast the relative availability of triphenylene decreased in the presence of benz[*a*]anthracene but no changes were observed in its disappearance rate. The authors noted that the AUC decreased in the order triphenylene > benz[*a*]anthracene > chrysene, and that their aqueous solubility decreased in a similar order. The group noted that these interactions were occurring at relatively high doses, the significance of these interactions at lower dose levels and for human risk assessment were unclear.

2.1.4 Enterohepatic cycling of PAH

Evidence for enterohepatic cycling was found after intratracheal administration of 1 µg/kg bw tritiated benzo[*a*]pyrene to bile-duct cannulated Sprague-Dawley rats. Studies in rats and rabbits showed the *in vivo* persistence of benzo[*a*]pyrene metabolites as a result of enterohepatic cycling. Chipman and colleagues demonstrated that bile was the major route of excretion in bile-duct cannulated rats in the initial 6 hours after intravenous administration of 3 µmol/kg bw ^{14}C-labelled benzo[*a*]pyrene, biliary excretion accounting for 60% of the dose whilst urinary excretion was 3%. These workers showed that mutagenic or potentially mutagenic derivatives may be excreted via bile into the intestine (Chipman *et al.,*1981, Chipman 1982, Boroujerdi *et al.,*1981 as cited in IPCS, 1998). The gastrointestinal microflora have been shown to hydrolyse glucuronic acid conjugates of biliary PAH metabolites resulting in the formation of potentially reactive compounds (Renwick and Drasar 1976, Chipman *et al.,*1981, Chipman 1982, Boroujerdi *et al.,*1981 as cited in IPCS, 1998). Other metabolites such as thio-ether conjugates have also been reported to undergo enterohepatic cycling (Hirom *et al.,*1983, Bakke *et al.,*1983 as cited in IPCS, 1998).

A study of the pharmacokinetics and bioavailability in rats following oral or intravenous administration of 2-15 mg/kg bw ^{14}C-labelled pyrene provided strong evidence of enterohepatic cycling (Withey *et al.,*1991 as cited in IPCS, 1998).

2.1.5 Metabolism

Studies of metabolic pathways in whole animals have largely been restricted to the simpler compounds whereas hepatic homogenates, microsomes, cultured cells and explants are the principal method for studying the metabolism of larger more complex compounds. Metabolism and excretion has been studied in whole animals for naphthalene, anthracene, phenanthrene, pyrene, benz[*a*]anthracene and chrysene and to a lesser degree with benzo[*a*]pyrene, dibenz[*a,h*]anthracene and 3-methylcholanthrene. The metabolism of benzo[*a*]pyrene is described in detail below.

The metabolism of PAH has been studied in a number of human cells and tissues including bronchus, colon, mammary cell aggregates, keratinocytes, monocytes, lymphocytes and bronchial macrophages. Whilst similar metabolites are formed in many of the *in vitro* tissue or cell preparations, both the relative levels and rate of formation are tissue or cell, species and strain of animal specific. The individual variability is marked with around 75-fold variation in the extent of PAH activation as measured by DNA adduct formation reported in human bronchus, mammary cell aggregates and macrophages.

What can metabolism do

Metabolism of xenobiotics is divided into two phases; phase 1 generally involves alteration of the structure of the compound to increase polarity and phase 2 addition of polar groups. Although metabolism was originally considered to be the inactivation or detoxification of foreign compounds, it has become increasingly clear that the actual situation is more complex and can result in either increased or decreased reactivity of the compound. Further phase 1 metabolism can occur when the initial introduction of a polar group does not alter physicochemical properties markedly and reactive metabolites can also occur from rearrangement or metabolism following conjugation. The general scheme of PAH metabolism involves oxidation to a range of primary (epoxides, phenols, dihydrodiols) and secondary (diol epoxides, tetrahydrotetrols, phenol epoxides) phase 1 metabolites followed by conjugation to phase 2 metabolites with glutathione, glucuronic acid or sulphate. Studies using simple PAH such as naphthalene demonstrated that similar phase 1 metabolites were produced following incubation with hepatic microsomes or tissue homogenates as after administration to animals. The metabolism of PAH has been extensively studied *in vitro* often in microsomal fractions of rat liver but also in many other tissue preparations. These *in vitro* methods are considered to adequately represent the *in vivo* metabolism of PAH.

Activation

- Phase 1

Phase 1 metabolism modifies the structure of xenobiotics by introducing into their structure more polar chemical groups such as hydroxy groups. An example would be the phenol metabolites of benzo[*a*]pyrene in the diagram below. These alterations can make compounds more electrophilic resulting in increased reactivity e.g. the epoxide metabolites of benzo[*a*]pyrene, in this example the metabolite can react readily with water to form a diol. However the diol and phenol metabolites of benzo[*a*]pyrene are still able to fit the active sites of the phase 1 enzymes and this results in the introduction of a second epoxide group into the metabolite. The reactivity of the resulting diol or hydroxy epoxide depends on the relative positions of the substituents since this affects the electrophilicity of the compound.

- Phase 2

The binding of conjugating groups especially sulphate or glutathione increases the bulk and aqueous solubility of compounds but can sometimes result in the formation of a reactive compound. This arises when alterations in the electrophilicity of the compound (e.g. 9-hydroxymethylanthracene; Flesher *et al.*, 1998) after conjugation result in formation of a leaving group, the loss of this group is thermodynamically favoured. This requires sequential metabolism involving methylation in the electron dense meso-region, hydroxylation of the methyl derivative, conjugation with a good leaving group such as

sulphate and subsequent leaving of the group resulting in formation of a highly reactive carbonium ion.

Deactivation
The modification of the structure can decrease the activity of compounds either directly (through modification of a reactive group e.g. formation of triols and tetraols from diolepoxides) or indirectly (through increasing the probability or rate of elimination e.g. introduction of epoxide or phenol groups allowing formation of conjugates).
The overall balance between the various reactions within an individual tissue will be a function of the specific enzymes found in that tissue, the availability of other substrates e.g. glutathione in the tissue and the levels of PAH within that tissue.

Adduct formation
An adduct is a chemical grouping which is covalently bound to a large molecule such as DNA or protein and covalent binding of PAH to proteins was first described in 1951. There is much more information on the covalent binding of electrophilic PAH metabolites to nucleic acids since characterisation of the products formed should be simpler, there is greater homogeneity of the nucleophilic nucleic acids than proteins and principally because such modifications could result in permanent alteration of cell phenotype. The covalent binding of dibenz[*a,h*]anthracene to DNA *in vivo* was first reported in 1961. Subsequently the levels of DNA binding of naphthalene, dibenz[*a,c*]anthracene, dibenz[*a,h*]anthracene, benzo[*a*]pyrene, 3-methylcholanthrene and 7,12-dimethylbenz[*a*]anthracene were correlated with their carcinogenic potency (Brookes and Lawley 1964 as cited in IPCS, 1998).

The majority of PAH metabolites shown to react with nucleic acids are vicinal diol epoxides, mainly diol epoxides of the “bay region”. Examples of other possibilities include activation of benzo[*j*]fluoranthene in mouse skin via a non-bay region diol epoxide and the production of hydroxymethyl derivatives of methyl substituted PAH which following conjugation can form electrophilic sulphate esters.

The usual sites of attack on nucleic acid bases are the extranuclear amino groups of guanine and adenine. Although many of the PAH-deoxyribonucleoside adducts formed in human cells and tissues have not been fully characterised, the available evidence from bronchial epithelium, colon, skin and cultured mammary cells suggests adducts formed are very similar to those from corresponding rodent tissues. The major adduct is formed on the N2 position of guanine. Diol epoxides are also thought to react frequently with the N7 position of guanine but these adducts are labile and their detection is difficult. N7 adducts are normally spontaneously released.

Interaction can result in DNA damage such as strand nicking, which may also be of significance in carcinogenesis. Studies on the biological effectiveness of PAH-nucleic acid adducts demonstrated that differences in the biological activities of 7-ethyl- and 7-methylbenz[*a*]anthracene were not due to differences in the mutagenic potential of the adducts formed. A similar conclusion was drawn from studies on a series of bay- and fjord-region epoxides. Therefore whilst data are not available on all PAH-nucleic acid adducts, currently they should all be considered as potentially damaging to the organism.

Table 2.1.2 DNA adduct formation following administration to rats of 10 mg/kg bw benzo[*a*]pyrene by oral gavage, dermal and intratracheal routes (Godschalk *et al.*, 2000).

Route of admin	**oral**	**Dermal**	**Intratracheal**
WBC adducts per 10^8 nucleotides	3.6 +/- 1.8	0.3+/- 0.2	4.3 +/- 0.5
Lung adducts at t_{max} (2 days) per 10^8 nucleotides	6.9 +/- 2.3	0.5 +/- 0.2	20.5 +/- 5.1
skin adducts at t_{max} (2 days) per 10^8 nucleotides	Not measured	70.3 +/- 14.0	Not measured
stomach adducts at t_{max} (1 day) per 10^8 nucleotides	1.6 +/- 0.5	Not measured	Not measured
C_{max} µg/24 h	4.5	4.8	11.5
AUC µg	12	18	31

Godschalk and colleagues (2000) measured DNA adduct formation in stomach, lung, skin and WBC following administration to rats of 10 mg/kg bw benzo[*a*]pyrene by oral gavage, dermal and intratracheal routes. The adducts were determined at the time of maximum generation in each tissue but skin and stomach adducts were only determined after dermal and oral gavage administration respectively. They also determined some pharmacokinetic parameters allowing comparison of the total and peak systemic exposure. These results demonstrate that adduct formation occurs at both the site of contact and systemically but adduct levels cannot readily be correlated with peak or total systemic benzo[*a*]pyrene exposure.

Arif and colleagues (1999) administered dibenzo[*a,l*]pyrene by intramammillary and intraperitoneal routes in DMSO or by the intragastric route in corn oil. At 6 hours, 2, 5 and 14 days post treatment DNA adducts were isolated from target (mammary epithelium) and non-target tissues (lung, liver, heart, bladder, pancreas). Levels in mammary epithelium were 2640 +/- 532 adducts/10^9 nucleotides were 10 to 65 fold higher than in lung, liver, heart, bladder and pancreas (ranked in decreasing order) and consisted of 1 major (30%) and at least 6 minor adducts except in liver where 4 additional adducts were formed.

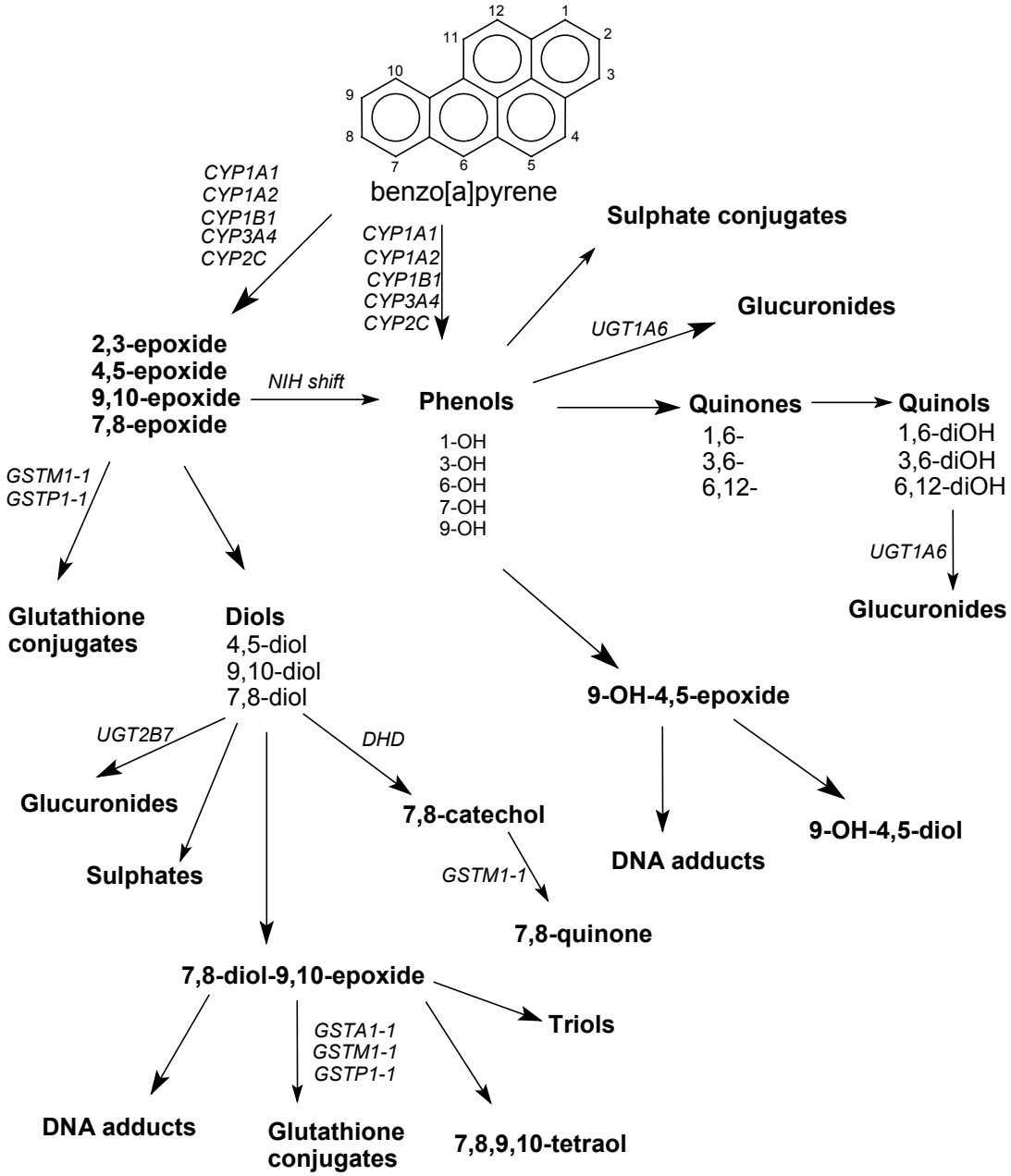
Benzo[a]pyrene as an example of metabolism

Benzo[*a*]pyrene, and PAH in general, can undergo a multitude of simultaneous and sequential metabolic transformations, which compounds the difficulty of ascertaining the biological effects of particular metabolites after benzo[*a*]pyrene treatment. Benzo[*a*]pyrene metabolism has been studied in a range of human tissue preparations. The metabolic profiles of human tissues are qualitatively similar to those in animal tissues and the identified metabolites are identical. The differences in susceptibility of different animal or human tissues to the carcinogenic properties of benzo[*a*]pyrene can be accounted for by quantitative differences in its metabolism or activation. However for some PAH such as benz[*a*]anthracene studies have indicated species dependent bioactivation.

The initial stage of benzo[*a*]pyrene metabolism is the formation of several epoxides by microsomal CYP-dependent mono-oxygenase. These epoxides can have several fates; spontaneous rearrangement to phenols, hydrolysis by epoxide hydrolase to dihydrodiols or covalent reaction with glutathione either chemically or catalysed by glutathione-S-transferase.

Dihydrodiols can also undergo further oxidative metabolism. The 4,5- dihydrodiol forms a range of metabolites. The 9,10-dihydrodiol predominantly forms its 1- and 3-phenol (triol) derivatives with only small quantities of the 9,10-diol-7,8-epoxide. The 7,8-dihydrodiol principally forms 7, 8-diol-9,10-epoxide with little or no triol. The diol epoxides and triols can be further metabolised to triol epoxides and pentols. Diol epoxides can undergo glutathione conjugation either chemically or catalysed by glutathione-S-transferase. Diol epoxides may spontaneously hydrolyse to tetraols. The oxidation of 9-hydroxybenzo[*a*]pyrene leads to the K-region 4,5-oxide which hydrates to the 9-hydroxy-4,5-dihydrodiol. Other enzymes such as prostaglandin H synthase, a myeloperoxidase system and lipoxygenases can also oxidatively metabolise the 7,8-diol to diol epoxides and tetraols. Further oxidation of 3- or 6-hydroxybenzo[*a*]pyrene may occur either spontaneously or metabolically by prostaglandin H synthase yielding 1,6-, 3,6- and 6,12-quinone. These enzymes may be of significance when levels of CYP are low such as in uninduced cells or in chronic irritation or inflammation.

Figure 2.1.1 Metabolism of benzo[*a*]pyrene (adapted from Besarati, 2001)



PAH compounds are stereoselectively metabolised to optically active products. Taking benzo[*a*]pyrene-7,8-diol-9,10-epoxide as an example, four isomers can be generated as there are two enantiomers of each diastereoisomer. In rat liver microsomes (+) 7,8-epoxide is formed in excess relative to the (-) isomer, the (+) isomer accounting for around 90% of the metabolites generated. The (+) 7,8-epoxide is stereospecifically metabolised by epoxide hydrolase to the (-) 7,8-dihydrodiol. This predominant isomer is metabolised primarily to a single diol epoxide isomer, (+)anti- benzo[*a*]pyrene-7, 8-diol-9,10-epoxide. This metabolically predominant isomer is also the isomer with the highest tumour inducing activity and that predominantly covalently bound to DNA following benzo[*a*]pyrene exposure in various mammalian cells and organs.

Metabolism of other PAH

The table below gives an overview on the dihydrodiols, diol-epoxides and other reactive metabolites, so far identified from the PAH covered in this opinion. The table includes identified dihydrodiols and their presumed reactive carcinogenic/mutagenic metabolites. The table is intended to identify which PAH can form adducts and should be considered in conjunction with table in the genotoxicity section. However much of the evidence with the more complex compounds comes from studies *in vitro* using hepatic homogenates, microsomes and cultured cells, this may not be representative of the preferred routes of *in vivo* metabolism. Thus the identification of a particular metabolite indicates the potential for its formation but not evidence that it is generated *in vivo*.

9-hydroxymethylanthracene is an example of a model compound which cannot be metabolised by phase 1 reactions to vicinal bay-region dihydrodiol-epoxides but is a potent electrophilic mutagen in the presence of hepatic cytosolic sulphotransferase and 3'-phosphoadenosine-5'-phosphosulphate. The dimethylbenz[*a*]anthracene metabolites 7-hydroxy-, 12-hydroxy- and 7,12-dihydroxy- dimethylbenz[*a*]anthracene were activated by cytosolic human hepatic dehydroepiandrosterone-steroid sulphotransferase to DNA binding metabolites.

Table 2.1.3 Potential reactive metabolites of PAH (IPCS, 1998)

PAH Compound	Dihydrodiols	Bay-region	Other	Other	DNA
3 rings:					
Aceanthrylene	1,2-			1,2-epoxide	+
Anthracene	1,2-				-
Fluorene					
Phenanthrene	1,2-	1,2-diol-3,4-			-
1-Methylphenanthrene	3,4-				
4 rings:					
Benz[*a*]anthracene	1,2-	3,4-diol-1,2-	7,8-diol-		+
Benzo[*a*]fluorene					
Benzo[*b*]fluorene					
Benzo[*c*]phenanthrene	3,4-	3,4-diol-1,2-			+
Chrysene	1,2-	1,2-diol-3,4-	3,4-diol-	9-hydroxy-	+
5-Methylchrysene	1,2-	1,2-diol-3,4-			+
Fluoranthene	2,3-		2,3-diol-		+
Pyrene	1,6-				-
Cyclopenta[*cd*]pyrene	3,4-			3,4-oxide	+
Triphenylene					
5 rings:					
Benzo[*b*]fluoranthene	1,2-	9,10-diol-		X-hydroxy-	+
Benzo[*j*]fluoranthene	4,5-	9,10-diol-		4,5-diol-	+
Benzo[*k*]fluoranthene	2,3-				
Benzo[*ghi*]fluoranthene					
Benzo[*a*]pyrene	4,5-	7,8-diol-		9-OH-4,5-	+
Benzo[*e*]pyrene	4,5-		9,10-diol-	4,5-diol-	
Dibenz[*a,h*]anthracene	1,2-	3,4-diol-1,2-		3,4:10,11-	+
Perylene					
6 rings:					
Anthanthrene					
Benzo[*ghi*]perylene					+
Dibenzo[*a,e*]pyrene	3,4-				+
Dibenzo[*a,h*]pyrene	1,2-	3,4-diol-1,2-			+
Dibenzo[*a,i*]pyrene	1,2-				+
Dibenzo[*a,l*]pyrene	8,9-				+
Indeno[1,2,3-*cd*]pyrene	1,2-			1,2-epoxide	+

Enzymes involved

One or more of each CYP family (1, 2 or 3) is capable of metabolising one or more PAH. Studies using a model compound, such as benzo[*a*]pyrene, demonstrated that the catalytic properties of different CYPs differ in PAH metabolism. The importance of a particular CYP in PAH metabolism is affected by two other factors; its mode of regulation and the tissue specificity in its expression.

CYP1A1 can metabolise a wide range of PAH molecules. It is present in many tissues but constitutive levels are generally low. Induction of CYP1A1 is controlled by the Ah receptor and since PAH can activate this receptor PAH are capable of inducing their own metabolism. Following induction CYP1A1 can reach high levels in some tissues (placenta, lung, peripheral blood cells) but hepatic expression levels remain low. Other CYPs are considered more important in the metabolism of benzo[*a*]pyrene. For a more detailed discussion of Ah receptor modulated effects see section 2.2.

CYP1A2 can also metabolise PAH, but its capacity to metabolise benzo[*a*]pyrene (to its 3-hydroxy metabolite) is only around 20% the metabolic capacity of CYP1A1. CYP1A2 is very active in forming benzo[*a*]pyrene-7,8-dihyrodiol and subsequently its diol epoxides. Evidence exists that CYP1A2 can activate 7,12-dimethylbenz[*a*]anthracene. CYP1A2 is also controlled by the Ah receptor but its hepatic levels are much higher than those of CYP1A1. Thus despite its low capacity its high expression could give it an important role in diol epoxide formation.

The CYP1B subfamily is capable of metabolising PAH and its expression is also controlled by the Ah receptor. There is limited information on its expression and catalytic properties.

The constitutive levels of CYP2B enzymes are extremely low in humans but they are strongly induced by phenobarbital type inducers. CYP2B6 was shown capable of metabolising benzo[*a*]pyrene to 3- and 9-phenols and trans-dihydrodiols by expressing human CYP2B6 cDNA in human lymphoblastoid cell line. CYP2B enzymes may have a role in 7,12-dimethylbenz[*a*]anthracene metabolism.

The CYP2C subfamily contains several members expressed at high level in liver and more than 1 member may be capable of metabolising PAH. Both CYP2C9 and CYP2C8, albeit to a lessed degree, can metabolise benzo[*a*]pyrene to 3- and 9-phenols and trans-dihydrodiols. The CYP2C enzymes may play a prominent role in the metabolism of benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene in phenobarbital induced liver.

The CYP3A subfamily is one of the most abundant CYP enzymes in human liver. CYP3A can metabolise benzo[*a*]pyrene and some of its dihydrodiols to several products. Human CYP3A4 was the most important enzyme in the 3-hydroxylation of benzo[*a*]pyrene.

A number of different enzymes are involved in conjugation reactions; UDP glucuronyl transferases, glutatione transferase, cytosolic 3'-phosphoadenosine-5'-phosphosulphate dependent sulphotransferase and S-adenosyl-L-methionine-dependent methyltransferase. In the case of benzo[*a*]pyrene at least two families of UDP glucuronyl transferases (UGTs) have been shown to undertake glucuronidation. Thus UGT1A6 has been shown to glucuronidate phenol and quinol metabolites whilst UGT2B7 glucuronidates diols. The specific families involved in some glucuronidations are unknown. At least three glutathione transferases (GSTA1-1, GSTM1-1 and GSTP1-1) have been shown to play roles in the metabolism of benzo[*a*]pyrene

Toxicokinetic influences on the toxicity of PAH
In assessing the effects observed after administration of PAH a number of factors concerning the compound and the enzymes involved in its metabolism need to be considered. As noted in the introduction to the metabolism section and the legend preceding the table of PAH metabolites, much of the data on metabolism has been generated using a range of *in vitro* experimental systems. These ranges in complexity from isolated enzymes to perfused tissues and identify the potential for generation of specific metabolites under defined conditions but are not capable of demonstrating the extent to which these are produced *in vivo*. Thus information on PAH metabolism is suitable for hazard identification. As previously noted there are *in vivo* data only for a very limited subset of PAH, these data mainly identify the final excreted products of metabolism. The intermediate steps in the generation of these final metabolites, particularly evidence for the occurrence of relatively short lived metabolites, have been determined though *in vitro* experiments. It is not currently possible to use data on the metabolism of PAH to assess or rank risk for most PAH. However these data can provide evidence that supports, supplements or clarifies observations from other hazard identification studies i.e. adds to the weight of evidence.

In considering the weight given to metabolism data as supporting evidence a number of factors need to be considered. The majority of studies on metabolism and toxicokinetics are on discrete compounds whereas exposure through food and the environment is to a mixture of different PAH. Since exposure is to a mixture of PAH there will be interaction between several of these factors. In addition exposures from other sources such as environment or smoking will also play a role.

These factors include;

- route of administration in a particular study and the relevance of this to PAH exposure,
- tissue specificity and tissue levels of a particular enzyme or enzyme family, whether this can account for the observed toxicity and the extent to which dietary PAH is distributed to these tissues,
- differences in the specificity and kinetics of the human forms of these enzymes compared to animal or recombinant forms,
- substrate specificity of the enzymes,
- induction or constitutive levels of the enzymes,
- effect of components of the diet or the PAH mixture affecting the rate or route of metabolism, and
- concentration and effect of co-factors or other reactants.

There is evidence that at high dose levels some PAH can affect the excretion of other PAH however the significance for humans at lower exposure levels is not known

2.1.6 Summary of absorption, distribution, metabolism and elimination of PAH

The toxicokinetic data indicates that the absorption of PAH following oral administration is affected by the other components of the diet. The evidence suggests that the oral bioavailabilty of PAH ranges from around 40-50% in highly lipophilic milieus to 20-25% in less lipophilic milieus and mixed food. The absorbed PAH are widely distributed particularly to fatty tissues. PAH are associated with toxic effects at the site of first contact and systemically. All PAH can be metabolised by phase 1 enzymes to electrophilic metabolites under experimental conditions. These metabolites are able to react with proteins and DNA, but these metabolites can also be deactivated by further metabolism. Effects are related to the extent of electrophile production and detoxification *in vivo*. Thus reactive metabolites are not formed to a significant extent from anthracene *in vivo* whereas for benzo[*a*]pyrene they represent a significant or major route of metabolism and this is reflected in the data on the genotoxicity and carcinogenicity of these compounds.

In using the metabolism data for the risk assessment of an individual PAH certain questions require an answer. Firstly the ability of the compound to produce an electrophile has to be established. The degree to which this represents a major route of metabolism and the characteristics (reactivity, stability, inactivation) of the electrophile require elicitation. Finally the possible effects of route of administration, species specificity or dose dependency on the metabolism needs addressing. In general all PAH have the potential to generate electrophiles, and all probably do produce electrophiles at some concentration or in some circumstances. However several PAH do not produce these to an appreciable

extent *in vivo* e.g. anthracene whereas for other compounds they represent a significant or major route of metabolism e.g. benzo[*a*]pyrene.

2.2 Significance of receptor mediated effects

2.2.1 Introduction

Polycyclic aromatic hydrocarbons (PAH) elicit many adverse biological effects, including immunosuppression, teratogenicity, tumour promotion and hormonal effects. All of these seemingly unrelated biological effects are believed to be mediated by the sustained activation of the arylhydrocarbon receptor (AhR) and the subsequent disturbance of cellular homeostase. The AhR is a ligand-dependent transcriptional regulator of several genes including enzymes involved in the metabolism of xenobiotics, e.g. cytochrome P4501A family as well as genes encoding factors involved in cell growth and differentiation. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most efficient ligand known for the AhR, that has subsequently been termed the dioxin receptor.

2.2.2 The Ah-receptor

The aromatic hydrocarbon receptor is a helix-loop-helix transcription factor that is member of the Per-Arnt-Sim family of transcription factors (Wilson and Safe, 1998). The AhR is located in the cytoplasma associated with two molecules of hsp90 (heat shock protein 90) and another protein. The hsp90 is important for protein folding and ligand recognition, i.e. dissociation of hsp90 from the unliganded AhR abolishes AhR ligand binding activity. The hsp90 is released following the formation of the AhR-ligand complex due to conformational changes in the AhR protein. Subsequently, the ligand-AhR complex enters the nucleus where it forms an active heterodimer with a nuclear protein AhR, nuclear translocator (ARNT). This AhR-ARNT-complex binds to specific DNA sequences located in the 5'-flanking regions of the genes regulated by the AhR. These consensus sequences, termed AhR response elements (AHRE) (also called dioxin responsive elements (DRE) or xenobiotica responsive elements (XRE)), are located in the promoter and enhancer regions of target genes. The four core nucleotides, CGTG, appears to be critical for the formation of ligand:AhR:ARNT:AHRE complex. In rodents, at least seven AHRE core consensus sequences in the CYP1A1 have been identified, but they bind the ligand:AhR:ARNT complex with varying affinities. Subsequently to the binding, it is assumed that the complexes can migrate to the higher-affinity AHRE enhancer sequences. Binding of the ligand:AhR:ARNT complex to an AHRE is in the major groove of DNA, resulting in an distortion of the DNA and a localized loss of nucleosomes at the promoter site resulting in increase in gene expression. The AhR also

control basal gene expression, suggesting that trans-activation occurs in the endogenous ligand or that an endogenous ligand occupies the binding site on the AhR allowing its interaction with certain AHREs.

Not only the core sequences but also the neighbouring bases are important for the binding. This requirement may explain differences in AhR-mediated toxicity by PAH and TCDD ligands. Exposure of mice to PAH induces the expression of *Bax* in oocytes. This effect is AhR dependent but is only induced by 7,12-dimethylbenz[*a*]anthracene and not by TCDD. This difference in the action between TCDD and PAH is conveyed by a single base pair flanking the AHRE in the *Bax* promoter region (Matikainen *et al.*, 2001).

Formation of the ligand:AhR:ARNT:AHRE complex results in an up-regulation of transcription and subsequent increases in mRNA and protein levels of genes containing the AHRE concensus sequence in their regulatory regions, e.g. CYP1A1 (see figure 2.2.1).

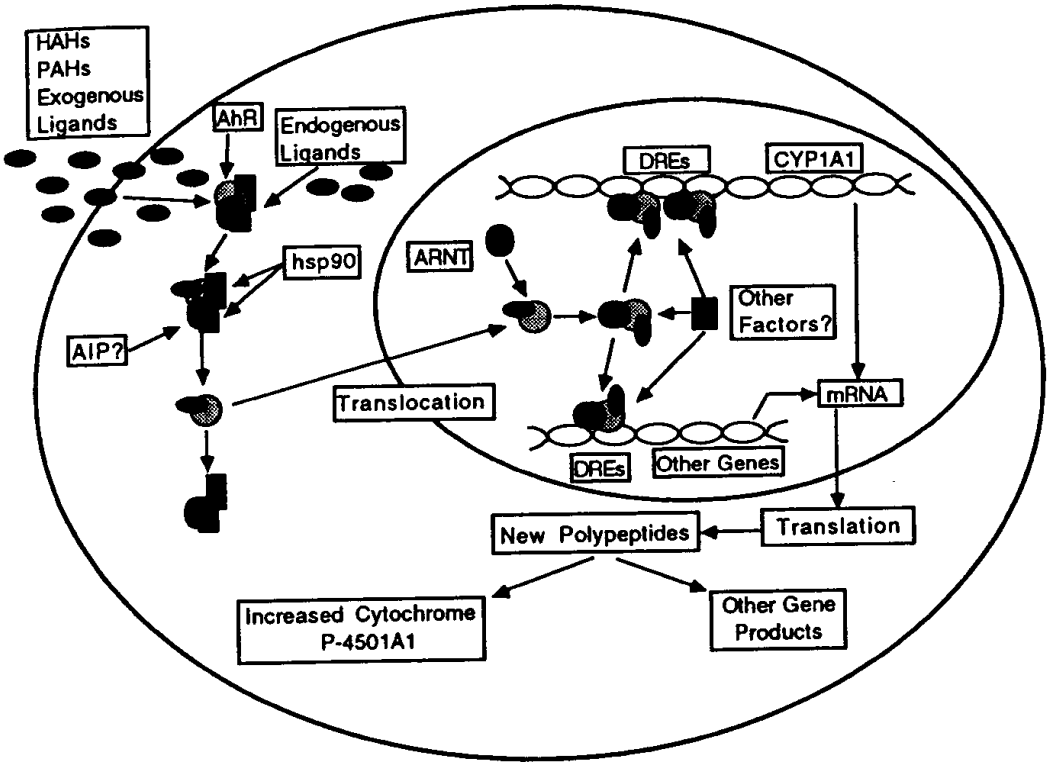


Figure 2.2.1 (Denison and Phelan, 1998).

Other proteins in addition to AhR and ARNT are required to mediate the action of TCDD and related chemicals by binding to the trans-activating domain of the AhR (Elferink and Whitlock,1994).

In addition to CYP1A1, a large number of other genes and gene products are known to be up-regulated, e.g., the Ah gene battery. Many of these genes are involved in critical metabolic and physiological events e.g. phase I and II enzymes, proteins involved in signal transduction, and growth factors. Some of the genes involved in the carcinogenic process and containing AHRE are shown in table 2.2.1 (Lai *et al.*,1996). The expression can thus be induced by PAH and consequently influence the carcinogenic process induced by PAH or other carcinogens present in the diet.

Table 2.2.1 AhR-receptor mediated increased gene expression

Xenobiotic metabolism	CYP1A1, CYP1A2, CYP1B1, GSTA, GSTM, NQO1, UGT, ALDH-3
Oncogenes	c-fos, c-jun, c-erb-A, bcl-2, bax
Growth factors and receptors	PAI-2, IL-1β, TGF-α, TNF-α

The mode of action of AhR-processes has been focused on genes with the AHRE consensus sequence. However, there are indications that AhR is likely to interact with other signalling pathways to cause the observed toxic response. Protein-protein interaction is an important mode of action for transcription factors. The activated AhR interacts with NF-κB, a key nuclear transcription factor, and this interaction is associated with mutual functional modulation of gene expression controlled by the transcription factors (Tian *et al.*, 1999). NF-κB controls genes that are involved in immunosuppression, carcinogenesis, cell growth and apoptosis. Therefore, some of the toxic outcomes of PAH exposure related to carcinogenesis may be mediated through this pathway.

In addition to up-regulation, the AhR is also involved in the PAH-induced down-regulation of various genes that play key roles in cell growth and differentiation, and their down-regulation by PAH may contribute to the toxic effects elicited by these compounds. CYP2C11, the predominant CYP enzyme expressed in human liver, was down-regulated by PAH, and the down-regulation correlated with the binding affinity of the PAH to the AhR and its transformation. The mechanism of this down-regulation is not clear, but lower CYP activity may decrease the ability to metabolize toxic compounds and hormones (Safa *et al.*, 1997). A down-regulation of liver methionine adenosyltransferase gene expression by PAH has also been reported. This enzyme is involved in syntheses of the most important biological methyl donor, and decreased activity will as such influence many important processes involved in cellular defence against toxic compounds, e.g. synthesis of GSH.

This effect may not be mediated solely by the AhR, as a similar effect was not observed with TCDD (Carretero *et al.*, 2001).

2.2.3 Variation in AhR levels

The AhR has been detected in most-cells and tissues, including the gastrointestinal tract. Large interspecies and interstrain differences in the concentration of AhR have been reported, the level in responsive mice (C57BL/6) being highest in the liver and lung. A single aminoacid change (A375V) in the ligand binding region is principally responsible for the high- to low-affinity AhR phenotype. In humans, the receptor has been found in, e.g. liver, lung, colon and placenta with the highest level in the lung. A 4-fold interindividual variation in expression has been reported (Hayashi *et al.*, 1994). Genetic polymorphisms have been observed in the human AhR gene, some of which results in aminoacid changes. However, none of these polymorphisms appears to be associated with either inducibility of the marker enzymes, CYP1A1, or binding affinity of the receptor-ligand complex to the AhRE (Wong *et al.*, 2001; Cauchi *et al.*, 2001).

2.2.4 PAH and AhR mediated effects

Many of the toxic responses elicited by PAH are mediated through the AhR. These effects have been observed mostly in experimental animal studies, *in vitro* toxicological studies and few human studies.

AhR and immunotoxicity
The immunosuppressive effect of PAH is partly dependent on the planarity of the molecule and its capacity to bind to the Ah-receptor (see chapter 3.6). A functional Ah-receptor is required for proper function of the immune system as has been demonstrated using transgenic mice lacking the AhR. The AhR-/- mice at 2 to 3 weeks of age contained 75% fewer lymphocytes in the spleen compared to mice expressing the receptor, but AhR deficiency does not affect the development of normal thymus subpopulations (Fernandez-Salguero *et al.*, 1995).

Furthermore, it has been shown that the AhR can influence PAH-mediated immunosuppression (Near *et al.*, 1999). The spectrum of PAH immunotoxic outcomes suggests that PAH interfere withthe lymphocyte programmed cell death/apoptosis machinery, e.g. induction of pre-B cell apoptosis (Quadri *et al.*, 2000). The induction of pre-B-cell apoptosis is not a direct effect of the PAH on the pre-B-cells but is influenced by local interactions with AhR-expressing marrow stromal cells (Yamaguchi *et al.*, 1997). The

induction of pre B-cell apoptosis may be dependent on PAH metabolism, as TCDD did not induce apoptosis and that metabolites of PAH, e.g. 7,12-dimethylbenz[*a*]anthracene-3,4-dihydro-diol was more potent (Mann *et al.*, 1999). However, PAH can also induce non-AhR mediated apoptosis, e.g. fluoranthene induces apoptosis in murine T-cells, but the mechanism of this process is currently not known (Yamaguchi *et al.*, 1996).

AhR and carcinogenicity

The AhR play an important role in PAH-induced carcinogenesis. Early animal studies showed a correlation between inducibility of arylhydrocarbon hydroxylase (AHH) activity and PAH-induced lung carcinogenesis. Using cultured human lymphocytes, it was shown that people expressing the high-inducibility phenotype had an increased risk of developing lung cancer (McLemore *et al.*, 1978). For a limited number of PAH, there was no link between their ability to induce AHH activity and lung cancer potency in the mouse lung adenoma carcinogenesis model (Ross *et al.*, 1995).

Recent studies have demonstrated that mutant AhR cell lines and AhR null allele mice are relatively resistant to chromosomal damage induced by cigarette smoke. Furthermore, benzo[*a*]pyrene induced tumours in wild type mice but not in the AhR null allele mouse (Shimizu *et al.*, 2000), suggesting an important role for AhR in PAH carcinogenesis. The role of AhR is not entirely clear, but using an AhR antagonist that did not influence CYP1A1 activity, the antagonist protects against benzo[*a*]pyrene-induced bone marrow cytotoxicity and genotoxicity which suggest the AhR signalling has a net potentiation effect on PAH genetic toxicity (Dertinger *et al.*, 2001).

Using biomarkers for exposure and early biological effects, it was demonstrated that following intra-gastric administration of benzo[*a*]pyrene, higher levels of benzo[*a*]pyrene-7,8-diol-9.10-epoxide-DNA adduct and SCE in bone marrow cells were observed in Ah-non responsive mice (DBA/2) than in Ah-responsive mice (Brauze *et al.*, 1997). Difference in susceptibility to PAH-induced carcinogenesis has been demonstrated in several studies using Ah-inducible and non-inducible mouse strains (see chapter 2.3).

In a multivariate analysis using data from assays that describe biological effects of PAH, i.e. bacterial mutagenicity, enhancement and inhibition of bacterial mutagenicity, AhR affinity, induction of CYP1A1, all the AhR affinity variables were statistically relevant to describe carcinogenicity. Strong mutagens like dibenzo[*a,l*]pyrene, cyclopenta[*cd*]pyrene and benzo[*a*]perylene were poor inducers. In the partial least square regression analysis a negative correlation between mutagenesis and enzyme induction was found (Sjögren *et al.*, 1996). Using the induction data from the CALUX assay, no correlation between mutagenicity and inducibility for the 25 PAH from which mutagenic potency data were available, was observed (Machala *et al.*, 2001a).

AhR and reproductive and developmental toxicity
Studies in B6(Ah-inducible) and D2 (non-inducible) mice have demonstrated that PAH-induced reproductive toxicity is mediated through the AhR. These effects are described in details in chapter 2.5.

2.2.5 Other receptor mediated effects

In addition to AhR mediated induction, the marker enzyme CYP4501A1 is also induced by another cytosolic receptor, the 4S PAH binding protein. The 4S protein specifically binds certain PAH with high affinity and undergoes nuclear translocation. This protein has been identified as glycine-N-methyltransferase, and the homodimer form of the enzyme acts as the receptor (Bhat *et al.*, 1997). TCDD does not bind to the 4S PAH binding protein. The human glycine N-methyltransferase gene is only expressed in the liver, pancreas and prostate (Chen *et al.*, 2000). The toxicological consequences of this altered expressing are not fully understood.

The steric resemblance of PAH to steroid molecules led to the postulation that they would be able to bind to the same receptor as steroid hormones, and both *in vivo* and *in vitro* studies have demonstrated that PAH has both oestrogenic and anti-oestrogenic activity. Using a model ER-α transactivation system it has been shown that phenolic metabolites rather than the parent compound are responsible for the effect (Charles *et al.*, 2000). The potency of 3-hydroxy-benzo[*a*]pyrene and 9-hydroxy-benzo[*a*]pyrene was equivalent to that of oestradiol.

2.2.6 Other PAH-mediated biological effects

Chemically induced carcinogenesis involves three operational stages of which promotion involves some reversible, nongenotoxic events, i.e., removal of the initiated cells from the homeostatic control mechanism. The mechanism of this tumour promoting activity and the role of the AhR is not clear, but it could be a relevant role of PAH-induced carcinogenesis. Down-regulation of gap junctional intercellular communication (GJIC) by tumour promoting compounds is considered to be a critical step in the carcinogenic process. Strong correlation has been found between tumour promoting activity in the two-stage skin carcinogenesis model and the ability of the compound to inhibit GJIC. In an *in vitro* test system it was found that different PAH do inhibit GJIC (table 2). It is interesting to note that several PAH considered to be non-mutagenic or possessing low carcinogenic activity belong to the most potent PAH inhibitors of GJIC, e.g. fluoranthene, 5-methylchrysene and picene. However,

the IC50 of PAH are 1000-fold higher than TPA, that is the reference promoting compound (Bláha *et al.*, 2002).

Apoptosis plays in important role in carcinogenesis, e.g. stimulation of apoptosis increases the removal of damaged cells whereas inhibition of apoptosis increases the probability for malignant transformation. An interesting difference in AhR mediated toxicological response between PAH and dioxins is that PAH induces the *Bax* gene and subsequent apoptosis in oocytes (Matikainen *et al.*, 2001).

2.2.7 Structure-activity correlations

The biologic activities of the PAH are dependent on the structures of the individual compounds, as the chemical structure is important for the binding of the compound to the AhR. The relationship between structure and effect has been studied in experimental studies and in modelling systems using the information obtained in the experimental studies.

Short term bioassays
Numerous short-term bioassays of AhR-mediated activity are available. They are based on the mechanism of AhR mediated responses: 1) induction of cytochrome P4501A-dependent 7-ethoxyresorufin O-dethylase (EROD) activity, 2) binding affinity of the ligand to the receptor, 3) binding of ligand:AhR to the consensus sequence measured by the gel retardation of AhR binding (GRAB) assay, and 4) AhR-mediated reporter gene expression.

The AhR activating potency of PAH can be tested in different test systems, animals and cell cultures. A dose-response relationship for receptor binding affinity and induction of AHH activity has been determined for 29 PAH in rat liver. It was apparent that the magnitude of effects was strongly dependent on the chemical structure, and compounds with the phenanthrene structure fused to at least one benzo ring had the strongests effects. However, no correlation between the two markers could be established (Piskorska-Pliszczynska *et al.*, 1986).

The various testsystems have different sensitivity and activity in relationship to the reference compound, TCDD. The difference in potency for CYP1A1 expression cannot be explained simply in terms of receptor affinity. The difference between TCDD and PAH may in part be due to a metabolic depletion of the PAH ligand whereas TCDD is not metabolized. Using TCDD as the inducer, large differences in response could be observed in cells from different species and of different origin, ranging from 6-fold induction in cells from hamster to 66-fold in human cells (Garrison *et al.*, 1996). A positive association was

established for the amount of ligand:AhR and induction of CYP1A1 activity for the potent ligand, TCDD.

New test systems to determine AhR mediated responses have been developed taking advantage of the knowledge on the molecular mechanism. Molecular constructs containing a reporter gene under the control of single or multiple AHRE have been developed. These contructs have been used to develop 1) cell lines, that are transfected with the reporter gene, and 2) transgenic animals that express the reporter gene in all tissues. The cell lines can either be transiently transfected or the reporter gene can be incorporated in the genome. Exposure of the cells to AhR agonists activates the production of the reporter gene products, the activity of which can easily be measured. Several different reporter genes and cell types have been used. The most frequently used assay to detect AhR-agonists in complex mixtures is the chemical-activated luciferase expression (CALUX) assay. An alternative reporter gene is the enhanced green fluorescent protein, providing a method with a higher capacity (Nagy *et al.*, 2002).

The CALUX assay has been used to determine AhR activation by PAH. The AhR inducing potencies of PAH were expressed as induction equivalency factors (IEF) relative to benzo[*a*]pyrene. The IEF was calculated as a ratio of EC50 and/or EC25 of the reference inducers and the concentration of the PAH inducing the same level of luciferase activity. The activity was significantly lower for the PAH than TCDD. Short exposure to PAH (6 hrs) leads to a significantly higher AhR-mediated activity than long exposure (24 hrs), suggesting that CYP1A1 mediated metabolism lowers the concentration of the active compound (Machala *et al.*, 2001a).

The AhR-inducing potency of many PAH is demonstrated and both in *in vivo* and *in vitro* systems. Induction equivalency factors (IEF) relative to the potency of benzo[*a*]pyrene have been established using the CALUX assay in rat hepatoma H4IIE cell line (Machala *et al.*, 2001a) and are listed in table 2.2.2.

Table 2.2.2 Induction potency of PAH relative to benzo[*a*]pyrene [1]

Compound	IEF $_{BaP}$	GJIC[2]
Flu	ni	2.6
Ant	ni	ni
Fla	1.05E-2	10.9
Py	7.57E-3	3.0
BaA	0.39	-
DBaiP	2.36E-2	ni
CPP	4.23E-3	3.5
BaPe	3.37E-2	ni
DBaeF	1.74E-3	ni
BcPhe	4.64E-3	3.4
BeP	2.27E-3	ni
DMBA	0.46	4.7
1-MePyr	8.54E-3	4.1
BbF	8.83	-
BaP	1	1
DBaeP	0.49	ni
DBahP	2.80	-
5-MeChry	3.07	8.2
Pic	0.12	7.5
Chry	3.25	-
IP	44.20	ni
NPyr	1.10	ni
DBaiP	2.59	ni
DBajA	2.16	ni
BjF	2.25	ni
DBacA	1.75	2.3
BkF	67.76	ni
DBahA	11.46	ni
DBakF	2.68	ni
TCDD	$1.11x10^5$	

ni = no induction/inhibition

[1]After 24 hrs incubation with the PAH

[2]Arbitrary Gap-Junctional intercellulat communication

The metabolism of PAH could play an important role in the determination of AhR-mediated activity as the hydroxylated metabolites may have a different affinity for the receptor than the parent compound. The transient induction of AhR-mediated activities is assumed to be a result of the disappearance of the inducing PAH following metabolism by the induced CYP1A1 activity.

Although the AhR demonstrates a strict specificity for planar PAH, non-planar compounds i.e. 7,8-dihydroxy-7,8-dihydro-benzo[*a*]pyrene also induce CYP1A1 activity but with a delayed time course. Planar PAH o-quinones were found to be potent inducers of AhR-mediated activities, e.g. CYP1A1 activity. The PAH quinones are formed by aldo-keto reductase activity that is induced by the AhR signalling pathway (Burczynski and Penning, 2000).

Even in cells expressing high level of AhR, no activation and translocation of the activated PAH:AhR complex to the nucleus were observed at low concentrations of the PAH, suggesting that a threshold may exist for the AhR mediated toxic responses. In the absence of a functional CYP1A1 activity or by inhibition of CYP1A1 activity, several genes in the Ah-gene battery are up-regulated (Alexander *et al.*, 1999). Some PAH act as mixed agonists facilitating PAH:AhR translocation but donot support transcriptional activation.
Some of these assays, especially the CALUX assay have been used to assess the level of inducers in complex mixtures e.g. river sediments, air pollution particles (Vondrácek *et al.*, 2001; Hamers *et al.*, 2000). For complex mixtures the results were expressed as benzo[*a*]pyrene equivalents.

In the analysis of complex mixtures there is significant discrepancy between the different assays, e.g., GRAB and CALUX, demonstrating that the *in vitro* bioassay alone is not a good predictor of the ability of a chemical to activate AhR.

Modelling
The computer-automated structure evaluation (CASE) program, that automatically selects relevant descriptors for structure-activity relationships has been used to analyse the binding of various PAH to the AhR. The binding activity was determined by an electrofocusing assay. The fragments from PAH that were most important for the AhR binding were found to contain the classical “bay” region and were as such identical to the same regions that were responsible for carcinogenicity (Rannug *et al.*, 1991).

2.2.8 Potentiation

Potentiation between different PAH on the effect of AhR can be mediated at many different levels. One of these is competitive inhibition at the 4S-PAH binding protein leaving a higher concentration of the more potent AhR-ligand to bind the receptor. In the case of benzo[*a*]pyrene and benzo[*ghi*]perylene, a potentiating effect in *in vitro* was only seen at high concentrations of benzo[*ghi*]perylene. The effect was seen both on the protein and mRNA level of CYP1A1. Benzo[*ghi*]perylene acted by activating theAhR, causing it to translocate to the nucleus and to form a ligand:AhR:ARNRT:AHRE complex. No effect was seen on any of the parameters at the concentrations of benzo[*a*]pyrene and benzo[*ghi*]perylene used (Cherng *et al.*, 2001).

2.2.9 Summary

In addition to their direct mutagenic activity, PAH can also influence many other processes involved in carcinogenesis. The main focus has been on the induction of enzymes involved in the metabolism of PAH and other carcinogens. This alteration will influence the level of the ultimate carcinogenic metabolite and as such the carcinogenic activity of the parent PAH. In addition to its effect on the initiation stage, the PAH also have an effect on the promotional stages, e.g. inhibition of intracellular communication, resulting in clonal expansion of initiated cells. The defence mechanism against tumour formation includes immunosurveillance and cell death of damaged cells, e.g. apoptosis, and PAH influence both these processes. All the latter processes are regulated through the AhR. Most of information is based upon studies involving a non-PAH AhR ligand, TCDD. The affinity of PAH to the AhR is significantly lower than that of TCDD, and this difference is positively correlated with differences in the toxic and biological activity of these chemicals. Differences in biological response between dioxins and PAH are not only a result of the affinity to the receptor but also the DNA-sequence at the binding site for the ligand-receptor complex.

2.3 Variation in the susceptibility of humans

2.3.1 General aspects

More than a 70-fold inter-individual variation in formation of carcinogen-DNA adducts was observed when human tissues were exposed to the same dose of carcinogenic PAH (Autrup,1986). This difference could be due to variation in uptake and biotransformation of the PAH or in the repair of the formed PAH-DNA adducts. Some of the enzymes

responsible for the biotransformation have been identified, e.g. cytochrome P-450, glutathione S-transferase, epoxide hydrolase (see chapter 3.1).

Many of the enzymes involved in the metabolism of PAH have a polymorphic distribution, and the genetic basis for some of these polymorphisms has been identified. The genetic polymorphisms can have different effect on the rate of metabolism depending on the type and location of the genetic alteration. A single nucleotide polymorphism in the coding sequences results in altered substrate specificity and enzyme kinetics, whereas a change in the regulatory region will results in an altered level of the normal enzyme. Lack of enzyme activity has been observed in case of deletion of the gene or when the genetic polymorphism is in the splice site. A genetic polymorphism has been defined as a genetic change that can be seen in at least 1% of the population.

However, activity of the enzymes will not only depend on the genotype but may change as a consequence of exposure to other chemicals that can either increase the expression of the gene, e.g. mediated by the Ah-receptor (see chapter 2.2) or inhibit the enzyme activity by binding to the active site of the enzyme. Many of the compounds present in fruit and vegetables that exhibit chemopreventive activity induce enzymes that are involved in the detoxification of PAH. Dietary habits characterized by frequent consumption of fresh fruit and vegetables and high intake of antioxidants were strongly associated with reduced level of PAH-DNA adducts (Palli *et al.*, 2000).

Aberrant metabolic capacity due to a genetic polymorphism may be more important at low levels of exposure than at high levels of exposure and could as such alter the threshold for the toxic response. At high doses of the toxic compound, the relevant enzymes are saturated both in efficient and poor metabolizers resulting in efficient metabolism, whereas this would not occur at low doses. It is therefore likely, that under exceptional exposure conditions (very high levels) that the individual susceptibility is not that important. However, low-dose exposure to carcinogens is widespread and its effects are likely to be modulated by genetic susceptibility (Vineis *et al.*, 1994).

Genetic polymorphism in xenobiotic metabolizing enzymes is not associated with an increased cancer risk by themselves as they are not involved in the carcinogenic process. However, they alter the risk of, e.g. PAH in the diet, by changing the ratio of activation/deactivation of the carcinogenic compound, and thus these genes are called “effect-modifying”.

In the following, the role of genetic polymorphism in xenobiotic metabolising enzymes is described. For details on the metabolism of PAH see chapter 2.1.

A nomenclature system for gene polymorphisms in the CYP1A1and the GSTM1 enzymes (amongst others) has been proposed by Garte and Crosti (1999). The gene name is followed by an asterisk, followed by an Arabic number (1 for the wild type) designating the specific polymorphism in chronological order of first publication. The final number may be followed by letters A, B, etc. when allelic subtypes exist.

2.3.2 Cytochrome P450 (CYP)

Members of the **CYP1A** family of enzymes play a central role in the activation of carcinogenic PAH. Four-point mutations have been detected in the CYP1A1 gene resulting in four variant alleles CYP1A1*2 (previously called CYP1A1*2A), CYP1A1*3 (previously called CYP1A1*2B), CYP1A1*4 and CYP1A1*5. The CYP1A1*2 and *3 genotypes have been associated with an increased risk of lung and renal cancer in smokers, and are associated with high CYP1A1 inducibility (Longuemaux *et al.*, 1999). The effect of *2 and *3 is especially pronounced in heavy smokers, suggesting that the PAH in tobacco smoke interacts with the Ah-receptor resulting in the induction of CYP1A1 enzyme in these genotypes and more of the ultimate carcinogen is formed. A slight increased risk for breast cancer was also associated with the *2 and *3 genotypes in smokers. Using benzo[*a*]pyrene-7,8-diol-9,10-epoxide-DNA adduct as a marker of exposure, a significantly higher level of adducts was observed in lymphocytes, lung and placenta from individual homozygotes in *2/2 than the other genotypes (Rojas *et al.*, 2000).

CYP1B1 is very active in the metabolism of PAH-diols to their ultimate carcinogenic forms. Transgenic animal experiments have shown that mice that do not express the CYP1B1 gene are almost completely protected against acute bone marrow toxicity and proleukemic effects when exposed to 7,12-dimethylbenz[*a*]anthracene. This suggests that in the absence of CYP1B1 activity, the biologically reactive metabolite of 7,12-dimethylbenz[*a*]anthracene is not formed (Heidel *et al.*, 2000). Seven different genetic polymorphims of the CYP1B1 gene have been identified, and these variants results in amino acid changes of the enzyme that result in altered enzyme activity. In an *in vitro* test system, only slight differences among these variants in ability to transform the PAH-diol into mutagenic activity was observed, the Ser119/Leu432 having slightly higher activity (1.2-1.5-fold) than the other variants including Ser119/Val432 (Shimada *et al.*, 1999). However, in a preliminary small epidemiological study the low activity Val 432 variant (CYP1B1*2) was associated with an increased risk of colorectal cancer (Fritsche *et al.*, 1999). However, this finding cannot be repeated in a larger case-control study (G. Smith, personal communication).

A number of allelic variants of **CYP1A2** have been identified, and the activity of the enzyme is polymorphically distributed in humans. However, the genetic basis for this polymorphism has not yet univocally been identified. CYP1A2 activity is highly inducible, and differences in exposure to inducers, e.g. smoking, food compounds, may be more important than any genetic differences. Relatively strong associations have been described suggesting that genetically determined high activity of CYP1A2 may be a risk factor for colon cancer (Brockmöller *et al.*, 2000).

CYP3A enzymes are the major CYP450 in human liver accounting for approximately 25% of the total CYP activity. This enzyme is involved in the metabolism of PAH to diols, but it has a lower Vmax and is less stereospecific than either CYP1A1 and CYP1A2 (Roberts-Thomson *et al.*, 1993). Large individual differences in the activity are reported, but the genetic basis for the variation is still under investigation. Two different isoenzymes of CYP3A, 3A4 and 3A5, have been found. Six different genetic variants of CYP3A4 has been identified, 3 of which are resulting in an lower activity, but the frequency of these polymorphisms is low in caucasian populations. A genetic polymorphism has been identified in the isoenzyme 3A5, a single nucleotide change at a splice site, the G6986A having normal splicing and thus normal activity. The frequency of this variant in Caucasian populations is 5% (Husterts *et al.*, 2001). No epidemiological studies to link this polymorphism and PAH exposure with cancer risk has been reported.

2.3.3 Glutathione S-Transferases (GST)

The GST are key phase II enzymes which play a critical role in protection against electrophilic PAH metabolites formed by phase I metabolism. At least five related gene families, mu, alpha, pi, theta and sigma, have been identified. Only GSTM and GSTP appear to play a role in the detoxification of PAH metabolites, and functional genetic polymorphism has been identified in both of these genes. Functional genetic polymorphisms have also been detected in GSTA. However, this enzyme is involved in the detoxification of aromatic amines, and the GSTA1*B genotype that show an intermediate level of expression, has an increased risk of colorectal cancer (Coles *et al.*, 2001).

Deletion of the **GSTM1** gene occurs in approximately 50% of the Caucasian population, and it is anticipated that the group lacking the gene (GSTM1*2/2) has a decreased ability to detoxify the ultimate carcinogenic form of PAH and subsequently an increased risk of cancer. A significantly increased risk of lung and bladder cancer was observed in individuals lacking the GSTM1 gene (GSTM1*2/2), and an interaction between smoking and the genotype was observed, i.e. the risk ratio was higher in the smokers than in non-smokers. The effect of GSTM1*2/2 was even more pronounced at low doses, i.e. few

cigarettes. In contrast to the PAH-related cancers, no clear association of GSTM1*2/2 and gastric cancer (Setiawan *et al.,* 2000) and colorectal cancer has been observed (Gertig *et al.*, 1998), and neither smoking nor smoking length had any effect.

It has been estimated that the lack of GSTM1 activity is responsible for approx 17% of bladder cancer cases in smokers, and nearly 50% of lung cancer cases in women exposed to environmental tobacco smoke (Bennett *et al.*, 1999).

Bulky carcinogen-DNA adducts are considered reliable biomarkers for exposure to PAH. These biomarkers have been used in many monitoring studies to assess total PAH exposure. At low exposure levels of PAH, the level of the adducts in lymphocyte DNA was significantly higher in the GSTM1*2/2 subgroup than in people expressing the gene. However, a protective effect of GSTM1 on the adduct level was not seen in people occupationally exposed for high doses of PAH (Autrup, 2000). These observations suggest that the GSTM1 genotype is more important at low dose exposures as those experienced with PAH in the diet than at high doses.

A higher level of PAH-DNA adducts was observed in breast tissue from patients lacking GSTM1 activity, whereas the GSTM1 genotype did not influence adduct level in normal people. This suggest that alternative pathways to detoxify the ultimate carcinogenic form exist in normal breast tissues, e.g. expression of GSTP in normal but not in cancerous tissues.

GSTM1 activity indirectly influences the induction of CYP1A enzymes. Frequent consumers of cruciferous vegetables with the GSTM1*2/2 genotype express a higher CYP1A2 activity than people expressing GSTM1. The compound present in the vegetables and responsible for the induction is metabolised by GSTM1, and enhanced the excretion of isothiocyanates. Thus GSTM1*2/2 individuals have a higher pharmacologically active dose of the inducer, and thus frequent consumers of cruciferous vegetables had higher CYP1A* activity (Probst-Hensch *et al.*, 1998). A protective effect of isothiocyanate in lung carcinogenesis was primarily seen in GSTM1*2/2 (London *et al.*, 2000). An increased risk of lung cancer has been observed in current smokers with a low intake of cruciferous vegetables but not in the high intake group (Spitz *et al.*, 2000). In the case of colorectal cancer a decreased risk was observed in the group with high broccoli intake and GSTM1*2/2 genotype. The hypothesis in this case is that the isothiocyanate present inhibits CYP450 activity and induces phase II enzymes, e.g. GST (Lin *et al.*, 1998).

These data indicate a complex interaction between dietary factors and genotype of xenobiotic metabolising enzymes, and thus the risk of PAH-induced carcinogenesis.

GSTP1 plays an important role in PAH-induced carcinogenesis, e.g. GSTP1 null mice has an increased risk for PAH-induced skin carcinogenesis (Henderson *et al.*, 1998). GSTP1 shows large variations in the ability to detoxify the various active metabolites of PAH and to reduce mutagenicity in an experimental model system, e.g. the mutagenicity of the + enantiomers of BPDE was reduced 65% whereas the activity of the – enantiomers was only reduced 15% (Seidel *et al.*, 1998). Large inter-individual variation in GSTP1 activity has been observed. Two genetic polymorphisms have been detected both of which resulted in amino acid change, Ile104Val and Ala113Val. The genetic changes are located within the region coding for the active site of the enzyme and the consequence of the amino acid change is altered enzyme kinetics and substrate specificity. The ValVal variant is the most active variant in the detoxification of the ultimate carcinogenic form of BPDE than the other variants (Hu *et al.*, 1999), whereas the IleAla form is the most efficient in detoxifying the diolepoxides of benzo[*g*]chrysene and benzo[*c*]phenanthrene (Hu *et al.*, 1998) which indicate that GSTP1 genetic polymorphism may be an important factor in differential susceptibility of humans for PAH-induced carcinogenesis. An increased risk of lung cancer was seen in the GSTP1*1/1 group when exposed for PAH (Ryberg *et al.*, 1994) and an increased bulky carcinogen-DNA adduct level was seen in people with the same high risk genotype (Autrup, 2000).

2.3.4 Others

Microsomal epoxide hydrolase (mEH) is involved in conversion of PAH-epoxides into diols, e.g. benzo[*a*]pyrene-7,8-oxide into benzo[*a*]pyrene-7,8-dihydrodiol. This reaction is in competition with GST mediated detoxification, and high mEH activity will increase the formation of the ultimate carcinogenic form of the PAH at the expense of detoxification. Two genetic polymorphisms that encode amino acid substitutions have been identified, i.e. exon 3 Tyr113 His and exon 4 His139Arg. The activity of the variants are not significantly different, but the expression of the corresponding proteins is changed, 40% decrease and 25% increase, respectively. It has been suggested that the amino-acid change may result in altered enzyme stability and consequently an altered enzyme activity. The his113his and his139his genotype are risk factors for colorectal adenomas in heavy smokers and in people with a frequent intake of fried and broiled meat. Using an imputed mEH genotype, the very slow metabolizers, 0 or 1 "fast" alleles, exposed for cigarette smoking had a significantly increased risk (Ulrich *et al.*, 2001). However, these results are in conflict with others as a slight but not statistically increased risk for colorectal adenoma was reported for carriers of the high activity alleles. However, when combined with exposure to PAH, smoking status or consumption of well-done cooked meat an increased risk was observed (Cortessis *et al.*, 2001). The “fast” phenotype was also associated with an increased risk for ovarian cancer in smokers, while it did not have any major impact on bladder cancer. Using chromosomal

aberrations in cultured human lymphocytes as the biomarker, BP induced higher level of damage in the carriers of the "fast" genotype, and this effect was especially significant in individuals that are classified as GSTM1*2/2 (Salama *et al.*, 2001).

Myeloperoxidase (MPO) is a phase I metabolic enzyme found in monocytes/macrophages and is involved in the metabolism of benzo[*a*]pyrene to the highly reactive benzo[*a*]pyrene-7,8-diol-9,10-epoxide. A genetic polymorphism has been detected in the SP1 transcription factors binding site in the regulatory region. The G to A nucleotide shift influences the binding of a transcription factor and thus influencing the basal level of the enzyme, the GG genotype having higher enzyme level. In vitro, the G-variants confers a 25-fold higher transcriptional activation compared to the A-variant. A lower risk for lung cancer was observed in the low activity phenotype, but only in people exposed to tobacco smoke and this was especially significant at high doses (Schabath *et al.*, 2000). A higher level of PAH adducts was also seen in atopic dermatitis patients with the GG genotype treated topically with coal tar ointment (Rojas *et al.*, 2001). This suggest an important role of MPO in the activation of PAH to DNA binding metabolites. An interesting sex difference was observed, as women with the GG genotype did not have an increased risk for lung cancer.

NAD(P)H:quinone oxidoreductase (NQO1) is important in the biotransformation of xenobiotica. The enzyme specifically prevents the formation of benzo[*a*]pyrene-quinone-DNA adducts. However, it is involved in the activation of nitro-PAH to mutagenic metabolites which makes interpretations difficult when co-exposure occurs. High NQO1 activity should as such prevent against PAH-induced carcinogenesis, and NQO1 deficiency does also increase susceptibility to benzo[*a*]pyrene-induced mouse skin carcinogenesis (Long *et al.*, 2000). A genetic polymorphism in NQO1 has been detected, a C to T transition at bp 609, that has been associated with a reduced enzyme activity. This polymorphism has also been linked to increased lung cancer risk in Caucasians when stratified according to exposure, e.g. smoking habits (Rosvold *et al.*, 1995).

2.3.5 DNA repair

An increased risk of cancer and malignant transformation in cellular systems have been associated with a decrease in repair capacity. Bulky DNA adducts, such as those induced by PAH , may be repaired by base excision repair (BER) and nucleotide excision repair (NER) pathways. Genetic polymorphism in DNA repair enzymes have been reported, e.g. ERCC1 where an amino acid substitution lys751Gln, where the Lys/Lys carriers have suboptimal DNA repair capacity, and a G to A mutation in XRCC1 resulting in aminoacid change Arg399Gln. The genetic polymorphisms influence the bulky carcinogen DNA adduct level and sister chromatid exchange in human mononuclear cells (Duell *et al.*, 2000). Carriers

with at least one Lys751 allele had a higher adduct level than the Gln/Gln carries when exposed to PAH in ambient air, whereas polymorphisms in XRCC1 and XRCC3 did not significantly alter the DNA adduct level (Palli *et al.*, 2001). These results suggest that individual with low DNA repair capacity may have an increased risk of PAH-induced cancers.

2.3.6 Conclusion

The exact influence of genetic polymorphism on risk for PAH carcinogenesis is not completely elucidated as compensatory mechanism, or alternative enzymatic pathways make take over. Genetic polymorphisms in genes involved in the metabolism of PAH have many faces – sometime beneficial, sometime detrimental. The actual effect may depend on the number of gene-gene and gene-environment interactions including interactions of polymorphic enzymes with the dose of the PAH, chemical nature of PAH and interaction with nutritional status. The current information suggests that the effect of these polymorphism depends on the level of exposure, i.e. the enzymes may be more important at low dose of exposure such as those experiences by food-borne PAH. Although CYP enzymes are involved in the phase I metabolism of many chemical carcinogens, it has not been possible to identify any specific relationship between a risk allele of a CYP and cancer development (Ingelman-Sundberg, 2001). However, the metabolism of PAH is very complex involving several competing pathways involving different enzymes, and it is the ratio between bioactivation and detoxification that determine the genotoxic effect. It is therefore important to define a high-risk population, e.g. GSTM1*2/2, the mEH fast phenotype and CYP1A1*2/ or CYP1A1*3/. The frequencies of high-risk genotypes in Caucasian populations is listed in table 3.3.1 (Garte *et al.*, 2001). The group having all three high-risk genotype will represent approximately 1 % of the European population, as the genes are not linked.

Table 2.3.1 Frequency of genetic polymorphisms in genes involved in PAH metabolism - Caucasians

Gene	High risk genotypes	Allele frequency
GSTM1	GSTM1*2/2 (null)	50%
GSTP1	GSTP1*2/2	5%
CYP1A1	CYP1A1*2/ and CYP1A1*3/	6%
CYP1B	CYP1B1*2/	40%
mEH	“fast“ phenotype	16%
MPO	G/G	56%
NQO1	“low” phenotype	38.6% (at least one low)

2.4 Short- and long-term toxicity and carcinogenicity

The text of this chapter is primarily based on IARC Vol 32 (1983), IARC Suppl 7 (1987), ATSDR (1995a,b), IPCS (1998) and IRIS (2002).

2.4.1 Toxicity after a single exposure

Except for naphthalene, there is only a limited number of studies available on the acute oral toxicity of PAH. The LD_{50} values (table 2.4.1) indicate that the acute oral toxicity is moderate to low.

Table 2.4.1 Acute oral toxicity (Adapted from IPCS, 1998)

Compound	Species	LD_{50} (mg/kg bw)	Reference
Anthracene	Mouse	18000	Montizaan *et al.* (1989)
Benzo[*a*]pyrene	Mouse	>1600	Awogi and Sato (1989)
Fluoranthene	Rat	2000	Smyth *et al.* (1962)
Naphthalene	Rat	1250	Sax and Lewis (1984)
	Rat (M)	2200	Gaines (1969)
	Rat (F)	2400	Gaines (1969)
	Rat	9430	US EPA (1978a)
	Rat	1110	Montizaan *et al.* (1989)
	Rat	490	Montizaan *et al.* (1989)
	Rat	1800	Montizaan *et al.* (1989)
	Mouse (F)	354	Plasterer *et al.* (1985)
	Mouse (M)	533	Shopp *et al.* (1984)
	Mouse (F)	710	Shopp *et al.* (1984)
	Guinea-pig	1200	Sax and Lewis (1984)
Phenanthrene	Mouse	700	Montizaan *et al.* (1989)
	Mouse	1000	Montizaan *et al.* (1989)

2.4.2 Short-term toxicity

In dogs a single oral dose of 3 or 9 g naphthalene or a total dose of 10.5 g per animal given over seven days induced anaemia. All three animals showed neurophysiological symptoms (Zuelzer and Apt, 1949).

After oral administration of 100 mg anthracene or phenanthrene/kg bw per day to rats for four days increased carboxylesterase activity in the intestinal mucosa was observed. Rats given 50 or 150 mg/kg bw per day of benzo[*a*]pyrene or 150 mg/kg bw of benz[*a*]anthracene showed suppressed carboxylesterase activity in the intestinal mucosa whereas renal microsomal carboxylesterase activity was moderately induced (Nousiainen *et al.*, 1984).

Wistar rats treated orally with 100 mg/kg bw per day of benzo[*a*]pyrene, benz[*a*]anthracene, anthracene, chrysene, or phenanthrene for four days showed induction of cytosolic aldehyde dehydrogenase activity. Benzo[*a*]pyrene and benz[*a*]anthracene were much more effective than the other substances, increasing liver weights by 27 and 19%, respectively (Törrönen *et al.*, 1981).

Death due to myelotoxicity was observed after daily oral administration of benzo[*a*]pyrene at 120 mg/kg bw to poor-affinity Ah receptor DBA/2N mice for one to four weeks, whereas high-affinity C57 Bl/6N mice survived with no myelotoxicity for at least six months under these conditions (Legraverend *et al.*, 1983).

After administration (gavage) of 175, 350 and 700 mg/kg bw per day acenaphthene to mice for 90 days increased liver weight and cellular hypertrophy were observed in the two highest dose groups. The NOAEL was 175 mg/kg bw per day (US EPA, 1989a).

With anthracene no treatment related effects were observed in CD-1 (ICR) BR mice following gavage doses up to 1000 mg/kg bw per day for at least 90 days. The NOAEL was the highest dose tested, 1000 mg/kg bw per day (US EPA, 1989b).

Fluorene was administered by gavage to CD-1 mice at a dose of 125, 250, or 500 mg/kg bw per day for 13 weeks. Haematological changes, an increase in the absolute and relative weight of liver, kidney and spleen, and an increase in the amount of haemosiderin in the spleen and in Kupffer cells of the liver was observed in mice treated with 250 or 500 mg/kg bw per day. The NOAEL was 125 mg/kg bw per day (US EPA, 1989c).

Dose-dependent nephropathy and liver toxicity (increased liver weight, increased ALAT activities, and microscopic lesions) were observed in CD-1 mice given 250, or 500 mg/kg

bw per day fluoranthene by gavage for 13 weeks. No adverse effects were observed at 125 mg/kg bw per day (US EPA, 1988).

In a 90-day study in mice, naphthalene administered at oral daily doses (gavage) of 5.3, 53 or 133 mg/kg bw caused a decrease in bodyweight and relative spleen weight in the highest dose group. Blood urea nitrogen (BUN) levels were decreased in all dosed females, but the clinical significance of this observation is unclear. The NOAEL was 53 mg/kg bw per day (Shopp *et al.*, 1984).

In Fisher 344 rats admistered 0, 25, 50, 100, 200 or 400 mg naphthalene/kg bw per day by gavage for 13 weeks (5 days a week), decreased bodyweight and microscopic kidney lesions were observed in the two highest dose groups. The NOAEL was 100 mg/kg bw per day (adjusted: 71 mg/kg bw per day) (BCL 1980).

In rats that ingested 150 mg/kg bw per day naphthalene for the first three weeks and 200-220 mg/kg bw per day for a further 11 weeks, reduced weight gain and food intake, enlarged livers with cell oedema and congestion of the liver parenchyma, and inflammation of kidney cells were observed (Kawai, 1979).

Oral administration (gavage) of 0.5-1 g/kg bw per day naphthalene for periods of 4-13 weeks induced the development of cataracts in rats, mice, and rabbits. Particularly in the eyes of rabbits cataracts developed within a few days of repeated oral administration. It was postulated that P450-dependent bioactivation of naphthalene to naphthoqninone or a free-radical derivative are essential for the induction of cataracts (Wells *et al.*, 1989). The effect could be reduced by pretreatment with P450 inhibitors and antioxidants and increased by pretreatment with P450 inducers or glutathione depletors (IPCS, 1998).

CD-1 mice were given 75, 125, or 250 mg/kg bw per day pyrene by gavage for 13 weeks. Dose-dependent nephropathy, characterized by the presence of multiple foci of renal tubular regeneration, often accompanied by interstitial lymphocytic infiltrates and/or foci of interstitial fibrosis. The renal lesions in all groups were described as minimal or mild. Relative and absolute kidney weights were lower in mice at the two higher doses. The NOAEL was 75 mg/kg bw per day (US EPA, 1989d).

Wistar rats (10 per dose, and sex) treated with benzo[*a*]pyrene (dissolved in soy oil) with doses of 0, 3, 10 or 30 mg benzo[*a*]pyrene/kg bw, 5 days a week were sacrficied after 3 month (additional sacrifice to the chronic carcinogenicity study carried out by Kroese *et al.*, 2001). A dose-related increase in liver weight was seen in male rats from 10 mg/kg bw onwards. In the highest dose group liver weight (females) and thymus weight (males and females) were significantly increased. A dose-related increase in basal cell hyperplasia in

the forestomach was found in males and females in the two highest dose groups. This effect was accompanied by an increase in the number of mitotic cells in the lamina muscularis mucosa of the forestomach in the same dose groups. An increase in thymus atrophy (males and females) was observed at 30 mg/kg bw.

2.4.3 Special short-term assays for induction of preneoplastic lesions

Benzo[*a*]pyrene, 100 and 200 mg per kg of body weight, was given to groups of 30 day old female CD1 mice and 21 day old female Sprague-Dawley rats by gavage twice with a four-day interval, and the animals were terminated 3 weeks later. Benzo[*a*]pyrene induced aberrant crypts foci of the colon in a dose-related fashion. The mouse was 15 times more sensitive than the rat (Tudek *et al.*, 1989).

A series of PAH were investigated for their ability to induce nuclear anomalies (NA) in the mouse gastrointestinal tract. Among the compounds tested, 7,12-dimethylbenz[*a*]anthrene was the most effective inducer of NA in all gastrointestinal tract tissues examined, with the relative potency in duodenum of 7,12-dimethylbenz[*a*]anthrene being much greater than benzo[*a*]pyrene, being greater than benzo[*b*]fluoranthene. Benzo[*a*]anthracene, pyrene and benzo[*e*]pyrene did not have any effect. When binary mixtures of some PAH were administered the yield of NA was less than that expected by simple additivity (Reddy *et al.*,1991).

Groups of six male C57/B6 mice, adapted to human diets, were treated by gavage with 200 mg benzo[*a*]pyrene per kg of body weight. After 24 hours, the frequency of colonic nuclear aberrations was increased by benzo[*a*]pyrene treatment; most markedly in mice fed a diet low in fibre and protein (O'Neill *et al.*,1991).

Nuclear anomalies were induced in crypts of duodenum and colon of a group of 10 male B6C3F1 mice treated with a single oral dose of 0.79 mmol benzo[*a*]pyrene per kg of body weight. In a similar group of mice pre-adapted to 1800 ppm chloroform for 30 days prior to the benzo[*a*]pyrene administration the level of nuclear anomalies induced in the proximal colon by benzo[*a*]pyrene was reduced by four-fold (Daniel *et al.*,1991).

Groups of 15 male F344 rats were initiated for liver carcinogenesis by a single intraperitoneal injection of diethylnitrosamine (200 mg/kg bw) and by two-thirds partial hepatectomy at week three. At week two benzo[*a*]pyrene was incorporated in the diet at a level of 33 mg/kg and this treatment continued for six weeks. Benzo[*a*]pyrene caused only marginal increases in the area of glutathione S-transferase P-form-positive foci in the liver (Hasegawa *et al.*,1989).

Benzo[*a*]pyrene, at a single dose of 200 mg/kg bw only initiated preneoplastic enzyme-altered islands (adenosine- 5'-triphosphatase-deficient and 7 gamma-glutamyltrans-peptidase-positive islands) in the livers of female Sprague-Dawley rats when the rats were both pre-treated and promoted with polychlorinated biphenyls at a dose which strongly induced aryl hydrocarbon hydroxylase (Deml *et al.*,1983).

Benzo[*a*]pyrene (80 mg/kg bw once a week by gavage or 150 mg/kg in the diet), pyrene (100 mg/kg bw once a week by gavage, benz[*a*]anthracene (180 mg/kg bw once a week by gavage), anthracene (180 mg/kg bw once a week by gavage), 3-methylcholanthrene (100 mg/kg bw once a week by gavage or 200 mg/kg in the diet, and 7,12-dimethylbenz[*a*]anthracene (30 mg/kg bw once a week) administered for 6 weeks were tested for initiation of the induction of liver hyperplastic nodules in male F344 rats (16-20 per group). Test chemicals were administered during the initiation stage after which rats were fed dietary N-2-fluorenylacetamide (2-FAA) for 2 weeks in conjugation with a necrogenic dose of CCl4 to enhance production of nodules initiated by test chemicals. Benzo[*a*]pyrene, benz[*a*]anthracene, 3-methylcholanthrene and 7,12-dimethylbenz[*a*]anthracene were all effective as initiators of preneoplastic liver lesions in the rat. 7,12-Dimethylbenz[*a*]anthracene was 20 times more potent than benzo[*a*]pyrene which was 10 times more potent than benz[*a*]anthracene. Administration by gavage was more effective than ingestion with the diet. Anthracene and pyrene had no effect (Tatematsu *et al.*,1983).

2.4.4 Long-term toxicity

A group of 28 BD I and BD III rats received anthracene in the diet from the age of about 100 days, at a daily dose of 5-15 mg per rat. Administration was terminated when a total dose of 4.5 g per rat had been achieved, on day 550. The rats were observed until they died (some lived for more than 1000 days). No treatment-related effects on lifespan or on gross or histological appearance of tissues were observed (Schmähl, 1955).

Non-responsive strains of mice (C57B1/6, C3H/HeN, and Balb/cAnN) had increased relative liver weights after they were fed for 180 days on a diet containing benzo[*a*]pyrene resulting in an intake of 120 mg/kg bw per day (Robinson *et al.*, 1975).

2.4.5 Oral carcinogenicity studies evaluated by IPCS (1998)

The vast majority of the long-term studies conducted on PAH were designed to assess their carcinogenicity. Most studies to assess the carcinogenic potential of single PAH were carried out following dermal, subcutaneous, or inhalation exposure (an overview is given in table 2.4.5). Only a limited number of studies dealt with oral administration, the route of exposure that is most relevant for the current report. The following will particularly concentrate on the results obtained in oral carcinogenicity studies. An overview of these studies is presented in table 2.4.4.

Anthracene orally administered to rats for 2-2.5 years did not show a clear carcinogenic response (IARC, 1983) (table 2.4.4).

A single administration of benz[*a*]anthracene by gavage did not produce tumours in mice whereas repeated oral administrations to mice produced hepatomas and lung adenomas, as well as a few forestomach papillomas (IARC, 1973). A single dose of 200 mg benz[*a*]anthracene administered by gavage to female Sprague-Dawley rats did not induce mammary tumours within 60 days, whereas 7-methylbenz[*a*]anthracene and 12-methylbenz[*a*]anthracene were reported to produce mammary tumours (Huggins and Yang, 1962) (Table 2.4.4).

Groups of 20 female albino mice were treated with a single intragastric dose of 0.2 mg benzo[*a*]pyrene per mouse (corresponding to 6 mg/kg of body weight). After 43 weeks a total of 14 tumours in the forestomach was observed in 5 of 11 surviving animals. Single doses of 0.05 mg (1.5 mg/kg of body weight) and 0.012 mg (0.36 mg/kg of body weight) produced 0/9 and 2/10 tumours, respectively. No tumours were seen in 9 surviving control animals. No tumours were seen in the glandular part of the stomach or other parts of the alimentary canal (Peirce 1961).

Nine female Sprague-Dawley rats (50-65 days of age) were given a single oral dose of 100 mg benzo[*a*]pyrene. Within 60 days 8/9 rats developed tumours of the mammary gland (papillary adenocarcinomas) (Huggins and Yang, 1962).

In a study with Sprague-Dawley rats of both sexes (3 1/2 months of age) daily doses of 2.5 mg benzo[*a*]pyrene per rat for 8-12 months induced papillomas of the oesophagus and forestomach in 3 out of 40 animals (Gibel, 1964).

Groups of male and female white Swiss mice, which were fed diets containing 250 or 1000 mg of benzo[*a*]pyrene per kg diet for various time periods, developed squamous papillomas and carcinomas of the forestomach in a dose dependent manner. All mice fed 1000 mg

benzo[*a*]pyrene per kg diet and examined after 86 days had tumours. In the group fed 250 mg benzo[*a*]pyrene per kg diet 25% developed gastric tumours when fed for more than 85 days. A high incidence of lung adenomas was also observed in these mice (Rigdon and Neal 1966). No increases in stomach tumours were found in mice after 110 days of treatment with diets containing up to 30 mg benzo[*a*]pyrene /kg diet, while 40-45 mg/kg diet for 110 days induced stomach tumours in about 10% of the mice, and more than 70% of mice fed 50-250 mg/kg diet for 122-197 days had stomach tumours. A diet containing 250 mg benzo[*a*]pyrene /kg fed for different time periods produced the following incidences of stomach tumours: one day, 0%; 2-4 days, 10%; 5-7 days, 30-40%; 30 days, 100% (Neal and Rigdon, 1967). The results of these two studies have been compiled in the following two tables (tables 2.4.2 and 2.4.3):

Table 2.4.2 Frequency of forestomach tumours in female mice after administration of benzo[*a*]pyrene (BaP) in the feed (Rigdon and Neal, 1966; Neal and Rigdon, 1967).

Dose: ppm BaP in feed	Number of mice	Age at start (days)	Age at termination (days)	Duration of tre-atment (days)	Percentage of mice with forestomach tumours
0	59	0	38-88		0
0	116	0	111-210		2
1	25	30	140	110	0
10	24	30	140	110	0
20	23	116	226	110	5
30	37	33-67	143-177	110	0
40	40	33-101	143-211	110	3
45	40	31-71	141-183	110	10
50	34	17-22	124-219	107-197	70
100	18	48	156	30	67
100	23	20-24	118-146	98-122	82
250	26	91	198	30	100
250	24	0	47-79	47-79	29
250	73	18-20	88-185	70-165	90
250	58	0	85-196	85-196	31
250	9	0	220-226	220-226	55
1000	31	0	23-83	23-83	26
1000	25	0	86-187	86-187	100
1000	3	0	221-238	221-238	100

In other studies in which mice of the same strain were fed benzo[*a*]pyrene, pulmonary adenomas, thymomas, lymphomas, and leukaemia occurred, remote from the site of primary exposure (Rigdon and Neal, 1969).

Table 2.4.3 Frequency of lung adenomas in female mice after administration of benzo[*a*]pyrene (BaP) in the feed (Rigdon and Neal, 1966).

Dose: ppm BaP in feed	Number of mice	Age at start (days)	Age at termination (days)	Duration of tre-atment (days)	Percentage of mice with lung adenomas*
0	55	0	62-88		11 (1.5)
0	30	0	119-124		23 (1.1)
0	58	0	177-182		28 (2.1)
0	8	0	210		50 (4)
250	55	0	56-95	31-77	45 (4.2)
250	8	0	117-222	47-90	63 (2.2)
250	14	0	178-226	135-153	86 (23.3)
1000	15	0	54-83	54-83	47 (2)
1000	6	0	124-141	124-141	67 (6.8)

* Number in parenthesis = average number of tumours per animal.

Daily administration of benzo[*a*]pyrene in olive oil in the diet of 70 days old female albino mice (strain not specified) for 14 months, in a concentration of 4 mg per kg feed, resulted in 13/160 animals with tumours of the forestomach (11 papillomas, two carcinomas). In control groups 0/117 (olive oil) and 0/158 (untreated controls) developed gastric tumours. When benzo[*a*]pyrene was administered in an ethanol solution instead of drinking water 5/81 tumours of the forestomach developed (Chouroulinkov *et al.*, 1967).

A vast number of studies have been performed in female mice where the development of forestomach neoplasia and lung adenomas after peroral administration of benzo[*a*]pyrene have been demonstrated. In general, these studies have been performed in order to investigate various dietary factors capable of inhibiting the tumourigenicity of benzo[*a*]pyrene , and therefore have normally used a dose regimen for benzo[*a*]pyrene , known to be effective in the mouse. Most often female mice, 7-10 weeks of age, have been used. Benzo[*a*]pyrene has typically been administered by gavage at doses of 1-4 mg/kg bw 1-2 times a week for 2-4 weeks and after 15-30 weeks the incidences of lung adenomas and/or forestomach papillomas (both benign tumours) have been recorded (IPCS 1998).

Oral benzo[*a*]pyrene doses estimated to be between 6 and 12 mg/kg body weight/day induced leukemia in non-responsive (Ahd/Ahd) mice after 100 or more days, but not in responsive mice (Ahb/Ahd). It was suggested that a higher dose is obtained in the bone marrow of non-responsive mice than in responsive mice (Nebert and Jensen, 1979a, 1979b; Nebert *et al.*, 1980).

Oral administration of a single dose of benzo[*a*]pyrene (100 mg) produced tumours of the mammary gland (papillary adenocarcinomas) in female Sprague-Dawley rats (Huggins and Yang, 1962). In another study using the same strain of rats daily doses of 2.5 mg benzo[*a*]pyrene per rat for 8-12 months induced a few papillomas of the oesophagus and forestomach (Gibel, 1964).

Benzo[*a*]pyrene was administered for 87-131 weeks to groups of 32 male and 32 female Sprague-Dawley rats either as an admixture to the diet or by gavage in an aqueous 1.5% caffeine solution. Benzo[*a*]pyrene in solution was given as doses of 0.15 mg/kg body weight either: 1) 5 days per week (annual dose: 39 mg/kg body weight), 2) every 3rd day (18 mg/kg body weight/year), 3) or every 9th day (6 mg/kg body weight/year). When mixed in the diet the doses were: 4) 0.15 mg/kg body weight 5 days a week (39 mg/kg body weight/year) or 5) 0.15 mg/kg body weight every 9th day (6 mg/kg body weight/year). Similar groups given either caffeine solution or control diet served as controls. A significant increased number of rats with forestomach papillomas was observed in group 1 to 4 (14, 25, 11, and 9 rats with tumours, respectively) compared to 1 in group 5) and 2 and 3 tumours in the control groups. No other tumours were found significantly different from control levels (Brune *et al.*, 1981).

A diet containing 500 ppm benzo[*a*]pyrene was given to 13 hamsters four days per week for up to 14 months. A total of 12 tumours (two in oesophagus, eight in the forestomach and two in the intestine) were seen in eight hamsters (Chu and Malmgren, 1965).

Bi-weekly administration of 2-5 mg benzo[*a*]pyrene by gavage produced 5 papillomas of the stomach in 67 hamsters treated for one to five months, seven papillomas and two carcinomas in 18 hamsters treated for six to nine months and five papillomas in eight hamsters treated for 10-11 months (Dontenwill and Mohr, 1962).

A low incidence of large bowel neoplasms was induced in a group of 30 male Syrian hamsters of the inbred strain BIO15.16 by intrarectal instillation of benzo[*a*]pyrene . The hamsters were given 0.8 mg benzo[*a*]pyrene once weekly for 30 weeks. The experiment was terminated after 1 year. The incidence of colon neoplasms was 6% after benzo[*a*]pyrene exposure (2/30 adenocarcinomas versus 0/15 in control hamsters). In a

similar group of hamsters 0.8 mg 3-methylcholanthrene, twice weekly induced tumours in 20% of animals (Wang *et al.*, 1985).

Dibenz[*a,h*]anthracene administered orally dissolved in PEG-400 or olive oil to mice induced forestomach papillomas or pulmonary adenomas or carcinomas (Table A). Also, mammary carcinomas and haemangioendotheliomas have been reported in mice following oral administration (Wattenberg, 1973; IARC, 1973).

Results obtained by Morris *et al.* (1960) feeding rats a diet containing 0.05% fluorene for up to 18 months did not show a clear increase in tumour incidence, though the results could not be interpreted as clearly negative. Therefore IPCS (1998) classified the study as "questionable" (see table 2.4.4).

Two studies in rats with oral administration of naphthalene or phenanthrene did not show an increase in tumour incidence.

It should be noted that most of the studies mentioned above were performed before 1970 and were carried out without using controls, using test compounds not clearly defined, or using inadequate experimental designs. The criteria used by WHO (IPCS,1998) to classify a study as "valid" (see table 2.4.4) were (i) an appropriate study protocol, i.e. use of concurrent controls (sham or vehicle), 20 or more animals per group, and study duration of at least six months; and (ii) sufficient documentation, including detailed description of administration, results, and the survival of animals.

Table 2.4.4 Overview of oral carcinogenicity studies with polycyclic aromatic hydrocarbons in experimental animals (Adapted from IPCS, 1998)

Species, strain	No./ group	Dosage	Duration at death/ sacrifice	Incidence and type of tumour	Result Stat/Val	Reference
Anthracene						
Rat	31	6 mg/animal/day, 7x/week (diet)	33 months	22/31 alive after 1 year; no tumours after 33 months	ld	Schmahl and Reuter, cited by Gerarde (1960)
Rat, BD I/BD III	28	5-15 mg/animal/day 6x/week, 78 weeks (diet)	700 days	2/28 malignant tumours,	ld	Schmahl (1955)
Benz[*a*]anthracene						
Mouse, C57/BL	8-19	0.5 mg/animal, 1x, 8x or 16 x (highest dose), $\leq$ 2 months	16 months	0/13, 1/19 and 1/8 with papillomas; no carcinomas observed; control: 0/12	q, ln	Bock and King (1959)
Mouse, m B6AF1/J, newborn	20 or 40 Check!	1.5 mg/animal, 3x/wk, 5 weeks	$\leq$ 547-600 days	100% hepatomas and 95 pulmonary adenomas; solvent only: 10% hepatomas and 35% pulmonary adenomas	val	Klein (1963)
Mouse, m B6AF1/J, newborn	20	1.5 mg/animal, 1x/day, 2 days	$\leq$ 568 days	80% hepatomas and 85% lung adenomas (inadequately reported)	val	Klein (1963)

Species, strain	No./ group	Dosage	Duration at death/ sacrifice	Incidence and type of tumour	Result Stat/Val	Reference
Rat, f SD	10	200 mg/rat, 1x	60 days	no tumours in treated animals; control: 8/164 after 310 days	ln, lc	Huggins and Yang(1962)
Benzo[*a*]pyrene						
Mouse, f A/HeJ	15	3 mg/animal in sesame oil, 2x	30 weeks	Increased pulmonary tumours (16.6); control: 0.3	yes/val	Wattenberg and Leong(1970)
Mouse, f A/J	15	2 mg/animal, 3x, every 2 weeks	26 weeks	15/15 with forestomach tumours and 15/15 with pulmonary adenomas; no control	yes/val	Sparnins et al. (1986)
Mouse, m/f CFW	25-73	0.004-1 mg/animal per day (diet), $\leq$ 110-165 days	140-200 days	Dose-dependent gastric tumours (0-90%); control: no tumours	no/val	Neal and Rigdon (1967)
Mouse, m/f CFW	9-26	1-20 mg/animal/day, (diet) $\leq$ 1-30 days	150-300 days	Dose-dependent gastric tumours (0-100%); control: no tumours	no/val	Neal and Rigdon, (1967)
Mouse, m/f White Swiss	60-175	0.25 and 1 mg/g food	$\leq$ 34 weeks	33 and 61 % with stomach tumours; 53 and 20% with lung tumours; controls: 1 and 21%	no/val	Rigdon and Neal (1966)
Rat, f SD	9	100 mg/kg, 1x	60 days	8/9 with mammary tumours; control: 8/164 in 310 days	no,ln,lc	Huggins and Yang(1962)

Species, strain	No./ group	Dosage	Duration at death/ sacrifice	Incidence and type of tumour	Result Stat/Val	Reference
Rat, f LEW/Mai	20	625 mg/animal, 1x/week, 8x; 50 mg/animal, 1x	90 weeks	67-77% with mammary tumours; control: 30%	yes/val	McCormick et al. (1981)
Hamster, m/f Malmgren Syrian	13	2.5 mg/animal (diet), 4 days/week,	≤ 14 months	9/13 with forestomach cancer; 2/13 with papillomas	no/val	Chu and (1965)
Dibenz[*a,h*]anthracene						
Mouse m Swiss	-	1.5 mg/anim in PEG-400, 1 x; initiation experiment	30 weeks	21 % forestomach papillomas; promotor only: 14%	q/no/lc	Berenblum and Haran (1955)
Mouse m/f DBA/2	21/21 control: 25/10	0.8 mg/day/animal in olive oil	8-9 months	14/14 m and 13/13 f with pulmonary adenomas; 14/14 m and 10/13 f with alveologenic carcinomas; control: 1 tumour	no/val	Snell and Stewart (1962)
Fluorene						
Rat, f Buffalo	20	0.05% diet; 4.3 mg/rat per day = 796 mg/rat (total intake) over 6 months	10.7 months	2/11 carcinomas (renal pelvis, ureter); control: 4/16 with carcinomas	q/no/ld	Morris *et al.* (1960)
Rat, f Buffalo	18	0.05% diet; 4.6 mg/rat per day = 2553 mg/rat (total intake) over 18 months	≤ 20.1 months	7/18 tumours; control: 4/18 or 15/18 tumours	q/no/val	Morris *et al.* (1960)

Species, strain	No./ group	Dosage	Duration at death/ sacrifice	Incidence and type of tumour	Result Stat/Val	Reference
Naphthalene						
Rat, BDI/BDI II	28	10-20 mg/animal (diet) (diet) 6x/week, 70 weeks	Life	No tumours	no/ld	Schmähl (1955)
Phenanthrene						
Rat, f SD	10	200 mg/rat, 1x; experiment on mammary tumours	60 days	No tumours at 60 days; controls: 8/164 after 310 days	no/ln	Huggins and Yang (1962)

lc, limited documentation; ld, limited design; ln, limited number of animals, q, questionable; Stat, statistical evaluation (yes or no); val, valid; f, female; m, male

Table 2.4.5 Overview of PAH carcinogenicity by route of administration (IPCS, 1998)

Common name	Oral	i.p.	i.p./s.c. new born	Resp. instill.	Skin init.	Skin compl.	s.c. or i.m.
Acenaphthylene							
Acenaphthene							
Anthanthrene				+, r	0, m	0, m	
Anthracene	0, r	0, r	+, m	0, r		0, m	0, m, r
Benz[a]anthracene	+, m		+, m	(+), m	(+), m		+, m 0, r
Benzo[a]fluorene					0, m	0, m	0, m
Benzo[b]fluorene					0, m		
Benzo[b]fluoranthene	+, m		+, m	+, r	+, m	+, m	+, m
Benzo[ghi]fluoranthene			+, m	+, r		0, m	
Benzo[j]fluoranthene			+, m	+, r	+, m	+, m	
Benzo[k]fluoranthene			+, m	+, r	(+), m	0, m	+, m
Benzo[ghi]perylene						(+), m	0, m
Benzo[c]phenanthrene					+, m	(+), m	0, m (+), rat
Benzo[a]pyrene	+, m,r,h,	+, m,r	+, m	+, r,h	+, m	+, m	+, m, r
Benzo[e]pyrene	0, m			0, r		0, m	
Chrysene			(+), m	(+), r	+, m	(+), m	+, m, r
Coronene			+, m			(+), m	
Cyclopenta[cd]pyrene			+, m		+, m	+, m	
Dibenz[a,h]anthracene	+, m		+, m	+, r	+, m	+, m	+, m, r
Dibenzo[a,e]pyrene					+, m	+, m	+, m
Dibenzo[a,h]pyrene			+, m		+, m	+, m	+, m
Dibenzo[a,i]pyrene					+, m	+, m	+, m
Dibenzo[a,l]pyrene					+, m	+, m	+, m
Fluoranthene			+, m		0, m	0, m	
Fluorene	0, r						0, m
Indeno[1,2,3-cd]pyrene			0, m	+, r	+, m	(+), m	+, m
5- Methylchrysene					+, m	+, m	
1- Methylphenanthrene					0, m		
Naphthalene							
Perylene					(+), m		
Phenanthrene	0, r		0, m	0, r	(+), m		0, m
Pyrene	0, m			(+), h	(+), m	(+), m	0, m
Triphenylene						0, m	

0 = no effect; (+) = weak effect; + = clear effect; m = mouse; r = rat; h = hamster; g = Guinea pig

2.4.6 Carcinogenicity of PAH following other routes of administration

The skin carcinogenicity of a number of PAH after dermal application to sensitive strains of mice is well established. In fact, the study of skin carcinogenicity of PAH, alone or in combination with tumour promoters, has provided much of the background for the initiation/promotion theory in chemical carcinogenesis (IPCS, 1998).

Benzo[*a*]pyrene is the only PAH that has been tested for carcinogenicity following inhalation. After long-term inhalation of 10 mg benzo[*a*]pyrene per m^3, cancer of the respiratory tract occurred in 35% of golden hamsters (Thyssen *et al.*, 1981; Pauluhn *et al.*, 1985). However, pulmonary carcinogenicity has been shown for a number of PAH in studies using direct application (instillation) of the PAH into the respiratory tract of rats and hamsters (IPCS, 1998).

Benzo[*a*]pyrene and many other PAH are potent inducers of liver and lung tumours (within half a year) following intraperitoneal or subcutaneous injection of newborn animals (ATSDR, 1994; IARC, 1973; Platt *et al.*, 1990; Busby *et al.*, 1988 and 1989; Lavoie *et al.*, 1987; IPCS, 1998).

In their evaluation of the data on the carcinogenicity of individual PAH, IPCS (1998) concluded that the following PAH should be considered carcinogenic: anthanthrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluor-anthene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*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 5-methylchrysene. In addition, benzo[*c*]phenanthrene and fluoroanthene were suspected of being carcinogenic. The carcinogenicity of acenaphthene, acenaphthylene, benzo[*ghi*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*e*]pyrene, coronene, naphthalene, phenanthrene and pyrene was considered questionable. Of these, naphthalene was considered to be not carcinogenic due to its negative genotoxicity, the others were further evaluated. In its final evaluation the IPCS found, that next to naphthalene, anthracene, benzo[*ghi*]fluoranthene, benzo[*ghi*]perylene, fluorene, 1-methylphenanthrene, perylene and triphenylene should be considered not carcinogenic (IPCS, 1998).

In most studies, the site of the tumour development was related to the route of administration, i.e. oral administration induced gastric tumours, dermal application induced skin tumours, inhalation and intratracheal instillation resulted in lung tumours, subcutaneous injection resulted in sarcomas. However, tumour induction is not restricted to the sites of application (IPCS, 1998; Kroose *et al.*, 2001).

In animal bioassays using the same route of exposure, dibenz[*a,h*]anthracene, dibenzo[*a,h*]pyrene, dibenzo[*a,l*]pyrene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and 5-methylchrysene seem to be the most potent carcinogenic PAH. Benzo[*j*]- and

benzo[*k*]fluoranthene are of moderate potency, while benz[*a*]anthracene and chrysene are relatively weak carcinogens.

Table 2.4.6 Overview of IARC evaluation (1983, 1987) of evidence for carcinogenicity to humans of PAH

PAH Compound	IARC conclusion	
	Animals	"Overall"
Anthanthrene	L	3
Anthracene	I	3
Benz[a]anthracene	S	2A
Benzo[b]fluoranthene	S	2B
Benzo[j]fluoranthene	S	2B
Benzo[k]fluoranthene	S	2B
Benzo[g,h,i]fluoranthene	I	3
Benzo[a]fluorene	I	3
Benzo[b]fluorene	I	3
Benzo[ghi]perylene	I	3
Benzo[c]phenanthrene	I	3
Benzo[a]pyrene	S	2A
Benzo[e]pyrene	I	3
Chrysene	L	3
Coronene	I	3
Cyclopenta[cd]pyrene	L	3
Dibenz[a,c]anthracene	L	3
Dibenz[a,h]anthracene	S	2A
Dibenz[a,j]anthracene	L	3
Dibenzo[a,e]pyrene	S	2B
Dibenzo[a,h]pyrene	S	2B
Dibenzo[a,i]pyrene	S	2B
Dibenzo[a,l]pyrene	S	2B
Fluoranthene	I	3
Fluorene	I	3
Indeno[1,2,3-cd]pyrene	S	2B
5-Methylchrysene	S	2B
1-Methylphenanthrene	I	3
Perylene	I	3
Phenanthrene	I	3
Pyrene	I	3
Triphenylene	I	3

I: inadequate evidence; L: limited evidence; S: sufficient evidence;
2A: probably carcinogenic to humans; 2B: possibly carcinogenic to humans;
3: not classifiable

Based on all available information judging data from studies concerning all different routes of administration (dermal, subcutaneous, intramusculary, intraperitoneal, inhalation, oral; see table 2.4.5) IARC (1983, 1987) has classified 32 different PAH with respect to the evidence for carcinogenicity in experimental animals and humans (table 2.4.6).

2.4.7 Carcinogenicity of complex mixtures containing PAH

In the recent feeding study that compared the tumourigenicity of coal tar with that of benzo[*a*]pyrene in female B6C3F1 mice it was shown that when benzo[*a*]pyrene was administered alone the major site of tumour formation (papillomas and squamous cell carcinomas) was the forestomach. When benzo[*a*]pyrene was part of coal tar mixtures the formation of forestomach tumours seemed to be in accordance with the benzo[*a*]pyrene content of the mixtures. However, in addition to the forestomach tumours the coal tar mixtures also produces increased incidences of alveolar and bronchiolar adenomas and carcinomas, liver tumours, tumours of the small intestine, and haemangiosarcomas. The overall carcinogenic potencies of the complex coal tar mixtures were 2-5 times higher than that of benzo[*a*]pyrene (Culp *et al.*, 1998) (see 2.4.7).

The 4-7 ring PAH fraction of condensate from car exhaust (gasoline, diesel), domestic coal stove emissions, and tobacco smoke contained almost all the carcinogenic potential of PAH. This was found in a series of studies using skin painting, subcutaneous injection and intrapulmonary implantation of different fractions. It was concluded from the skin painting tests of different condensates that benzo[*a*]pyrene represented about 5-15% of the carcinogenic potency of the exhaust condensates from petrol-driven vehicles and coal-fired domestic stoves. When tested by lung implantation in the rat, benzo[*a*]pyrene contributed a somewhat lower percentage of the total carcinogenicity (<1 - 2.5%) (Pott and Stober, 1983; Grimmer *et al.*, 1983, 1984a, 1984b; 1985, 1987a, 1987b, 1988; Deutsch Wenzel *et al.*, 1984).

A dose-related lung carcinogenic effect was observed in rats exposed by inhalation to a coal tar/pitch condensation aerosol at exposure levels corresponding to either 20 or 46 µg benzo[*a*]pyrene/m^3. The lifetime lung tumour risk for rats exposed to 1 µg benzo[a]pyrene/m^3 as a constituent of this complex mixture was calculated to be 2% ($2x10^{-2}$). In comparison, the WHO (2001) estimated a unit lung cancer risk for humans of nearly 9% ($8.7x10^{-2}$) per µg benzo[*a*]pyrene/m^3 in ambient air based on epidemiological data on workers exposed to coke-oven emissions from coking plants. It was suggested that in the evaluation of the lung carcinogenicity of PAH adsorbed on inhaled fine particles the likely enhancing properties of the inflammatory effects of particles on lung tissue should be considered (Heinrich *et al.*, 1994).

2.4.8 Recent oral carcinogenicity studies on benzo[*a*]pyrene not evaluated by IPCS (1998)

Two recent oral carcinogenicity studies with benzo[*a*]pyrene have become available since the evaluation in 1998 by WHO.

In a study to compare the tumourogenicity of coal tar with that of benz[*a*]pyrene female B6C3F1 mice were fed diets containing 0, 5, 25 or 100 mg/kg benz[*a*]pyrene (dissolved in acetone) for 2 years. Equivalent doses are 0, 0.7, 3.6 or 14 mg benzo[*a*]pyrene/kg bw. Papillomas and squamous cell carcinomas were observed in the forestomach: 1/48, 4/47, 36/47, 46/47, with significant and dose-related increased incidences at 25 and 100 mg/kg. The incidences of papilomas and carcinomas in the oesophagus were 0/48, 0/48, 2/45, 27/46, and in the tongue 0/48, 0/48, 2/46, 23/48. In the latter two tissues only the highest dose group differed significantly from the solvent control (Culp *et al.*, 1998). DNA adducts determined with the ^{32}P-postlabelling method in the forestomach of a subset of mice fed benzo[*a*]pyrene for 4 weeks, were increased in a linear manner as function of the dose. The major adduct has been characterized as dG-N2- benzo[*a*]pyrene-7,8-diol-9,10-epoxide (Culp et al, 1996).

In the same experiment groups of 48 female B6C3F1 mice were fed diets containing 0, 0.01, 0.03, 0.1, 0.3, 0.6 or 1.0% of coal tar mixture I containing 2240 mg benzo[*a*]pyrene/kg as determined by HPLC (dose levels equivalent to 0.03, 0.09, 0.32, 0.96, 1.92 or 3.2 mg benzo[*a*]pyrene/kg bw), and 0, 0.03, 0.1 or 0.3% of coal tar mixture II containing 3669 mg benzo[*a*]pyrene/kg as determined by HPLC (equivalent to 0.16, 0.52 or 1.1 mg benzo[*a*]pyreneP/kg bw). A significantly increased incidence of alveolar and bronchiolar adenomas and carcinomas was found at 0.3, 0.6 and 1.0% of mixture I (27/47, 25/47 and 21/45 versus 2/47 in the control), and at 0.1 and 0.3% of mixture II (10/48 and 23/47 versus 2/47 in the control). A significant increase in tumours of the forestomach was found at 0.3 and 0.6% of mixture I (14/46, 15/45 versus 0/47 in the control) with a distinct lower incidene (6/41) in the highest dose (1%). Forestomach tumours were also observed at 0.3% of mixture II (13/44). Also the incidence of liver tumours (14/45 at 0.3% mixture I), tumours of the small intestine (22/36 and 36/41 at 0.6 and 1.0% mixture I, respectively) and haemangiosarcomas (0.3 and 0.6% mixture I and 0.3% mixture II) was significantly increased.

In a subset of mice fed coal tar mixture I for 4 weeks, DNA adducts were determined in forestomach and small intestine. In the forestomach adduct levels increased in a dose-related manner. In the small intestine adduct levels increased until the 0.6% dose, with a sharp decrease in the highest dose (1.0%), where the tumour incidence

was greatest. Therefore the authors concluded that coal-tar induced cytotoxicity and cell proliferation may be critical factors for tumour induction in this tissue (Culp et al, 1996).

Table 2.4.7 Composition of the coal tar mixtures used in the mice carcinogenity study (Culp et al, 1998)

Compound	Mixture I (mg/kg)	Mixture II (mg/kg)
Acenaphthene	2049	1270
Acenapthylene	3190	5710
Anthracene	2524	2900
Benz[a]anthracene	2374	3340
Benzo[b]fluoranthene	2097	2890
Benzo[k]fluoranthene	699	1010
Benzo[g,h,i]perylene	1493	2290
Benzo[a]pyrene	1837	2760
Chrysene	2379	2960
Dibenz[a,h]anthracene	267	370
Dibenzofuren	1504	1810
Fluoranthene	4965	6370
Fluorene	3692	4770
Indan	1133	490
Indeno[1,2,3-*cd*]pyrene	1353	1990
1-Methylnaphthalene	6550	5660
2-Methylnaphthalene	11 289	10 700
Naphthalene	22 203	32 300
Phenanthrene	7640	10 100
Pyrene	5092	7220

Wistar rats (52 per dose, and sex) were treated with benzo[*a*]pyrene (dissolved in soy oil) with doses of 0, 3, 10 or 30 mg benzo[*a*]pyrene /kg bw, 5 days a week for 104 weeks. The most prominent carcinogenic effects were observed in the liver and the forestomach. In the forestomach the incidence of combined papilloma and carcinoma was respectively 1/52, 6/51, 30/51, 50/52 for females, and 0/52, 8/52, 43/52, 52/52 for males. The incidence of combined adenoma and carcinoma in the liver was respectively 0/52, 2/52, 39/52, 51/52 for females, and 0/52, 4/52, 38/52, 49/52 for males. Besides these major target sites, benzo[*a*]pyrene treatment also induced soft tissues sarcomas at various sites (oesophagus, skin, mammary), as well as tumours of the auditory canal, skin, and oral cavity, small intestine and the kidney (Kroese *et al.*, 2001).

A separate experiment in the rat chronic carcinogenicity assay concerns DNA adduct formation. DNA adducts detected by using the ^{32}P-postlabelling method are found in all tissues, with remarkably high levels in organs devoid of any tumour development (i.e. lungs, kidneys). This leads the authors to conclude that "factors additional to DNA adducts formation apparently are critical to tumour development by benzo[*a*]pyrene " (Kroese *et al.*, 2001).

2.5 Reproductive and developmental toxicity of PAH

2.5.1 Introduction

The reproductive and developmental toxicity of PAH has been reviewed by IARC (1983), ATSDR (1995b) and IPCS (1998). The information below is taken from these reviews, supplemented by a literature search from 1998 onwards.

Many PAH metabolised by the cytochrome P-450 system are also inducers of that enzyme system via activation of arylhydrocarbon receptors (AhR). As with other aspects of PAH toxicity, enzyme induction generally enhances the toxic response. Inducibility of the cytochrome P-450 system is determined by genotype. Thus, in reproduction and developmental toxicity studies both the maternal and the fetal genotype are relevant to outcome. Benzo[*a*]pyrene, chrysene, dibenz[*a,c*]anthracene, fluoranthrene, perylene and phenanthrene all induce the cytochrome P-450 enzyme, benzo[*a*]pyrene hydroxylase, in rat placenta (IARC, 1983).

2.5.2 Placental transfer of PAH and formation of placental PAH-DNA adducts

It has been shown that benzo[*a*]pyrene readily crosses the placenta in mice and rats and that dibenz[*a,j*]acridine (or its metabolites) and dibenz[*a,h*]anthracene also cross the placenta (IARC, 1983). It is reasonable to assume that because of their lipid solubility, most PAH are likely to pass into the embryo and fetus.

PAH-DNA adducts are found in the human placenta and in fetal tissues (umbilical artery and vein, liver and lung) indicating that PAH are transferred to and activated by the human fetus (Autrup, 1993). Placental and fetal adducts are found in both smokers and non-smokers, but relative adduct levels are significantly higher in smokers than in non-smokers (Hansen *et al.*, 1993; Šrám *et al.*, 1999). They are also higher in women living in an area with high airborne pollution compared with women in a less polluted area (Šrám *et al.*, 1999)

2.5.3 Reproductive toxicity in experimental animals

The majority of investigations for reproductive and developmental toxicity in animals have been on benzo[*a*]pyrene.

Benzo[a]pyrene
The main observations on mice are summarised in table 2.5.1, taken from the IPCS review (IPCS, 1998).

Benzo[*a*]pyrene was administered via the diet to Swiss mice, at doses corresponding to 33, 67 or 133 mg/kg bw/day, for for varying time periods of 20-30 days before mating, during gestation and parturition. No effects were found on the numbers of offspring or on numbers of sperm in the testicular lumen (Rigdon and Neal, 1965).

Administration of benzo[*a*]pyrene by oral gavage to CD-1 mice for 10 days from day 7-16 of pregnancy at doses of 10, 40 and 160 mg/kg bw/day, reduced the numbers of pregnant females reaching parturition at 160 mg/kg bw/day and reduced the fertility of F_1 offspring in all dose groups when bred with untreated animals (see table 2.5.3) (Mackenzie and Angevine, 1981). There were significant alterations in gonadal morphology and germ cell development and almost complete sterility of F_1 offspring at 40 mg/kg bw/day.

PAH can affect female fertility by destruction of oocytes. In studies in DBA/2N mice, a single intraperitoneal (ip) injection of benzo[*a*]pyrene given 14 days before mating in doses ranging from 10-500 mg/kg bw reduced pup numbers in a dose-dependent manner. In a second experiment primordial oocytes were destroyed in a dose-dependent manner by single doses of 5-500 mg/kg bw given 21 days before sacrifice. The threshold for these effects was estimated to be around 2.7-3.4 mg/kg bw (Mattison *et al.*, 1980). Destruction of oocytes by PAH has been shown to be an AhR-mediated effect, involving increased expression of the *Bax* gene in oocytes (see section on "Significance of receptor-mediated effects").

In experiments with B6 *(Ah*-inducible) and D2 (non-inducible) mice given a single ip injection of 100 mg benzo[*a*]pyrene/kg bw 13 days before sacrifice, primordial oocytes of B6 mice underwent more rapid destruction than those of D2 mice (Mattison and Nightingale, 1980). This effect corresponded to a two- to threefold increase in ovarian arylhydrocarbon hydroxylase (AHH) activity in B6 mice after treatment. This correlation was not found in analogous experiments with $D2B6F_1$ mice, in which AHH activity was increased by two- to threefold, but the oocyte destruction was similar to that observed in D2 mice (Mattison and Nightingale, 1980). The IPCS review (IPCS, 1998) commented that this demonstrates an inconsistent consequence of strain differences in genotype and that the sum of activation, detoxification, and repair seems to be decisive for the process of oocyte destruction.

Benzo[*a*]pyrene and its three metabolites, the benzo[*a*]pyrene 7,8-oxide, 7,8-diol, and diol epoxide, were administered by injection at a single dose of 10 µg into the right ovary of B6, D2, and $D2B6F_1$ mice. Various reductions were observed in ovarian volume, weight, and follicle numbers after 2 weeks, with compensatory hypertrophy in the left ovary, in all strains given benzo[a]pyrene or its metabolites. This indicates the ovary can metabolise benzo[*a*]pyrene into reactive metabolites (Mattison *et al.*, 1989).

There are no recent studies in rats. Dietary administration of benzo[*a*]pyrene to female rats for 28-days had no effect on the oestrous cycle and no effect on fertility when these rats were bred with untreated males (Rigdon and Rennels, 1964). When male and female rats were given benzo[*a*]pyrene in the diet at a dose equivalent to around 50 mg/kg bw and bred there was said to be fewer pregnancies and increased stillborn and resorbed fetuses, but the data are poorly reported (Rigdon and Rennels, 1964).

Other PAH

More recent studies by the US EPA on other PAH are described in the review by the ATSDR (1995b). Mice were treated orally by gavage for 13 weeks with acenaphthene up to 700 mg/kg bw/day, anthracene at 1000 mg/kg bw/day, and fluoranthene or fluorene each at 500 mg/kg bw/day. There were no effects on male or female reproductive organ weights or histology, except in the case of acenaphthene, where reduced ovary weights were observed at 700 mg/kg bw/day but not at 350 mg/kg bw/day.

2.5.4 Developmental toxicity in experimental animals

Developmental toxicity (embryolethality, reduced fetal weight and malformations) has been reported in response to benz[*a*]anthracene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and naphthalene. The effects are summarised in table 2.5.2, modified from the IPCS review (IPCS, 1998). The important studies are discussed below.

Benzo[a]pyrene

Benzo[*a*]pyrene is embryotoxic to mice and the size of the effect is partly dependent on the genetically determined induction of the cytochrome P-450 mono-oxygenase receptor, *Ah*, of the mother and fetus by PAH (IARC, 1983; IPCS, 1998). In the case of an inducible mother *(Ah^b/ Ah^b* and *Ah^b/ Ah^d*, B groups), the genotype of the fetus is not crucial because the active metabolites formed in the mother appear to cross the placenta, causing fetal death or malformation. In contrast, when the mother is non-inducible *(Ah^d/ Ah^d*, D group), the genotype of the fetus is important; one litter may contain both inducible fetuses that are affected and non-inducible fetuses that are not.

Another influential factor is the route by which benzo[*a*]pyrene is given to the mother. The three studies described below and in table 2.5.2 illustrate these aspects.

Injection of benzo[*a*]pyrene ip at 50 or 300 mg/kg bw on day 7 or 10 of gestation was more toxic and teratogenic in genetically inducible C57Bl/6 *(Ah*^{b}/ *Ah*^{b}) than in non-inducible AKR inbred mice *(Ah*^{d}/ *Ah*^{d}). In AKR × (C57Bl/6)(AKR)F_1 and (C57Bl/6)(AKR)F_1 × AKR back-crosses (father × F_1 mother), allelic differences at the *Ah* locus in the fetus correlated with dysmorphogenesis. The inducible fetal *Ah*^{b}/ *Ah*^{d} genotype results in more stillborn and resorbed fetuses, decreased fetal weight, increased frequency of congenital anomalies, and enhanced P-450-mediated covalent binding of benzo[*a*]pyrene metabolites to fetal protein and DNA, when compared with fetuses of the non-inducible *Ah*^{d}/ *Ah*^{d} genotype from the same uterus. In the case of an inducible mother *(Ah*^{b}/ *Ah*^{d}), however, these parameters do not differ in *Ah*^{b}/ *Ah*^{d} and *Ah*^{d}/ *Ah*^{d} fetuses in the same uterus, presumably because of the increased benzo[*a*]pyrene metabolism in maternal tissues and placenta (Shum *et al.*, 1979).

An inducible genotype is not the only factor involved in the reproductive toxicity of benzo[*a*]pyrene. In a study in which C57Bl/6 female mice *(Ah* inducible) were mated with C57Bl/6, DBA/2, or BDF_1 male mice (B groups), and DBA/2 females (non-inducible) were mated with C57Bl/6, DBA/2, or BDF_1 males (D groups) and received ip injections of benzo[*a*]pyrene, fetal mortality increased dose-dependently in all groups except the DBA/2 × DBA/2. Fetal body weight was reduced dose-dependently in all experimental groups, but the effect was more pronounced in D than B groups, as was a dose-dependent increase in the frequency of cervical ribs (Hoshino *et al.*, 1981). These results suggest that *Ah*-inducible fetuses are more sensitive to lethal events, whereas those of non-inducible dams are more susceptible to a decrease in body weight and an increased incidence of cervical ribs. The incidence of external malformations may, however, differ in mice of different genotypes after treatment with benzo[*a*]pyrene, even if both dams and fetuses are inducible.

The toxicity of benzo[*a*]pyrene was investigated in pregnant *Ah*^{d}/ *Ah*^{d} × *Ah*^{b}/ *Ah*^{d} F_1 and *Ah*^{b}/ *Ah*^{d} × *Ah*^{d}/ *Ah*^{d} F_1 back-crossed mice fed benzo[*a*]pyrene in the diet at 120 mg/kg daily on days 2-10 of gestation (Legraverend *et al.*, 1984). Embryos of D females *(Ah*^{d}/ *Ah*^{d} genotype; non-inducible) showed more signs of toxicity and malformations than *Ah*^{b}/ *Ah*^{d} embryos. Fetuses of B females *(Ah*^{b}/ *Ah*^{d} genotype) did not show these changes. The authors suggested that reduced benzo[*a*]pyrene metabolism in the intestine had caused high concentrations in the embryos, and more toxic metabolites (benzo[*a*]pyrene-1,6- and -3,6-quinones) were detected in the *Ah*^{d}/ *Ah*^{d} embryos than in *Ah*^{b}/ *Ah*^{d} embryos. These results were in contrast to those reported after ip injection by Shum *et al.* (1979) and Hoshino *et al.* (1981). The route of administration can thus affect the magnitude of the observed effects.

Three studies on the postnatal effects of *in utero* exposure to benzo[*a*]pyrene on mouse offspring, with maternal administration dermally, ip or orally, showed adverse effects on offspring after birth, including an increased incidence of tumours, immunological suppression, and reduced fertility (see table 2.5.3) (Andrianova, 1971; Urso and Gengozian, 1980; MacKenzie and Angevine, 1981). Benzo[*a*]pyrene given to pregnant rats on day 15 or 19 of gestation caused alterations at the thymic glucocorticoid receptors in the offspring, suggesting binding to the pre-encoded hormone receptors and interference with receptor maturation (Csaba *et al.*, 1991; Csaba and Inczefi-Gonda, 1992). Strong suppression of immunological parameters was found in the progeny of mice that had been treated ip with benzo[*a*]pyrene at mid-gestation (Urso and Johnson, 1987). Benzo[*a*]pyrene is also a transplacental carcinogen, producing lung and/or liver tumours in the offspring of mice and rabbits given benzo[*a*]pyrene during gestation (IARC, 1983).

PAH would not be expected to cause malformations, when given during embryogenesis, via DNA adduct formation. Mechanistic studies in the mouse suggest that benzo[*a*]pyrene may act as an embryotoxic and teratogenic agent via generation of reactive oxygen species (Nicol *et al.*, 1995; Winn and Wells, 1997) .

There are few studies in rats suggesting embryolethality and developmental neurotoxicity from benzo[*a*]pyrene exposure during gestation. Hood and co-workers have investigated the effects of nose-only inhalational exposure to a benzo[*a*]pyrene:carbon black aerosol in the rat. Controls received carbon black only. Exposure to the aerosol at 100 μg/m^3 for 4h on day 15 of gestation resulted in augmentation of the developmental expression profile of the Sp1 gene, which is actively expressed in developing brain, in various regions of the brain in pups 3-15 days postnatally (Hood *et al.*, 2000). More recent work, published in abstract only (Nayyar *et al.*, 2001) has confirmed increased expression of Sp1 in pups from rats given a single oral gavage dose of 0.70 μg TCDD/kg bw on day 14 of gestation and/or inhalational exposure to benzo[a]pyrene:carbon black aerosol at 100 μg/m^3 of for 4h per day on days 14-17 of gestation. Inhalational exposure to benzo[*a*]pyrene:carbon black aerosol at 25, 75 or 100 μg/m^3 for 4h per day from days 10-19 of gestation caused dose-related reductions in embryonic survival of 27%, 62% and 69% respectively, associated with reductions in maternal plasma prolactin concentrations (Archibong *et al.*, 2001). Sp1 expression in the brain was increased in the pups from all treated groups at 3-20 days of age in association with raised brain tissue levels of the tetrahydro-benzo[*a*]pyrene metabolite (Hood *et al.*, 2001).

Naphthalene

Studies on naphthalene in mice are summarised in table 2.5.2. Administration of 300 mg/kg bw/day by oral gavage to pregnant CD-1 mice during organogenesis caused maternal toxicity (deaths and reduced weight gain) and reduced numbers of live offspring but no malformations (Plasterer *et al.*, 1985; Hardin *et al.*, 1987).

Naphthalene was administered orally by gavage at 50, 150, or 450 mg/kg bw per day to pregnant Sprague-Dawley rats on days 6-15 of gestation. The dams showed signs of toxicity including lethargy, slow breathing, prone body posture, and rooting, and these effects persisted after the end of dosing with the high dose. The bodyweight gain of treated animals was reduced by 31 and 53% at the two higher doses. Naphthalene did not induce developmental toxicity. The maternal NOAEL was < 50 mg/kg bw per day (National Toxicology Program, 1991). In a second study, doses of 0, 20, 80, or 120 mg/kg bw per day were given to rabbits by gavage during days 6-19 of gestation. There were no signs of maternal toxicity or developmental toxicity (National Toxicology Program, 1992). Administration of 395 mg/kg bw/day naphthalene ip to Sprague-Dawley rats on days 1-15 of gestation had no effects on mothers or fetuses (Hardin *et al.*, 1981).

2.5.5 Reproductive and developmental toxicity in humans

Since PAH nearly always occur in combination with other pollutants, there are few specific investigations on the reproductive or developmental effects of exposure to PAH in humans. The few studies on populations with high exposures to airborne pollutants, in which PAH exposure was assessed as one of the variables, are reviewed below. Epidemiological studies on cigarette smokers, who are exposed to many different smoke components including PAH, are not reviewed here since PAH have not been specifically identified as playing a causal role in the known effects of smoking in pregnancy.

Czech studies

An extensive investigation of the human health effects of airborne environmental pollutants has been conducted in the Czech Republic (Šrám, 2001). Teplice, a heavily polluted mining area in Northern Bohemia, was selected as a model district and the district of Prachatice in Southern Bohemia, an agricultural area, was selected for comparison. In the late 1980s the mining districts of Northern Bohemia were considered among the most heavily polluted in Europe. The potentially significant pollutants involved included particulate matter ($PM_{2.5}$ and PM_{10}), sulphur dioxide, oxides of nitrogen, metals, volatile organic compounds and PAH. Amelioration of pollution began in the early 1990s.

The impact of air pollution on fecundability and pregnancy outcome was studied between 1994 and 1999 (Dejmek *et al.*, 2001), based on self-administered maternal questionnaires and medical records on all singleton births in Teplice and Prachatice. Daily data on air pollution came from continuous monitoring of $PM_{2.5}$, PM_{10}, SO_2 and carcinogenic PAH. The proportion of couples conceiving in the first unprotected menstrual cycle was used as the measure of fecundability and infants below the 10^{th}

centile of birth weight for gestational age was used as a measure of intrauterine growth retardation (IUGR). A reduction in fecundability was found in association with increasing exposure to SO_2 in the second month before conception but there was no association with exposure to carcinogenic PAH. After adjustment for confounding variables, a significantly increased risk for IUGR was established for mothers from Teplice exposed to PM_{10} levels >40 $\mu g/m^3$ during the first month of gestation. A similar but weaker association was found for $PM_{2.5}$. No increased risk of IUGR was found in Prachatice in relation to PM_{10} or $PM_{2.5}$ concentrations. A highly significant and exposure-related response for IUGR was found in Teplice in association with exposure to carcinogenic PAH at medium or high concentrations during the first month of gestation; the adjusted Odds Ratios (OR) were 1.59 (95% Confidence Interval 1.06-2.39) for exposure to 15 to <30 ng/m^3 ($P<0.025$) and 2.15 (95% CI 1.27-3.63) for exposure to $\geq$30 ng/m^3 ($P<0.0043$). No significant associations were observed in later months of gestation. A similar relationship between IUGR and exposure to carcinogenic PAH in the first month of gestation was also seen in Prachatice, despite lower exposures to particulate matter.

In another study, enzyme activities were measured in human placental samples from Teplice and Prachatice (Machala *et al.*, 2001b). The activity of aromatase (CYP19), which converts androgens to oestrogens, was measured as a biomarker of possible endocrine disruption; placental-fetal production of oestrogens in humans is important in the maintenance of pregnancy and is an indicator of embryonic well-being. The activity of 7-ethoxyresorufin O-deethylase (EROD), a marker of CYP1A1 induction, is used as a biomarker of exposure to compounds interacting with AhR; activation of the AhR by dioxin-like compounds has been shown to suppress oestrogen receptor (ER)-mediated responses. This study showed reduced aromatase activity in placental samples from Teplice and an association between reduced aromatase and IUGR ($P<0.05$). EROD activity was increased in Teplice samples compared with Prachatice ($P<0.02$).

A study of neurobehaviour has also been conducted in children aged 7 years or older in Teplice, Prachatice and Znojmo (a district where natural gas rather than brown coal is used for heating and power generation) (Otto *et al.*, 2001). This showed poorer neurobehavioural performance in Teplice children. While the results suggest that *in utero* exposure to air pollutants may be associated with subsequent neurobehavioural impairment, it is not known which, if any, of the several air pollutants present might be involved.

Studies on human semen quality in Teplice and Prachatice showed an association between high levels of air pollution during the 90 days before sperm collection and increased percentage of abnormal sperm (morphology, head shape and chromatin structure) and reduced sperm motility (Perreault *et al.*, 2001). However, no analysis

has been undertaken as yet on whether the effects are related to any particular pollutant.

Polish studies

Whyatt and co-workers have published a series of studies on 70 mother-infant pairs from Krakow in Poland, a city with high air pollution including PAH, and 90 from Limanowa, an area with lower ambient pollution but greater indoor coal use. They measured PAH-DNA adduct formation in maternal and umbilical white blood cells. They found a dose-related increase in maternal and newborn PAH-DNA adduct levels with increasing ambient pollution at the women's place of residence in those who were not employed away from the home ($p \leq 0.05$) (Whyatt *et al.*, 1998).

They also investigated whether genetic differences in activation and detoxification capabilities may modify PAH-induced DNA damage in maternal and newborn white blood cells (Whyatt *et al.*, 2000). There was no association between maternal DNA adduct levels and polymorphisms of either the CYP1A1 enzyme or the glutathione S-transferase P1 enzyme (GSTP1). However, adduct levels were higher among newborns with the CYP1A1 *Msp*I restriction site (heterozygotes and homozygotes combined), which has been linked with increased enzyme activity, compared with newborns lacking the restriction site ($p=0.06$). Similarly, adduct levels were higher among GSTP1 *ile/val* and *ile/ile* newborns, compared with *val/val* newborns, the *val* allele having more catalytic efficiency towards PAH diol epoxides. The presence of both CYP1A1 *Msp*I and GSTP1 *ile/ile* gave a higher increase in adduct levels, than the presence of either alone in newborns.

The same group (Perera *et al.*, 1999) also found that the newborns with PAH-DNA adducts greater than the median value had significantly reduced birth weight ($p=0.05$), birth length ($p=0.02$) and head circumference ($p=0.0005$). The reduction in birth weight was 147 g in those with adducts above the median value compared with those below the median. These effects are known to be associated with maternal smoking and indeed birth weight and length in this study correlated significantly with newborn plasma cotinine levels. Thus there is no evidence that the effects were attributable to the PAH component of tobacco smoke.

Since exposure to cigarette smoke rather than ambient air pollution may be important in PAH-DNA adduct formation, Whyatt *et al.* (2001) also investigated in the same white blood cell samples the influence of cigarette smoking on adduct formation using plasma cotinine as a biomarker of exposure to cigarette smoke. There was a significant correlation between paired newborn/maternal samples for plasma cotinine and aromatic-DNA adduct levels but not PAH-DNA adduct levels. In 80 mother-newborn pairs for which the blood samples were drawn within 1 h of each other, levels of PAH-DNA adducts were significantly higher in the newborn than in the paired maternal sample ($p<0.05$). Since fetal exposures to PAH via blood are 10-fold

lower than paired maternal exposures, the authors suggest that there is reduced detoxification of PAH in the fetus and increased susceptibility of the fetus to DNA damage, which would have implications for risk assessment.

Ukrainian study

In a Ukrainian study, 7 different PAH (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenz[*ah*]anthracene, indeno[1,2,3-*cd*]pyrene) were measured in the placentas of 200 women from the general population living in two industrialised cities in the Ukraine, Kyiv and Dniprodzerzhinsk (Gladen *et al.*, 2000). All 7 PAH were detected in all placentas, at concentrations ranging from 0.06 - 13.05 ng/g dry weight, except for a single placenta in which benzo[*a*]pyrene was not found. Total PAH concentrations ranged from 2.16 - 43.50 ng/g dry weight. There was no relationship between mean birth weight and tertiles of concentrations of any individual PAH or total PAH. Adjustment for known predictors of birth weight did not affect this result.

US study

A US investigation, described by the authors as a pilot study, has used information on mothers and their pregnancy outcomes from 222,406 live birth certificates and 1,591 fetal death certificates from 1990 and 1991 combined with air quality data from the US Environmental Protection Agency Cumulative Exposure Project in New Jersey, a highly urban state (Vassilev *et al.*, 2001 a,b). Exposure categories were based on tertiles of modelled average "polycyclic organic matter" (POM) for each census tract in New Jersey. Plural births and births with chromosomal anomalies were excluded. After adjustment for confounders, ORs for small for gestational age births were significantly raised for the highest POM exposure tertiles for both term and pre-term births. The ORs for all births were 1.22, 95% CI 1.17-1.27 (individual-level confounders) and 1.13, 95% CI 1.07-1.18 (group-level confounders). Comparing highest with lowest tertiles, the ORs were 1.31 (95% CI 1.15-1.51) for very low birth weight, 1.19 (95% CI 1.02-1.39) for fetal death and 1.25 (95% CI 1.19-1.31) for premature birth.

2.5.6 Discussion and conclusions

There is limited or no evidence in animals on the reproductive toxicity of individual PAH, other than benzo[*a*]pyrene and naphthalene. In oral studies, benzo[*a*]pyrene was without effects on reproductive capacity in a single generation study in mice up to 133 mg/kg bw/day via the diet, but impaired fertility was seen in the offspring of female mice given $\geq$10 mg/kg bw/day by gavage. A NOAEL for this effect has not been established. A single, poorly reported study in the rat, in which 1000 ppm benzo[*a*]pyrene (c.50 mg/kg bw/day) was given in the diet, reported an effect on fertility. Intraperitoneal administration of benzo[*a*]pyrene resulted in toxicity to the

ovary (destruction of primordial oocytes, reduced ovarian weight). An oral study with acenaphthene has shown reduced ovarian weight at a high dose of 700 mg/kg bw/day.

There is clear evidence for developmental toxicity of benzo[*a*]pyrene in mice from oral and ip administration in the form of embryonic and fetal death, reduced fetal weight and malformations. The occurrence and extent of the developmental toxicity is dependent in part on maternal and fetal genotypes. In mice of a susceptible genotype 120 mg benzo[*a*]pyrene/kg bw/day via the diet was developmentally toxic. A NOAEL for the oral route has not been established. In the rat sc administration of benzo[a]pyrene caused fetal deaths and reduced fetal weight and inhalational administration of benzo[*a*]pyrene:carbon black aerosol at 25-100 μg/m^3 caused dose-related embryonic deaths. However, there are no oral developmental toxicity studies on benzo[*a*]pyrene in the rat. Naphthalene given orally was without any developmental toxic effects in well-reported studies in the rat and rabbit and was not teratogenic in the mouse.

Information on the possible effects of PAH in human pregnancy is very limited. A Czech study has reported an association between an increased incidence of intrauterine growth retardation and airborne exposure to high levels of PAH (>15 ng/m^3) (Dejmek *et al.*, 2001). However, a significant association was only present in the first month of gestation. This observation is difficult to reconcile with observations from other agents causing IUGR, such as cigarette smoke, which usually exert their main effects during the last trimester of pregnancy. A study from Poland also reported lower birth weight in association with levels of PAH-DNA adducts in cord blood above the median (Perera *et al.*, 1998). However, the findings on birth weight in this study were correlated with exposure to tobacco smoke and were not necessarily due to the PAH component of tobacco smoke. The Czech study findings also contrast with those of Gladen *et al.* (2000), who found no association between birth weight and placental tissue concentrations of 7 different PAH in 200 women from two cities in the Ukraine. A pilot study in the USA has indicated possible adverse effects of high exposure to polycyclic organic matter on birth weight, prematurity and fetal death, which the authors suggest should be further examined to identify specific hazardous air pollutants (Vassilev *et al.*, 2001 a,b). As with studies on cigarette smokers, evidence from these studies is difficult to interpret because of the possible or known co-exposure to other pollutants.

Thus the data from animals and humans are insufficient for risk assessment. Although adverse effects on reproduction and development in animals have generally been seen only at relatively high doses of benzo[*a*]pyrene (compared to doses inducing carcinogenic effects), the experimental database for oral risk assessment is sparse and NOAELs for reproductive and developmental effects have not been established.

Table 2.5.1 Effects of benzo[*a*]pyrene on fertility in experimental animals

Species (strain)	Sex/No. per group	Route of administration	Duration, dose	Effects	Reference
Mouse White	M 5	Diet	Up to 30 days before mating, 33, 67, or 133 mg/kg bw/ day	NOEL: 150 mg/kg bw per day Parameters: sperm in lumen of testes; number of offspring	Rigdon and Neal (1965)
Mouse White Swiss	F 5-65	Diet	20 days before mating 33, 67, or 133 mg/kg bw/ day	NOEL: 150 mg/kg bw per day Parameter: number of offspring	Rigdon and Neal (1965)
Mouse DBA/2N	F 15	Intraperitoneal	Day 14 before mating, 10, 100, 200, or 500 mg/kg bw once	10, 100 mg/kg bw: dose-dependent decrease in number of pups 200, 500 mg/kg bw: completely infertile; threshold: 3.4 mg/kg bw; 50% effect dose: 25.5 mg/kg bw	Mattison *et al.* (1980)
Mouse DB/2N	F	Intraperitoneal	Day 21 before sacrifice, 5, 10, 50, 100, or 500 mg/kg bw once	Dose-dependent increase in primordial oocyte destruction; 500 mg/kg: 100% destruction; threshold: 2.7 mg/kg bw; 50% effect dose: 24.5 mg/kg bw	Mattison *et al.* (1980)

Species (strain)	Sex/No. per group	Route of administration	Duration, dose	Effects	Reference
Mouse B6 and D2	F 5	Intraperitoneal	Day 13 before sacrifice, 100 mg/kg bw once	100 mg/kg bw: significant increase in primordial oocyte destruction in both genotypes; effects in B6 mice greater than in D2 mice	Mattison and Nightingale (1980)
Mouse C57BI/6N (B6), DBA/2N (D2), D2B6F_1 (F_1)	F	Intra-ovarian injection	Day 14 before sacrifice, 10 µg/right ovary once	10 µg: decreased ovarian weight (D2); decreased ovarian volume (D2 and F_1); decreased antral follicles (F_1); decreased number of small follicles (D2 and F_1)	Mattison *et al.* (1989)
Mouse C57BI/6N	F 5	Intraperitoneal	1, 2, 3, and 4 weeks before sacrifice; 1, 5, 10, 50, 100, or 500 mg/kg bw	500 mg/kg: 35% mortality 1-500 mg/kg bw: dose- and time-dependent decrease in ovarian volume, total volume and number of corpora lutea/ovary (for last parameter, after 1 week threshold was about 1 mg/kg bw and ED_{50} 1.6 mg/kg bw); effect transitory in low-dose groups, but not reversible in two highest by four weeks	Swartz and Mattison, (1985); Miller *et al.* (1992)

Table 2.5.2 Developmental toxicity of PAH in experimental animals

Species (strain)	No. per group	Route of administration	Duration, dose	Effects	Reference
Anthracene					
Rat Sprague-Dawley		Gavage	GD 19 60 mg/kg bw	No induction of Benzo[a]pyrene-hydroxylase in fetal liver compared with controls	Welch *et al.* (1972)
Benz[a]anthracene					
Rat	2	Subcutaneous	GD 1-11 or 1-15 5 mg/animal/day	Day 10 and 12: severe maternal vaginal haemorrhage; Day 14: intraplacental haemorrhage; fetal death and resorption up to day 18	Wolfe and Bryan (1939)
Rat Sprague-Dawley		Gavage	GD 19 60 mg/kg bw	Induction of Benzo[a]pyrene-hydroxylase in fetal liver	Welch *et al.* (1972)
Benzo[a]pyrene					
Mouse, White Swiss	9	Diet	GD 5 or 10 until delivery 150 mg/kg bw	No malformations	Rigdon and Neal (1965)
Mouse C57Bl/6N, AKR/J, and back-crosses (reciprocal)	6-17	Diet	GD 2-10 120 mg/ kg bw/day	Increased intrauterine toxicity and malformations in Ah^d/Ah^d embryos compared with Ah^b/Ah^d embryos in pregnant Ah^d/Ah^d mice (effect not seen in pregnant Ah^b/Ah^d mice)	Legraverend *et al.* (1984)

Species (strain)	No. per group	Route of administration	Duration, dose	Effects	Reference
Benzo[a]pyrene (cont'd)					
Mouse C57Bl/6, AKR and back-crosses (reciprocal)	5-30	Intraperitoneal	GD 7, 10, or 12 50-300 mg/kg bw	200 mg/kg bw: increase in stillbirths, resorptions, malformations (4-fold higher in pregnant C57Bl than in AKR mice)	Shum *et al.* (1979)
Mouse C57Bl/6, DBA/2, and back-crosses (reciprocal)	20	Intraperitoneal	GD 8 150 or 300 mg/kg	150 and 300 mg/kg bw: increased fetal mortality (except DBA/2 × DBA/2 offspring); reduced fetal body weight; increased number of cervical ribs 300 mg/kg: increased malformations (C57Bl/6 × C57Bl/6)	Hoshino *et al.* (1981)
Mouse CD1		Gavage	GD 7-16 10, 40, 160 mg/kg bw/day	No maternal or embryofetal toxicity	MacKenzie and Angevine (1981)
Rat	17	Subcutaneous	GD 1-11 or 16 5 mg/animal/day	Days 10 and 12: profuse maternal vaginal haemorrhage; Day 14: intraplacental haemorrhage; fetal death and resorption up to day 18	Wolfe and Bryan (1939)
Rat Sprague-Dawley		Gavage	GD 19 60 mg/kg	Induction of Benzo[a]pyrene-hydroxylase in fetal liver	Welch *et al.* (1972)

Species (strain)	No. per group	Route of administration	Duration, dose	Effects	Reference
Benzo[a]pyrene (cont'd)					
Rat Sprague-Dawley	10-15	Subcutaneous	GD 6-8 or 6-11 50 mg/kg bw/day	Significant increase in resorptions and fetal wastage (dead fetuses plus resorption); fetal weight reduced	Bui *et al.* (1986)
Chrysene					
Rat Sprague-Dawley		Gavage	GD 19 60 mg/kg bw	Induction of Benzo[a]pyrene-hydroxylase in fetal liver	Welch *et al.* (1972)
Dibenzo[a,h]anthracene					
Rat Sprague-Dawley		Gavage	GD 19 60 mg/kg bw	Induction of Benzo[a]pyrene-hydroxylase in fetal liver	Welch *et al.* (1972)
Rat	38	Subcutaneous	GD 1-8 or 1-18 5 mg/animal/day	Days 10 and 12: profuse maternal vaginal haemorrhage; Day 14: intraplacental haemorrhage fetal death and resorption up to day 18	Wolfe and Bryan (1939)
Naphthalene					
Mouse CD-1	50	Gavage	GD 7-14 300 mg/kg bw/day	Significant increase in maternal mortality, significant reduction in weight gain Significant reduction in live offspring, no malformations	Plasterer *et al.* (1985)

Species (strain)	No. per group	Route of administration	Duration, dose	Effects	Reference
Naphthalene (cont'd)					
Mouse CD-1		Gavage	GD 6-13 300 mg/kg bw/day	Increased maternal mortality, significant reduction in weight gain Significant reduction in liveborns per litter	Hardin *et al.* (1987)
Rat Sprague-Dawley	10-15	Intraperitoneal	GD 1-15 395 mg/kg/day	No maternal or embryofetal toxicity	Hardin *et al.* (1981)

GD = Gestation Day

Table 2.5.3 Effects of benzo[*a*]pyrene on postnatal development in experimental animals

Species (strain)	Sex/No per group	Route of Administration	Duration, dose	Effects	Reference
Mouse non-inbred	F	Dermal	Entire gestation period 1 drop of 0.5% solution, twice per week; F_1-F_4 treated with Benzo[a]pyrene, m 1x/week, f 2x/week	F_1- F_4: sensitisation of offspring: increased incidence of papillomas and carcinomas in all generations compared with animals not treated *in utero*	Andrianova (1971)
Mouse C3H/Anf	F 25	Intraperitoneal	GD 11-13 or 16-18 100 or 150 mg/kg bw	F_1: no difference in birth rate, litter size of progeny compared to controls; severe suppression of anti-SRBC PFC response up to 78 weeks of life; 11-1 fold increase in tumour incidence (liver, lung, ovaries) after 56-78 weeks	Urso and Gengozian (1980)
Mouse CD-1	F	Gavage	GD 7-16 10, 40, 160 mg/kg bw/day	F_1: 10 mg/kg markedly impaired fertility (by 20%) and reduced testis weight (by 40%), 34% sterility of females; 40 and 160 mg/kg: fertility impaired by 90 - 100%; testis weight reduced by > 80%; 100% sterility of females	MacKenzie and Angevine (1981)

anti-SRBC PFC, anti-sheep red blood cell antibody (plaque)-forming cells
GD = Gestation Day

2.6 Effects of PAH on the immune system

The immunotoxic effects of PAH have been investigated for many years (Malmgren *et al.*, 1952). The majority of the reports that are quoted and discussed hereafter have been published after the IPCS evaluation of PAH (IPCS, 1998). Whatever the route of exposure, the resulting effects have been considered mostly at the systemic level, very few studies have looked for alterations of the local gut immune system.

Immunosuppression is associated with an increased susceptibility of the exposed individuals to the development of cancers or of infectious diseases, whereas immunopotentiation results in an increased secretion of cytokines by immune cells, thus leading to inflammation. This may in turn, and under specific circumstances, facilitate tumour development, expression of hypersensitivity (allergy, contact hypersensitivity) or auto immunity. Depending on various parameters in the design of the protocol such as route of exposure, end point, high or low level of dosage, model used, immunosuppression or immunopotentiation can be observed (Burchiel and Luster, 2001). However, in the available literature, immunosuppression is the most frequent effect reported after exposure to PAH, immunopotentiation being only reported to occur after either atmospheric or topic exposure, or by using in vitro systems.

Most of the reports published in the literature have used either subcutaneous and intraperitoneal injection or inhalation, as the route of exposure. Immunotoxicity resulting from ingestion of PAH in contaminated food can be considered by taking into account only the experimental studies in which immunotoxicity have been evaluated after oral intake of a diet contaminated with PAH. Alternatively, given the possible links between the various local mucous immune systems, it can be of some interest to consider alterations of the pulmonary immune system that have been observed after inhalation for assessing the possible impact of ingested PAH on the gut immune system. In addition, because it has been reported that some PAH when taken into the diet may induce DNA adducts in the lungs, translocations from one organ to another may result in “at distance” effects. Finally, it must be noted that most of the immunotoxic effects that are reported for PAH are not thought to be due to parent compounds but rather to their reactive epoxide metabolites (Davila *et al.*, 1996; Miyata *et al.*, 2001; Pessah *et al.*, 2001).

2.6.1 Immunosuppressive effects possibly leading to either increased susceptibility to cancer or infectious diseases

Oral exposure to 7,12-dimethylbenz[*a*]anthracene (gavage with 50 to 150 mg/kg bw in corn oil) induced suppression of the gut-associated and splenic mitogen responsiveness of lymphoid cells in female B6C3F1 mice. The total administered dose of 7,12-dimethylbenz[*a*]anthracene seems to be a more important determinant of immunotoxicity than the length of exposure (Davies *et al.*, 1991).

In the absence of any clinical evidence of toxicity, benzo[*a*]pyrene treatment (50 or 100 mg/kg bw per day for 5 days by intraperitoneal injection) of female B6C3F1 mice induced a reduction in the thymic cellularity as well as an alteration of thymocyte differentiation, a reduced cellularity of the bone marrow was also noted (Holladay and Smith, 1995a).

The immunosuppressive (to T-cell-dependent and –independent antigens) effect of a mixture of 17 congeners including PAH with 2 (indan, naphthalene, 1- and 2-methylnaphtalene), 3 (acenaphtylene, acenaphtene, dibenzofuran, flmuorene, phenantrene and anthracene), or 4 or more rings (pyrene, fluoranthene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene) were studied after administration to B6C3F1 mice. Female mice were gavaged with 0 to 312 mg/kg of the 17 congeners mixture. Immunotoxicity was assessed by measuring the effects of the substances on splenocyte responses to sheep red blood cells (a T-cell dependent antigen) and trinitrophenyl-lipopolysaccharide (a T-cell independent antigen) using the plaque forming cell (PFC) assay. Both PFC responses were inhibited, serum anti TNP IgM titers were also reduced. PAH with 4 rings or more were primarily responsible for the effect. (Harper *et al.*, 1996).

Treatment of male Wistar rats by gavage for 35 days with 3, 10, 30, or 90 mg/kg bw benzo[*a*]pyrene (extended OECD 407 protocol) induced various immunotoxic effects such as decrease in thymus and lymph nodes weights, decreased absolute and relative B cell numbers in the spleen, decreased RBC and WBC numbers. Decreased serum IgM and IgA levels were noted after treatment of the animals with 30 and 90 mg/kg, respectively. The highest dose of treatment resulted in a significant decrease of NK cells activity in the spleen. Thymus weight changed and spleen B-cell populations were affected at a dose of 10mg/kg, a level where no overt toxicity was noted (De Jong *et al.*, 1999).

2.6.2 Mechanistic studies

Two main mechanisms are usually suggested as promoting immunosuppression. One involve the reactivity of PAH with the Ah receptor and the other their capacity to modulate the intra cellular calcium concentration of immune cells. In any case, antigen and mitogen receptor signaling pathways are altered (Burchiel and Luster, 2001) leading to proliferation and/or death (apoptosis) of immune cells.

Role of the Aromatic Hydrocarbon Receptor (AhR)
Although broadly recognized, the importance of the role of the AhR may vary given the PAH in cause. As an example, it has been shown that bone marrow stromal cell CYP1B1 metabolism of 7,12-dimethylbenz[*a*]anthracene, but not AhR activation is required for 7,12-dimethylbenz[*a*]anthracene-induced pre B-cell apoptosis (Heidel *et al.*, 1999).

As it is the case with dioxins, the immunosuppressive effect of PAH is dependent on their capacity to bind the Aromatic Hydrocarbon Receptor (Silkworth *et al.*, 1984; Silkworth *et al.*, 1995; Quadri *et al.*, 2000). As controlled by the AhR, immunotoxicity of PAH can be attributed to induction of apoptosis in bone marrow derived pre-B cells (Near *et al.*, 1999). This apoptosis closely resembles the process by which developing immune systems are purged of pathologic autoimmune lymphocytes. (Mann *et al.*, 1999). Using a bone marrow derived pre-B cell line (BU-11) cultured on a monolayer of the AhR+ bone marrow derived stromal cell line (BMS2), it has been shown that pre B-cell death is controlled by the Aryl hydrocarbon receptor/transcription factor (AhR) and/or genes regulated by the AhR (Near, *et al.*, 1999).

In a mitogenesis assay human peripheral blood T lymphocytes, benzo[*a*]pyrene, 7,12-dimethylbenz[*a*]anthracene and 3-methylcholanthrene were the most effective in suppressing T-cell proliferation; dibenz[*a,c*]anthracene and dibenz[*a,h*]anthracene were of intermediate toxicity, 9,10-dimethylanthracene, benzo[*e*]pyrene and benz[*a*]anthracene were mildly immunotoxic and anthracene not immunotoxic at the concentrations tested. Using the same assay system, alpha-naphthoflavone, an Ah receptor antagonist and an inhibitor of cytochrome P-450 was able to block the immunosuppressive effect of benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene but not 3-methylcholanthrene which indicated a different mechanism of action (Davila *et al.*, 1996)

Role of Ca^{++}
Intracellular Ca^{++}-concentration signaling is often associated with cell proliferation and cell death (apoptosis) in lymphocytes (Krieger *et al.*, 1994; Davila *et al.*, 1995). It should be noted that alterations in calcium mobilization have mostly been observed in in vitro studies.

When added at a dose of 10micromolar for 16 min. in the culture medium, various PAH have been shown to increase the cytosolic Ca^{++} concentration in the HPB-ALL human T-cell line. Protein tyrosine kinase activation may partially explain the increase in Ca^{++} mobilization induced by PAH. The most immunosuppressive PAH (7,12-dimethylbenz[*a*]anthracene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and 9,10-dimethylanthracene produced a sustained increase in intracellular Ca^{++}, whereas PAH with moderate to minimal immunosuppressive properties (dibenz[*a,c*]anthracene, benz[*a*]anthracene, benzo[*e*]pyrene and anthracene) produced small to transient Ca^{++} increase in the HPB-ALL human cell line. (Krieger *et al.*, 1994). A similar observation has been reported with human peripheral blood mononuclear cells (Mounho *et al.*, 1997).

In vitro metabolic bioactivation of benzo[*a*]pyrene is important in its capacity to mediate disruption of Ca^{++} homeostasis through mediating ryanodine receptor dysfunction, the effect being mostly associated with the o-quinone metabolite benzo[*a*]pyrene-7,8-dione (Pessah *et al.*, 2001).

2.6.3 Immunological effects observed in offsprings after treatment in utero

Benzo[*a*]pyrene given to pregnant rats on day 15 of gestation caused a relative decrease in the number but not in the binding affinity of thymic glucocorticoïds receptors in the offsprings. This suggests binding to the pre-encoded hormone receptors and interference with receptor maturation (Csaba and Inczefi-Gonda, 1992).

Fetus from pregnant mice injected intra-peritoneally with 7,12-dimethylbenz[*a*]anthracene at 10 or 25 mg/kg daily on gestational days 13 to 17 were collected on day 18. Significant thymic atrophy was noted and fetal liver cellularity was reduced to 80 and 49% of control. The percentage of liver cells expressing CD44 antigen was reduced by 77% of control by treatment with 25 mg 7,12-dimethylbenz[*a*]anthracene. The percentage of Mac-1+ and CD45+ fetal liver cells was reduced at both 10 and 25 mg/kg dose level by 61 and 50% and 45 and 40%, respectively (Holladay and Smith, 1995b).

Progeny from benzo[*a*]pyrene exposed (150 µg/g bw) primiparous mice injected during the second trimester of pregnancy may be immunologically compromised as a consequence of the profound alterations that are observed in the maternal T-cell populations in the thymus and the spleen.

In mice, progeny from benzo[*a*]pyrene exposed (150 mg/kg bw) primiparous mothers, injected during the second trimester of pregnancy, were severely compromised immunologically. Immunodeficiency (abnormalities in the T cell-mediated responses caused

by disruption of T cell differentiation) occurred early after birth (1 week) and persisted for 18 months (Urso and Johnson, 1987; Urso *et al.*, 1992; 1994). After 12-18 months the progeny developed high incidences of hepatomas, lung adenomas and adenocarcinomas, reproductive tumours, and lymphoreticular tumours (Urso and Gengozian, 1980; 1982; 1984; Urso and Johnson, 1988; Rodriguez *et al.*, 1999, 2000). When benzo[*a*]pyrene was administered postnatally (after 1 week) both immune suppression and tumour incidence were substantially lower (Urso and Gengozian, 1982). Benzo[*a*]pyrene-induced splenic disruption could be due to changes in the differentiation potential of T precursor cells (Urso and Johnson, 1988; Rodriguez *et al.*, 1999 and 2000). Mice exposed in utero to benzo[*a*]pyrene have a persistent defective T-cell differentiation which results in an abnormal T-cell behaviour possibly leading to a defective recognition of antigens (Urso *et al.*, 1994).

Whether prenatal exposure to certain immunotoxicants such as PAH may play a role in postnatal expression of autoimmunity in humans is still discussed (Holladay, 2001).

2.6.4 Observations in humans

The status of humoral immunity has been evaluated in 274 male workers in Poland including 199 coke oven workers and 76 cold-rolling mill workers. Coke oven workers were exposed chronically to complex mixtures of air pollutants composed primarily of PAH. Exposure was 3-5 magnitudes higher in the coke oven group than in the cold-rolling one, it ranged from 0.2 to 50 µg benzo[*a*]pyrene/m^3. As compared to the cold-rolling mill employees, a marked depression of mean IgG and IgA was observed in the coke oven group together with a trend to decreased IgM and increased IgE values (Szceklik *et al.*, 1994).

In an other study conducted on 24 male coke oven workers compared to 20 healthy workers without any known history of exposure to PAH, a series of immunological tests were carried out on blood samples. The total dust exposure ranged from 3.0 to 52.2 µg/m^3 of dust, PAH most present were phenanthrene, fluoranthene, benzo[*b*]fluoranthene, benzo[*e*]pyrene and indeno1,2,3-*c,d*]pyrene. The alterations found were significant but slight. They include: decreased mitogenic reaction of T-cells to phytohemagglutinin, reduced expression of the interleukin-2 receptor, impairment of B-cell activity (decreased proliferation and low synthesis rate of IgM) and a decreased oxidative burst in monocytes after challenge. No significant differences were observed between the number of lymphocytic subpopulations in peripheral blood and in immunoglobulin levels of serum (Winker *et al.*, 1997).

In a report on 16 male workers employed in road paving operations, PAH exposure was assessed using urinary 1-hydroxy-pyrene levels; it was found to be significantly higher than in 12 unexposed male controls. The $CD4^+$ percentage and the $CD4^+/CD8^+$ ratio were significantly higher in the PAH exposed group, the percentages of $CD8^+$ and $CD19^+$ cells being unchanged. A significant enhancement in serum IgM levels and the percentage of monocytes was noted in the workers compared to the control group (Karakaya *et al.*, 1999).

2.6.5 Immunostimulation leading to allergy or contact hypersensitivity

Exposure to low doses of PAH may augmenT-cell signaling pathways, resulting in immune enhancement or an adjuvant effect (Burchiel and Luster, 2001). The metabolic status of the host seems to be a key determinant of individual susceptibility to the development of allergic contact dermatitis. Contact hypersensitivity to 7,12-dimethylbenz[*a*]anthracene was observed only in strains of mice that are capable to metabolize the compound, inhibitors of PAH metabolism reduced DMBA contact hypersensitivity. Depletion of CD8 but not CD4 T-cell populations inhibited contact sensitivity to 7,12-dimethylbenz[*a*]anthracene. It was also observed that among PAH, only those capable to induce aryl hydrocarbon hydroxylase were immunogenic (Anderson *et al.*, 1995).

Pyrene, anthracene, fluoranthene and benzo[*a*]pyrene when injected intraperitoneally with the allergen have an adjuvant activities on the production of anti IgE antibodies to ovalbumin and Japanese cedar pollen allergen in mice (Suzuki *et al.*, 1993).

Diesel exhaust particulates enhance eosinophil adhesion to nasal epithelial cells and cause degranulation (Terada *et al.*, 1997) and enhanced human IgE production (Takaneka *et al.*, 1995). Diesel exhaust particles, their organic extract (in methylene chloride) and the core carbon particles co injected with ovalbumin in mice induced a rapid and marked elevation of albumin specific IgE, IgG1 and also IgG2a compared with albumin alone. Phenantrene (the most prevalent PAH in diesel exhaust particles) and anthracene are both capable of enhancing IGE and IgG 1 production (Heo *et al.*, 2001).

Organic extracts from diesel exhausts enhances mRNA expression and the production by peripheral blood mononuclear cells from healthy subjects of various chemokines involved in the recruitment of inflammatory cells (IL-8, MCP-1, and RANTES) (Fahy *et al.*, 1999).

In vitro treatment of human T-cells with pyrene induces the transcription of IL-4 messenger RNA and expression of IL-4 a potent mediator in allergic diseases in primary human T-cells. Pyrene but not related PAH enhanced basal transcription of the human and mouse IL-4 promoter (Bommel *et al.*, 2000).

2.7 Genotoxicity of PAH

2.7.1 Introduction

Polycyclic aromatic hydrocarbons undergo multiple metabolic transformations which may lead to the formation of electrophilic derivatives (e.g. diolepoxides, quinones, conjugated hydroxyalkyl derivatives, see section 6.4) capable of covalent interaction with nucleophilic centers of macromolecules. The mechanism of mutagenicity of PAH has been mainly investigated using benzo[*a*]pyrene and benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BaPDE) as model compounds. Binding of benzo[*a*]pyrene and BaPDE to nucleic acids mainly occurres at exocyclic amino groups of purines (Meehan *et al.*, 1977). The mutational spectrum induced by BaPDE in bacteria shows a prevalence of G>T transversions, due to the rotation of adducted guanine to *syn* conformation and its pairing with adenine (Eisenstadt *et al.*, 1982). A similar spectrum of base-pair substitutions is induced by BaPDE in mammalian cells *in vitro* (Keohavong and Thilly, 1992; Yang *et al.*, 1999), and by benzo[*a*]pyrene *in vivo* in transgenic mice (Kohler *et al.*, 1991; Miller *et al.*, 2000) and in the *Ha-ras* oncogene in mouse skin tumours (Wei *et al.*, 1999; Prahalad *et al.*, 1997). Molecular analysis of p53 mutations in lung cancers of smokers shows a similar prevalence of G>T transversions (Hainaut and Pfeifer, 2001), possibly reflecting the contribution of PAH to tobacco smoke carcinogenesis. In addition to base pair substitutions, bulky adducts of PAH to DNA bases can induce frameshift mutations, deletions, S-phase arrest, strand breakage and a variety of chromosomal alterations.

2.7.2 Genotoxicity of selected polycyclic aromatic compounds

The genotoxic profiles of the 33 non-heterocyclic polycyclic aromatic compounds considered by IPCS in 1998 have been updated and reevaluated on the basis of literature data available at the Environmental Mutagen Information Center[2].

Qualitative results obtained in *in vitro* and *in vivo* assays addressing different end-points are summarized in table 2.7.1. Overall evaluations on genotoxicity of individual compounds are listed in table 2.7.2. For classification, the same criteria used under the Directive 91/325/EEC (Labelling Guide) were applied herein. In practice, only PAH with evidence of mutagenicity/genotoxicity (e.g. DNA binding) *in vivo* were evaluated as established genotoxic agents.

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[2] Available on internet at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?EMIC.

Fifteen compounds (namely benz[*a*]anthracene, benzo[*b*]-, benzo[*j*]- and benzo[*k*]-fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]-, dibenzo[*a,h*]-, dibenzo[*a,i*]-, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene and 5-methylchrysene) met this criteria and were classified as "genotoxic" (table 2.7.2). These PAH may represent a priority group for the assessment of the risks of genotoxic and carcinogenic effects.

Concerning the induction of germ cell effects, benzo[*a*]pyrene, benzo[*a*]anthracene and chrysene gave positive results in chromosome aberrations and/or dominant lethals in rodents. No quantitative estimation of the genetic risk associated to PAH exposure can be done on the basis of such data, which do not concern transmissible effects. However, the relatively high dosages required to elicit a significant positive response in these assays (in the order of hundreds of mg/kg bw) suggest that the risk of transmissible effects due to the dietary intake of minute amounts of PAH is low.

For other six compounds (anthranthene, benzo[*ghi*]fluoranthene, benzo[*c*]phenanthrene, 1-methylphenanthrene, perylene, triphenylene) the evidence of genotoxicity is limited and mainly based on results obtained in *in vitro* systems. Further studies, especially *in vivo*, are warranted to clarify the genotoxic potential of these PAH.

Equivocal or contradictory data are available for another eight compounds (acenaphthene, acenaphthylene, benzo[*b*]fluorene, benzo[[pyrene, coronene, fluoranthene, fluorene, phenanthrene), which cannot be properly evaluated for genotoxicity.

Finally, four compounds (anthracene, benzo[*a*]fluorene, naphthalene, pyrene) gave totally or mainly negative results in a variety of short term tests. Although the strength of the supporting evidence is variable, in first instance these four PAH can be considered as not genotoxic or to pose a relatively minor risk of irreversible effects.

2.7.3 Mutagenicity of PAH mixtures

PAH contaminated foodstuffs have not been directly evaluated in mutagenicity studies, possibly because of practical problems related to their occurrence in relatively tiny amounts. On the other hand, numerous mutagenicity studies have been carried out on environmental samples containing complex PAH mixtures, such as air particulate, combustion emissions and water sediment extracts. However, in only a few cases has the mutagenicity of crude PAH fractions been correlated to the content of genotoxic PAH. In coal gasification residues, for example, both increasing (Donelly *et al.*, 1996; Booth *et al.*, 1998) and decreasing (Donnelly *et al.*, 1993; Randerath *et al.*, 1999) levels of genotoxicity

with increasing benzo[*a*]pyrene (or benzo[*a*]pyrene-equivalents) content have been observed, highlighting the difficulties in the toxicological evaluation of complex mixtures, where the final genotoxic effect may be modulated by toxic or inhibitory components present in the mixture. For this reason, caution should be used in the quantitative comparison of genotoxic potencies of different PAH mixtures to extrapolate cancer risks according to the so-called comparative potency approach (Lewtas, 1988).

2.7.4 Relationship of genotoxicity with carcinogenicity

Several experimental data show a mechanistic link between DNA adducts formation, mutations, and cancer outcome following PAH exposure (You *et al.*, 1994; Nesnow *et al.*, 1995; 1998). Also the evidences of genotoxicity and carcinogenicity of the compounds considered in this report show considerable overlapping (table 2.7.3), highlighting the pivotal role of genetic alterations in the mechanism of carcinogenicity of PAH. However, even though genotoxicity is a most plausible mechanism for PAH carcinogenicity, the correlation between DNA adduct, mutagenesis and carcinogenesis of PAH is not straightforward. In fact, comparable levels of DNA adducts (Goldstein *et al.*, 1998) and gene mutations (Hakura *et al.*, 2000) have been detected in tumour target and non target tissues of mice treated with benzo[*a*]pyrene, suggesting that genotoxic end-points alone may not adequately predict tumour outcome due to organ/tissue-specific mechanisms. Moreover, the carcinogenicity of PAH is modulated by the promoting activity of parent compounds which may play a major role, at least at the high doses applied in cancer bioassays. Therefore, mutagenicity/genotoxicity data should be used with caution in cancer risk characterization. On the other hand, it is conceivable that at the low doses corresponding to PAH uptake from air or food , the initiating effect of PAH may be more important than the promoting activity, and that in vivo mutagenicity data might be modelled to estimate cancer risk (Helleberg *et al.*, 2001).

Table 2.7.1 Synopsis of genotoxicity test results with selected polycyclic aromatic hydrocarbons

common name (CAS no.)	gene mutation in bacteria	DNA damage/ repair *in vitro*	genotoxicity in lower eukaryotes	gene mutation in mammalian cells *in vitro*	*in vitro* cytogenetics in mammalian cells	DNA binding in mammalian cells *in vitro*	DNA binding *in vivo*	mutagenicity/ genotoxicity *in vivo*
acenaphthene (83-32-9)	pos [a,b] (1, 2, 3)	neg [d] (4)				neg [c] (1)		
acenaphthylene (208-96-8)	neg [a] (1, 3) pos [b] (1)							
anthanthrene (191-26-4)	pos [a,b] (1)	pos [d,e] (1)						
anthracene (120-12-7)	mainly neg [a] (1, 5)	neg [d] (1)	neg [f,g, j, n] (1) inconcl [i] (1) neg [m] (6) pos [m] (7)	mainly neg [o,p,q,r] (1)	neg [s, t,] (1) neg [u] (8, 9)		neg (1)	neg [w,y] (1)
benz[*a*]anthracene (56-55-3)	pos [a, b] (1)	pos [d, e] (1)	pos [i] (1) neg [j] (1) pos [l, m, n] (1) neg [m] (6, 11)	mainly pos [o, p] (1, 12)	inconclusive [t u] (1, 13)	pos [c] (1, 10)	pos (1)	pos [w, x] (1)
benzo[*b*]fluoranthene (205-99-2)	pos [a] (1)	pos [d] (1)		neg [o] (1) weakly pos [p] (12)		pos [c] (1, 10)	pos (1, 14-16)	pos [w] (1)
benzo[*j*]fluoranthene (205-82-3)	pos [a, b] (1)	pos [d] (1)		weakly pos [p] (12)		pos [c] (1)	pos (1)	
benzo[*k*]fluoranthene (207-08-9)	pos [a] (1)			weakly pos [p] (12)		pos [c] (1)	pos (1)	
benzo[*ghi*]fluoranthene (203-12-3)	pos [a] (1) pos [b] (17)	pos [d] (1)		neg [p] (12)				
benzo[*a*]fluorene (238-84-6)	neg [a] (18, 19)	neg [d] (20)		neg [p] (12)				

common name (CAS no.)	gene mutation in bacteria	DNA damage/ repair *in vitro*	genotoxicity in lower eukaryotes	gene mutation in mammalian cells *in vitro*	*in vitro* cytogenetics in mammalian cells	DNA binding in mammalian cells *in vitro*	DNA binding *in vivo*	mutagenicity/ genotoxicity *in vivo*
benzo[*b*]fluorene (243-17-4)	pos/neg [a] (1) pos [b] (1)			neg [p] (12)				
benzo[*ghi*]perylene (191-24-2)	pos [a, b] (1)	pos [d] (1)		weak pos [p] (12)			pos (1, 21)	
benzo[*c*]phenanthrene (195-19-7)	pos [a] (1)	pos [d] (22)	pos [p] (12)				weak pos (22)	
benzo[*a*]pyrene (50-32-8)	pos [a, b] (1) neg [i] (1)	pos [d, e] (1)	pos [g, m, n] (1) neg [f, j, l] (1)	pos [o, p, q, r] (1)	pos [s, t, u] (1)	pos [c] (1)	pos (1)	pos [v, w, y, z] (1) neg [x] (1)
benzo[*e*]pyrene (192-97-2)	pos/neg [a] (1) neg [i] (1)	weak pos/neg [d] (1)	neg [n] (1)	pos [p] (1, 12) neg [o, q] (1)	neg [s, t] (1) neg [u] (8)		inconclusive (1)	pos/neg [w] (1)
chrysene (218-01-9)	pos [a, b] (1, 23) neg [i] (1)	pos [d] (1, 24) neg [e] (1)	neg [f, j] (1) neg [m] (25)	pos [d] (1, 12) neg [o, r] (1)	weak pos [t] (26)	pos [c] (1)	pos (1)	pos [w, x] (1)
coronene (191-07-1)	pos [a] (1) neg [b] (1)	neg [d] (1)		neg [p] (12)				
cyclopenta[*cd*]pyrene (27208-37-3)	pos [a, b] (1)			pos [o,p] (1, 12, 27, 28)	pos [t] (1)	pos [c] (1)	pos (29-31)	
dibenz[*a,h*]anthracene (53-70-3)	pos [a, b] (1)	pos [d, e] (1)	neg [f] (1) neg [m] (6)	pos [o] (1) pos [p] (12)	pos [u] (9)	pos [c] (1, 10)	pos (1, 31)	pos [w] (1, 32, 33)
dibenzo[*a,e*]pyrene (192-65-4)	pos [a] (1) pos [b] (34)			pos [p] (12, 34)			pos (1)	
dibenzo[*a,h*]pyrene (189-64-0)	pos [a] (1) neg [b] (34)	pos [d] (1)		pos [p] (34)	pos [t] (1)		pos (35)	
dibenzo[*a,i*]pyrene (189-55-9)	pos [a] (1) pos [b] (34)	pos [d] (1) neg [e] (1)		weak pos [p] (34)			pos (1)	pos [w] (1)

common name (CAS no.)	gene mutation in bacteria	DNA damage/ repair *in vitro*	genotoxicity in lower eukaryotes	gene mutation in mammalian cells *in vitro*	*in vitro* cytogenetics in mammalian cells	DNA binding in mammalian cells *in vitro*	DNA binding *in vivo*	mutagenicity/ genotoxicity *in vivo*
dibenzo[*a,l*]pyrene (191-30-0)	pos [a] (1) pos [b] (34)	pos [d] (1)		pos [p] (12, 34)		pos [c] (36)	pos (37, 38)	
fluoranthene (206-44-0)	mainly pos [a] (1) pos [b] (1)	mainly neg [d] (1) pos [e] (39)		mainly neg [p] (1, 12) pos [o] (1, 39)	pos [t] (1, 39)		pos (40)	neg [v, w] (1, 41)
fluorene (86-73-7)	neg [a, b] (1)	neg [d] (4) neg e (1)	neg [n] (1)	neg/pos [p] (1, 42)	pos [s] (1)	pos [c] (1)		
indeno[1,2,3-*cd*]pyrene (193-39-5)	pos [a] (1)	pos [d] (1)		weak pos [p] (12)			pos (1, 43)	
5-methylchrysene (3697-24-3)	pos [a] (44)			weak pos [p] (12)			pos (1, 15, 31, 45, 46)	
1-methylphenathrene (832-69-9)	pos [a b] (1)	pos [e] (1)		pos [o] (1) pos [p] (12)		pos [c] (1)		
naphthalene (91-20-3)	neg [a, b] (1)	neg [d] (1)	pos [m] (7)		pos [s] (1)			neg [w] (1)
phenanthrene (85-01-8)	pos/neg [a] (1)	pos/neg [d] (1)	neg [f, j] (1) neg [m] (7)	pos/neg [p] (1, 12) neg [o, q] (1)	neg [t] (1) mainly neg [s] (1) neg [u] (1)			neg/margilally pos [w] (1)
pyrene (129-00-0)	pos/neg [a] (1) pos [b] (1) neg [i] (48)	neg [d] (1) mainly neg [e] (1)	neg [g, h, j] (1, 48) neg [l, m] (6, 48)	neg [o] (1, 47) mainly neg [p] (1, 12, 47) pos [q] (1)	mainly neg [s, t] (1) neg [u] (1, 8, 9)		neg (1, 15)	neg [v, w, y, z] (1, 48)
triphenylene (217-59-4)	pos [a] (1)	pos [d] (1)		pos [p] (1, 12)		pos [c] (1)		

Notes table 2.7.1

pos/neg: contrasting results reported in independent studies;

Assay codes:

a, reverse mutation (Ames test, several strains); b, forward mutation (strain TM677); c, DNA binding in mammalian cells *in vitro*; d, DNA damage/repair in bacteria; e, DNA damage/repair in mammalian cells; f, mitotic gene conversion in yeast; g, mitotic recombination in yeast; h, forward mutation in yeast; i, host mediated assay (bacteria); j, host-mediated assay (yeast); l, sex-linked recessive lethals (Drosophila); m, somatic mutation and recombination (Drosophila); n, DNA repair (Drosophila); o, HPRT system; ; p, thymidine kinase system; q, ouabain resistance; r, diphteria toxic resistance; s, chromosomal aberrations; t, sister chromatid exchanges; u, micronucleus test; v, DNA damage /repair *in vivo* (various tissues); w, cytogenetic effects in somatic cells; x, cytogenetic effects in germ cells; y, sperm abnormalities; z, dominant lethals.

References quoted in table 2.7.1:

(1) IPCS, 1998; (2) Zeiger. *et al.*1992; (3) Gatehouse, 1980 (4) Mersch-Sundermann *et al.*, 1993; (5) Mortelmans *et al.*, 1986; (6) Vogel and Nivard, 1993; (7) Delgado-Rodrigues, 1995; (8) Fritzenschaf *et al.*, 1993; (9) Crofton-Sleigh *et al.*, 1993; (10) Topinka *et al.*, 1998; (11) Rodriguez-Arnaiz *et al.*. 1993; (12) Durant *et al.*, 1996; (13) Warshawsky *et al.*, 1995; (14) Ross, *et al.*, 1992; (15) Ross *et al.*, 1995; (16) Mass *et al.*, 1996; (17) Lafleur *et al.*, 1993; (18) Salamone *et al.*, 1979; (19) Hermann, 1981; (20) Mersch-Sundermann *et al.*, 1992; (21) Hughes and Phillips, 1993; (22) Einolf *et al.*, 1996; (23) Cheung *et al.*, 1993; (24) Verschaeve *et al.*, 1997; (25) Perez-Chiesa and Rodriguez, 1993; (26) Tucker *et al.*, 1993; (27) Penman *et al.*, 1994; (28) Keohavong *et al.*, 1995; (29) Beach and Gupta,1994; (30) Garg *et al.*, 1993; (31) Nesnow *et al.*, 1995; (32) Morita *et al.*, 1997; (33) Whong *et al.*, 1994; (34) Busby Jr. *et al.*, 1995; (35) Marsch *et al.*, 1992; (36) Nesnow *et al.*, 1997; (37) Prahalad *et al.*, 1997; (38) Arif *et al.*, 1999; (39) Vaca *et al.*, 1992; (40) Wang, *et al.*, 1995; (41) Stocker *et al.*, 1996; (42) Oberly *et al.*, 1984; (43) Hughes *et al.*, 1993; (44) Kier *et al.*, 1986; (45) You *et al.*, 1994; (46) Misra *et al.*, 1992; l. (47) Oberly *et al.*, 1993; (48) WHO, 1988

Table 2.7.2 Genotoxicity of selected polycyclic aromatic hydrocarbons: overall evaluation of individual compounds

common name (CAS no.)	**conclusion of evaluation**
acenaphthene (83-32-9)	database **inadequate** for evaluation (mixed results from few *in vitro* studies)
acenaphthylene (208-96-8)	database **inadequate** for evaluation (mixed results from bacterial studies)
anthanthrene (191-26-4)	**limited** evidence of mutagenicity (positive results from *in vitro* assays, no *in vivo* data)
anthracene (120-12-7)	**not genotoxic** (negative results in the majority of *in vitro* test systems and in all *in vivo* assays)
benz[*a*]anthracene (56-55-3)	**genotoxic** (positive results *in vitro* and *in vivo* for multiple end-points; positive also at germ cell level)
benzo[*b*]fluoranthene (205-99-2)	**genotoxic** (positive results in assays *in vitro* and *in vivo* for different end-points)
benzo[*j*]fluoranthene (205-82-3)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
benzo[*k*]fluoranthene (207-08-9)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
benzo[*ghi*]fluoranthene (203-12-3)	**limited** evidence of mutagenicity (mainly positive results *in vitro*, no data *in vivo*)
benzo[*a*]fluorene (238-84-6)	**probably not genotoxic** (negative results from several *in vitro* assays)
benzo[*b*]fluorene (243-17-4)	database **inadequate** for evaluation (mixed results from few *in vitro* studies)
benzo[*ghi*]perylene (191-24-2)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
benzo[*c*]phenanthrene (195-19-7)	**limited** evidence of mutagenicity (positive results *in vitro*, limited evidence of DNA binding *in vivo*)
benzo[*a*]pyrene (50-32-8)	**genotoxic** (positive results *in vitro* and *in vivo* for multiple end-points; positive also at germ cell level)
benzo[*e*]pyrene (192-97-2)	**equivocal** (mixed results *in vitro*, inconsistent results *in vivo*)
chrysene (218-01-9)	**genotoxic** (positive results *in vitro* and *in vivo* for multiple end-points; positive also at germ cell level)
coronene (191-07-1)	database **inadequate** for evaluation (mixed results from few *in vitro* studies)

common name (CAS no.)	**conclusion of evaluation**
cyclopenta[*cd*]pyrene (27208-37-3)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
dibenz[*a,h*]anthracene (53-70-3)	**genotoxic** (positive results in assays *in vitro* and *in vivo* for multiple end-pointsl)
dibenzo[*a,e*]pyrene (192-65-4)	**genotoxic** (positive results assays *in vitro* and for DNA binding *in vivo*)
dibenzo[*a,h*]pyrene (189-64-0)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
dibenzo[*a,i*]pyrene (189-55-9)	**genotoxic** (positive in assays *in vitro* and *in vivo*)
dibenzo[*a,l*]pyrene (191-30-0)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
fluoranthene (206-44-0)	**equivocal** (mixed results *in vitro*; evidence of DNA binding *in vivo* after i.p. administration, negative in mutagenicity/ genotoxicity tests by oral route)
fluorene (86-73-7)	database **inadequate** for evaluation (mixed results from few *in vitro* studies; no *in vivo* data available)
indeno[1,2,3-*cd*]pyrene (193-39-5)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
5-methylchrysene (3697-24-3)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)
1-methylphenathrene (832-69-9)	**limited** evidence of mutagenicity (positive results from *in vitro* assays, no *in vivo* data available)
naphthalene (91-20-3)	**probably not genotoxic** (mainly negative results *in vitro*; limited negative data *in vivo*)
perylene (198-55-0)	**limited** evidence of mutagenicity (positive results in some vitro assays, negative for DNA binding *in vivo*)
phenanthrene (85-01-8)	**equivocal** (mixed results *in vitro*; negative or borderline positive *in vivo* cytogenetics)
pyrene (129-00-0)	**not genotoxic** (mainly negative results *in vitro*; extensive negative database *in vivo*)
triphenylene (217-59-4)	**limited** evidence of mutagenicity (positive results from *in vitro* assays, no *in vivo* data available)

Table 2.7.3 Evaluations of genotoxicity and carcinogenicity of selected polycyclic aromatic hydrocarbons

common name (CAS no.)	**genotoxicity (SCF, 2002)**	**carcinogenicity (IPCS, 1998)***	**carcinogenicity (IARC)[$]**	**carcinogenicity (UE, 2001)^**
acenaphthene (83-32-9)	inadequate data	questionable		
acenaphthylene (208-96-8)	inadequate data	no studies		
anthanthrene (191-26-4)	limited evidence	**positive**		
anthracene (120-12-7)	**not genotoxic**	**negative**		
benz[*a*]anthracene (56-55-3)	**genotoxic**	**positive**	**2A**	**cat. 2**
benzo[*b*]fluoranthene (205-99-2)	**genotoxic**	**positive**	**2B**	**cat. 2**
benzo[*j*]fluoranthene (205-82-3)	**genotoxic**	**positive**	**2B**	**cat. 2**
benzo[*k*]fluoranthene (207-08-9)	**genotoxic**	**positive**	**2B**	**cat. 2**
benzo[*ghi*]fluoranthene (203-12-3)	limited evidence	negative ?		
benzo[*a*]fluorene (238-84-6)	probably not genotoxic	questionable		
benzo[*b*]fluorene (243-17-4)	inadequate data	questionable		
benzo[*ghi*]perylene (191-24-2)	**genotoxic**	negative ?	3	
benzo[*c*]phenanthrene (195-19-7)	limited evidence	positive ?	3	
benzo[*a*]pyrene (50-32-8)	**genotoxic**	**positive**	**2A**	**cat. 2**
benzo[*e*]pyrene (192-97-2)	equivocal	questionable		**cat. 2**
chrysene (218-01-9)	**genotoxic**	**positive**	3	**cat. 2**
coronene (191-07-1)	inadequate data	questionable		
cyclopenta[*cd*]pyrene (27208-37-3)	**genotoxic**	**positive**	3	
dibenz[*ah*]anthracene (53-70-3)	**genotoxic**	**positive**	**2A**	**cat. 2**
dibenzo[*a,e*]pyrene (192-65-4)	**genotoxic**	**positive**	**2B**	
dibenzo[*a,h*]pyrene (189-64-0)	**genotoxic**	**positive**	**2B**	
dibenzo[*a,i*]pyrene (189-55-9)	**genotoxic**	**positive**	**2B**	
dibenzo[*a,l*]pyrene (191-30-0)	**genotoxic**	**positive**	**2B**	
fluoranthene (206-44-0)	equivocal	positive ?		
fluorene (86-73-7)	inadequate data	**negative**		

common name (CAS no.)	**genotoxicity (SCF, 2002)**	**carcinogenicity (IPCS, 1998)***	**carcinogenicity (IARC)$**	**carcinogenicity (UE, 2001)^**
indeno[1,2,3-*cd*]pyrene (193-39-5)	**genotoxic**	**positive**	**2B**	
5-methylchrysene (3697-24-3)	**genotoxic**	**positive**	**2B**	
1-methylphenathrene (832-69-9)	limited evidence	negative ?		
naphthalene (91-20-3)	probably not genotoxic	questionable		
perylene (198-55-0)	limited evidence	negative ?	3	
phenanthrene (85-01-8)	equivocal	questionable	3	
pyrene (129-00-0)	**not genotoxic**	questionable	3	
triphenylene (217-59-4)	limited evidence	negative ?	3	

* as tabulated in Environmental Health Criteria 202, Table 2 (corrigendum), p.13 (IPCS, 1998)

$ IARC Monographs volumes 1-79

^ Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC

2.8 Special studies on cardiovascular effects of PAH

It has been hypothesised that a mechanism could be that PAH from cigarette smoke tars or combustion products could cause endothelial injury and changes in smooth muscle cells leading to clonal expansion of these in the arterial walls and thereby might contribute to the development of arteriosclerosis.

It is unequivocal that tobacco smoking is a major risk factor for cardiovascular disease and there is also some evidence that occupational exposure to combustion products including PAH might also be associated with an increased risk of cardiovascular disease (Ström *et al.*, 1993; Gustavsson *et al.*, 2001).

Following induction with 3-methylcholanthrene microsomes from rat aorta transformed benzo[*a*]pyrene into active carcinogenic and toxic metabolites (Thirman *et al.*, 1994). In humans, PAH adducts have been detected in the endothelium of the internal mammary artery of smokers. DNA adducts has also been detected by ^{32}P-postlabelling in smooth muscle cells of human abdominal aorta affected by arteriosclerotic lesions. The levels of adducts were correlated to current smoking and other atherogenic risk factors. In individuals with the null GSTM1 had higher adduct levels in both non-smokers and smokers (Izzotti *et al.*, 2001a). It is not established whether the DNA adducts are formed from PAH. Similar adducts in the aorta was also observed in smoke exposed rats (Izzotti *et al.*,2001b). PAH adducts determined by immunohistochemistry were also found in the endothelium of microvessels in the muscular layer of large blood vessels (Zhang *et al.*, 1998). In human umbilical vein endothelial cells cultured *in vitro,* and induced with β-naphthoflavone, benzo[*a*]pyrene induced DNA damage as measured by alkaline single cell gel electrophoresis (Annas *et al.*, 2002).

Benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene caused a decreased secretion of newly synthesised collagen from bovine arterial smooth muscle cells *in vitro* without reducing the cell number. Total cellular DNA was decreased and the relative collagen secretion increased. When the cells had been preincubated with platelet factors similar effects were observed. Benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene could also induce cell death and release of mitogenic factors, dependingen on the concentration and mode of administration (Stavenow and Pessah-Rasmussen, 1988). Dimethylbenz[*a*]anthracene induced proliferation of smooth muscle celles. This effect was independent of the Ah-receptor (Pessah-Rasmussen *et al.*, 1991). Glutathione transferase (GST) negative human fibroblasts produced relatively more collagene than GST positive fibroblasts (Pessah-Rasmussen *et al.*, 1992).

Although PAH related adducts have been observed in blood vessels in humans and some effects of PAH on vascular cells *in vitro*, this does not prove that the increased cardiovascular risk following tobacco smoking and occupational exposure to combustion products is due to exposure to PAH.

2.9 Observations in humans

2.9.1 Biomarkers of exposure to PAH

Several methods have been developed to assess internal exposure to PAH after exposure in the environment and in workplaces. In most studies, metabolites of PAH were measured in urine, 1-hydroxypyrene being widely used. It should be noted that the concentration or excretion of parent PAH compound or metabolites in body fluids or urine not only is dependent on the external exposure, but also on absorption, biotransformation and excretion, which can vary considerably between individuals. Genotoxic endpoints of PAH have been used, but they are unspecific and will not be further discussed. Adducts of benzo[*a*]pyrene with DNA in peripheral lymphocytes, and other tissues and with proteins such as albumin have been used as an indicator of the dose of reactive metabolite.

Urinary metabolites
The metabolites mostly used have been hydroxylated phenanthrenes and 1-hydroxypyrene. Also total thioether excretion has been used, but this is unspecific.

1-Hydroxypyrene
1-Hydroxypyrene, a metabolite of pyrene, has been widely used as urinary biomarker of PAH exposure since 1986. Its advantages are that pyrene is present in all PAH mixtures at relatively high concentrations (2-10%) of the total PAH, and in certain environments the pyrene content of the total PAH is fairly constant. However, the relationship between pyrene and benzo[*a*]pyrene may vary considerably beteen different exposures. Coal tar contains 2-10 % pyrene and 0.4-0.6 % benzo[*a*]pyrene. In studies at different workplaces, a strong correlation was found between the pyrene concentrations in air and those of benzo[*a*]pyrene, other selected PAH, and total PAH (Jongeneelen *et al.*, 1990; Tolos *et al.*, 1990; Zhao *et al.*, 1990; Van Rooij *et al.*, 1992; Ferreira *et al.*, 1994a,b; Jongeneelen, 1994; Elovaara *et al.*, 1995; Levin *et al.*, 1995; Øvrebo *et al.*, 1995; Quinlan *et al.*, 1995a, all cited in IPCS, 1998). Pyrene is metabolised predominantly to 1-hydroxypyrene, which can be measured easily. In contrast to other PAH metabolites, which are excreted mainly in faeces, 1-hydroxypyrene is excreted in urine.

The background concentrations of 1-hydroxypyrene in urine of persons from different countries range from 0.06 to 0.23 µmol/mol creatinine (Kang *et al.*, 1995). For non-smokers and non-occupationally exposed individuals, food accounted for 99% of the total daily pyrene intake (Van Rooij *et al.*, 1994a, cited in IPCS, 1998). Five volunteers who ate low-PAH meals and high-PAH meals showed 100- to 250-fold increases in benzo[*a*]pyrene dose, accompanied by a four- to 12-fold increase in 1-hydroxypyrene excretion in urine (Buckley and Lioy, 1992, cited in IPCS, 1998). Ten volunteers eating charbroiled beef for five days had a 10-80 fold increase in 1-OH-pyrene glucuronide excretion in urine above background returning to background within 24-72 hours (Kang et al 1995). The intake of pyrene from cigarette smoking (12 nmol/day) is about the same as the dietary intake from normal food (9.4 nmol/day). Tobacco smokers who are not otherwise exposed to PAH have

about twice the level of 1-hydroxypyrene in their urine as non-smokers (Jongeneelen *et al.*, 1990; Sherson *et al.*, 1992; Van Rooij *et al.*, 1994a; Levin *et al.*, 1995, all cited in IPCS, 1998).

1-Hydroxypyrene concentrations in the urine of persons occupationally exposed to PAH at various workplaces are usually increased. Dermal uptake can be a significant pathway in many cases. 1-Hydroxypyrene cannot predict exposure to benzo[*a*]pyrene or other carcinogenic PAH as the relative content of these two compounds can vary considerably.

DNA adducts

DNA adducts with reactive metabolites (mainly diol epoxides) of benzo[*a*]pyrene and other PAH have been identified in humans exposed to smoking or living in polluted areas in numerous studies (Kyrtopoulos *et al.*, 2001). PAH- DNA adducts has also been detected in peripheral white blood cells following human exposure to charbroiled meat (Kang *et al.*, 1995). Cigarette smokers have higher levels of adducts with PAH in their lungs than non-smokers. As binding of electrophilic PAH metabolites to DNA is thought to be a key step in the initiation of cancer, measurement of DNA adducts could be an indicator of exposure to PAH and also of the dose of the ultimate reactive metabolite. An increased lung cancer risk has been found among smoking individuals with a higher level of aromatic DNA adducts in white blood cells (Tang et al 2001).

The methods for measuring DNA adducts include immunoassays with polyclonal and monoclonal antibodies (enzyme-linked immunosorbent assay [ELISA] and ultrasensitive enzymatic radioimmunoassay), ^{32}P-postlabelling, and synchronous fluorescence spectrophotometry. Direct comparisons of adduct levels determined by different techniques may be misleading, however, because different end-points are measured.

In the general population the levels of DNA adduct in control subjects range from 0.2 to about 10 adducts per 10^8 nucleotides in leukocytes (Dell'Omo and Lauwerys, 1993). There are many studies on the effect of adduct level in leukocytes of tobacco smokers and also on populations living in areas polluted by industry; the latter with adduct levels up to 5-13 adducts per 10^8 nucleotides. Eating charcoal-grilled beef resulted in a 1.9-3.8-fold increase above the individual baseline adduct levels in four of 10 subjects (Kang *et al.*, 1995). Workers exposed to PAH in general had elevated levels of adducts (5-70 adducts per 10^8 nucleotides) (IPCS, 1998).

DNA-adducts and 1-hydroxypyrene

In general, exposures that lead to the excretion of high concentrations of 1-hydroxypyrene in urine also lead to elevated DNA adduct levels in white blood cells and significant correlations have been found. Although the concentrations of PAH that occur under different exposure conditions differ by orders of magnitude, the differences in DNA adduct levels are quite small, in contrast to the results of experiments on excretion of 1-hydroxypyrene. In all populations studied, exposed by inhalation or orally, there is substantial inter-individual variation in PAH-DNA adduct levels, which is greater than that

described for 1-hydroxypyrene excretion in urine. This is probably due to differences e.g. in biotransformation, excretion, DNA adduct removal etc. (Autrup, 2000, Lee et al 2002). Such interindividual variation result in a wide overlap in the ranges of values between exposed and unexposed subjects in all studies.

Protein adducts
Because genotoxic compounds can bind to haemoglobin and serum protein, the assessment of PAH-blood protein adducts has also been considered as a possible marker of exposure to PAH. However, studies show conflicting results using such adducts as a marker of exposure.

Biomarkers and exposure via inhalation and diet
In many cases exposure to PAH from food is a confounder when using biomarkers in the evaluation of exposure to PAH by inhalation. Although increased levels in urinary 1-hydroxypyrene have been found in people living in polluted areas exposure from ambient air, large changes in atmospheric levels of PAH is not reflected in urinary 1-hydroxypyrene and DNA-adducts. This indicates that ambient air is relatively unimportant in comparison with dietary PAH and tobacco smoking (Phillips, 1999; Kyrtopoulos *et al.*, 2001). In a study of forest fire-fighters in the USA levels of PAH-DNA adducts in blood cells were not found to correlate with recent fire-fighting activity, but with recent consumption of charbroiled meat. Surprisingly the PAH DNA-adduct levels were lower in US army personnel fighting oil field fires in Kuwait, possibly because a lower intake of charbroiled meat (Phillips, 1999).

2.9.2 Effects of PAH exposures observed in humans

Unfortunately there are almost none published studies on health effects in humans following oral exposure to PAH. In the majority of studies humans have been occupationally exposed to PAH via inhalation and in a few studies the exposure has been dermal. There is little information on human exposure to single, pure polycyclic aromatic hydrocarbons (PAH) except for accidental exposure to naphthalene and some data from defined short-term studies of volunteers which are not relevant for the human exposure to PAH via food. All other reports are on exposure to mixtures of PAH, which also contained other potentially carcinogenic chemicals, in occupational and environmental situations. Information on the health effects of these mixtures is in practically all cases confined to their carcinogenic potential, for which there is evidence from a number of epidemiological studies, especially for lung cancer and, in some cases, cancers of the skin and of the urinary bladder.

Oral exposure to PAH
Only one fully reported study on oral PAH exposure and health effects has been identified (Lopez-Abente *et al.*, 2001). In some rural areas in Spain wine has traditionally been stored in leather bottles sealed with a tar-like substance (i.e. pez) obtained through boiling and

destilation of fir and pine wood, and which contain PAH. In order to assess this exposure with regard to risk of gastric cancer, 59 cases and 53 controls all of who were residents in the Province of Soria, were selected from a case control multi-center study from Spain on gastric cancer. The multi-center study consisted of 354 incident cases and 354 controls matched by age, sex and place of residence. The exposure to wine stored in tar impregnated leather bottles was assessed by a self-administered questionnaire. A total of 85 questionnaires were returned of which 78, i.e. 38 cases and 40 controls, could be analysed. In the analysis odds ratios (OR) were calculated by logistic regression taking into account also variables that had been found to be associated with gastric cancer in the multi-center study. Several variables for consumption of wine from leather bottles were reported. Although an increased OR was reported, the study population was to small to achieve statistically significant increases. An exception was consumption of more than 2 litres of wine/week, which appeared to be associated with gastric cancer particularly in males OR and 95 % confidence interval = 10.5, 1.13-97.76, p value for trend 0.02. No change in effect estimator was observed upon inclusion of other risk variables in the model.

Studies on intake of cooked meat

In a published abstract Sinha and co-workers (2001) described the risk of colorectal adenomas and dietary benzo[*a*]pyrene intake in a case-control study of 146 newly diagnosed cases and 226 controls. In this study dietary intake of red meat, well-done red meat, grilled red meat and exposure to heterocyclic amines such as 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and benzo[*a*]pyrene were estimated using a food frequency questionnaire with detailed questions also on meat-cooking methods in combination with a heterocyclic amine and benzo[*a*]pyrene database (Kazerouni *et al.*, 2001). Increased risks were found with a high intake of red meat, well-done and grilled and heterocyclic amines. The median (10^{th} and 90^{th} percentile) exposure to benzo[*a*]pyrene in the controls was 5 ng (0.2-66) ng/day from meat and 73(35-140) ng/day from all foods. In cases, median benzo[*a*]pyrene intake was 17 (0.5-101) ng/day from meat and 76 (44-163) ng/day from all foods When multivariate analysis were carried out adjusting for MeIQx and established colorectal adenoma risk factors, the odds ratios (95 % confidence interval) for dietary benzo[*a*]pyrene from meat with the first quintile as the referent group were: 1.5 (0.7-3.4, 2.0 (0.9-4.3), 2.6 (1.2-5.7) and 3.3 (1.5-7.4) for the second, third, fourth and fifth quintile. The p-value for trend was 0.01. Increased risk of colorectal adenomas was also associated with benzo[*a*]pyrene intake estimated from all foods, p-value for trend 0.01.

In a short feeding study ten healthy adults were fed a diet enriched with chargrilled meat for 7 days (Fontana *et al.*, 1999). The meat contained from 8.35 to 15.64 ng benzo[*a*]pyrene/ g and also heterocyclic amines: 100 and 50 ng PhIP/g. Whereas the intake of PhIP was estimated to 13-28 µg/day the intake of benzo[*a*]pyrene was not given. The chargrilled meat intake resulted in an induction of CYP1A enzymes both in the liver and the small intestine. No induction of CYP3A4, CYP3A5 or P-glycoprotein in the small or large intestine and CYP3A4 in the liver was observed. There was an inverse correlation between the level of PAH DNA adducts in peripheral blood mononuclear cells and both liver

CYP1A2 activity and enterocyte CYP1A1 protein concentration on day 11. It is not clear whether the enzyme induction was due to heterocyclic amines or PAH as Sinha and co-workers (1994) in a previous study feeding of pan-fried meat containing high levels of heterocyclic amines, but unchanged and low levels of PAH, also found an induction of CYP1A2.

PAH in cigarette smoke

Tobacco smoke contains a mixture of PAH in addition to numerous other carcinogens. For example levels of 11 ng per cigarette benzo[*a*]pyrene were found in mainstream smoke and 103 ng per cigarette in sidestream smoke; the corresponding values were 6.8 and 76 ng per cigarette for benzo[*e*]pyrene, 20 and 497 ng per cigarette for chrysene and triphenylene, and 13 and 204 ng per cigarette for benz[*a*]anthracene for mainstream and sidestream smoke, respectively (Grimmer *et al.*, 1988). Other data on intake by smoking are reported in the section 'Intake estimates'.

A large volume of literature exists on the effects of tobacco smoke on human lungs (see IARC, 1986). On the basis of large body of studies in many countries, cigarette smoke has been shown to be by far the most important single factor contributing to the development of lung cancer. Other types of cancer caused by cigarette smoking include cancers of the oral cavities, larynx, pharynx, oesophagus, bladder, renal pelvis, renal adenocarcinoma, and pancreas. Based upon studies using implantation into the lungs of rats Grimmer *et al.*, 1988 (cited in IPCS, 1998) estimated that PAH with four or more rings were responsible for 83% of the total carcinogenic activity of sidestream smoke (IPCS,1998).

PAH and occupational exposure

Many workplaces have atmospheres with heavy loads of PAH. In general, industrial workers using or producing coal or coal products are exposed to mixtures of PAH

The first cancer that might be attributed to an occupational exposure was reported by Pott in 1775, who described the susceptibility of English chimney sweeps to scrotal cancer (Pott, 1775, cited in IPCS, 1998); a second was published by Butlin in 1892 (cited in IPCS, 1998).

Epidemiological studies have been conducted on workers exposed at coke ovens in coal coking and coal gasification, at asphalt works, at foundries, and at aluminium smelters and to diesel exhaust. Details of the most recent and most important cohort and case-control studies are given in IPCS (1998) (see also IARC 1983, 1984, 1985).

Most important for an evaluation of the possible risk of cancer due to exposure to PAH are studies of workers exposed at coke ovens in coke plants or in coal-gasification processes. Particularly at coke ovens, the PAH concentrations are considerable, with levels up to 200 μ/m^3 total PAH, but values between some tens of μ/m^3 are more common; extreme values up to 1 mg/m^3 total PAH and 100-300 $\mu g/m^3$ benzo[*a*]pyrene have been reported in the 60s and 70s in eastern European countries (IARC, 1984). Values reported from coal

gasification plants are lower. In most epidemiological studies on PAH exposed workers, however, the concentrations to which workers were exposed are not available. Significantly increased risks of lung cancer was found among coke oven workers including workers in coal-gasification particularly in the large cohort studies (IPCS, 1998).

Several epidemiological studies have been performed on the potential risks of handling asphalt. In the meta-analysis of Partanen and Boffetta (1994, cited in IPCS, 1998), increased risks for lung tumours were seen for both pavers and roofers; tumours of the stomach, bladder, and skin and leukaemia were also observed.

Workers are exposed dermally to very high concentrations of PAH when impregnating wood with creosote. Increased risk for skin and lip cancer has been observed. Since the work involves some time outdoors, it cannot be ruled out that exposure to sunlight contributed to the risks for cancers of the skin and lip.

The PAH concentrations in iron, steel, and other ferroalloy foundries reach levels of 50 $\mu g/m^3$ and that of benzo[*a*]pyrene about 10 $\mu g/m^3$. Increased mortality from lung cancer has been observed consistently in many studies of foundry workers (Andjelkovich *et al.*, 1990, cited in IPCS, 1998).

Information on the possible risks for cancer due to exposure to PAH can also be obtained from studies of workers in aluminium plants. During Söderberg electrolysis, workers may be exposed to 3-35 $\mu g/m^3$ benzo[*a*] pyrene; workers in the carbon area are exposed to 0.4-1.2 $\mu g/m^3$ benzo[*a*] pyrene. An increased risk for lung cancer, but also urinary bladder cancer has been found in several studies with exposure in a Söderberg potroom. The risk of bladder cancer may be due to exposure not only to PAH but also to aromatic amines, which have been detected in the potrooms.

Increased risks for lung cancer were found in several studies of workers exposed to diesel exhausts (WHO, 1996). In comparison with the occupations described above, the concentrations of PAH to which these workers are exposed are usually relatively low. The benzo[*a*]pyrene concentrations in automobile repair shops and garages reach about 70 ng/m^3, and truck drivers are exposed to less than 10 ng/m^3.

The increased risk is seen for workers in several occupations, which have exposure to PAH in common. Although other carcinogenic chemicals were present, they differed with each occupation. Furthermore, tobacco smoking is a confounder in several studies, but this factor in general cannot explain the excess risk. Airborne high-molecular-mass PAH, which are considered to be the most carcinogenic, are adsorbed mainly onto particulate matter, and it was often difficult to distinguish the toxicological effects caused by particles from those caused by the PAH themselves.

PAH exposure from unvented coal combustion in homes

Interdisciplinary studies were conducted to investigate exposure to PAH and the high lung cancer rates in a rural county, Xuan Wei, located in Yunnan Province, China (Mumford *et al.*, 1987, cited in IPCS, 1998). Mortality from lung cancer in this county is five times the Chinese national average, especially among the women, who have the highest rate in China. The mortality rate from lung cancer was correlated with domestic use of 'smoky' coal (medium-volatile bituminous coal with low sulphur and high ash) as fuel for cooking and heating, but not with use of wood or smokeless coal. Monitoring of air during cooking inside the homes showed that women were exposed to extremely high levels of PAH, with a mean benzo[*a*] pyrene concentration of 14.7 μg/m^3, comparable to the levels to which coke-oven workers were exposed. Urine samples from Xuan Wei residents confirmed that they were exposed to high concentrations of alkylated PAH. Thus, alkylated PAH may play an important role in the etiology of lung cancer in Xuan Wei (Mumford *et al.*, 1995, cited in IPCS, 1998).

3 REFERENCES

Alexander, D.L., Zhang, L., Foroozesh, M., Alworth, W.L., Jefcoate, C.R. (1999). Metabolism-based polycyclic aromatic acetrylene inhibition of CYP1B1 in 10T½ cells potentiates aryl hydrocarbon receptor activity. Toxicol. Appl. Pharmacol., 161, 123-139.

Anderson, C., Hehr, A., Robbins, R., Hasan, R., Athar, M., Mukhtar, H., and Elmets, C.A., 1995. Metabolic requirements for induction of contact hypersensitivity to immunotoxic polyaromatic hydrocarbons. J. Immunol., 155, 3530-3537.

Andersson, K., Levin, J.-O., and Nilsson, C.-A., 1983. Sampling and analysis of particulate and gaseous polycyclic aromatic hydrocarbons from coal tar sources in the working environment. Chemosphere, 12, 197-207.

Andrianova, M.M., 1971. Transplacental action of 3-methylcholanthrene and benz[a]pyrene on four generations of mice. Bull. Exp. Biol. Med., 71, 677-680.

Annas, A., Brittebo, E., and Hellman, B., 2000. Evaluation of benzo(a)pyrene-induced DNA damage in human endothelial cells using alkaline single cell gel electrophoresis. Mutat. Res., 47, 145-155.

Archoibong, A.E., Inyang, F.E., Ramesh, A., Hood, D.B., Greenwood, M., Nunes, M.G., Nayyar, T., Kopsumbut, P., and Nyanda, A.M., 2001. Reduction in luteotropic activity and embryonic survival in pregnant rats by inhaled benzo[a]pyrene. Toxicologist, 60, 274, Abstract No.1303.

Arif, J.M., Smith, W.A., and Gupta, R.C., 1999. DNA adduct formation and persistence in rat tissues following exposure to the mammary carcinogen dibenzo[a,l]pyrene. Carcinogenesis, 20, 1147-1150.

ATSDR, 1994. Toxicological profile for polycyclic aromatic hydrocarbons (PAH): update. U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substances and Disease Registry, Atlanta.

ATSDR, 1995a. Toxicological profile for naphthalene (update). U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substances and Disease Registry, Atlanta.

ATSDR, 1995b. Toxicological profile for polycyclic aromatic hydrocarbons (update). U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substances and Disease Registry, Atlanta.

Autrup, H., 1986. Host factors in carcinogenesis: Carcinogen metabolism and DNA damage. In: Biochemical Molecular Epidemiology Cancer (C.C. Harris, Ed.), Alan R. Liss, New York, pp. 359-372.

Autrup, H., 2000. Genetic polymorphisms in human xenobiotica metabolising enzymes as susceptibility factors in toxic response. Mutat. Res., 464, 65-76.

Autrup, H., 1993. Transplacental transfer of genotoxins and transplacental carcinogenesis. Environ. Health Perspect., 101 (Suppl.2), 33-38.

Awogi, T., and Sato, T., 1989. Micronucleus test with benzo[a]pyrene using a single peroral administration and intraperitoneal injection in males of the MS/Ae and CDl-1 mouse strains, Mutat. Res., 223, 353-356.

Bartosek, I., Guaitani, A., Modica, R., Fiume, M., and Urso, R., 1984. Comparative kinetics of oral benzo[a]anthracene, chrysene and triphenylene in rats: Study with hydrocarbon mixtures. Toxicol. Lett., 23, 333-339.

Baum, M., Amin, S., Guengerich, F.P., Hecht, S.S., Kohl, W., and Eisenbrand, G., 2001. Metabolic activation of benzo[c]phenanthrene by cytochrome P450 enzymes in human liver and lung. Chem. Res. Toxicol., 14, 686-693.

BCL, 1980. Unpublished subchronic toxicity study: Naphthalene (C52904), Fisher 344 rats. By Bartelle Columbus Laboratories (cited in IRIS, 2002).

Beach, A.C., and Gupta, R.C., 1994. DNA adducts of the ubiquitous environmental contaminant cyclopenta[cd]pyrene. Carcinogenesis, 15, 1065-1072.

Beckman Sundh, U., Thuvander, A., and Andersson, C., 1998. Review of PAH in food – potential health effects and contents in food. Report 8/98. Livsmedelsverket, National Food Administration, Uppsala.

Bennett, W.P., Alavanja, M.C., Blomeke, B., Vahakangas, K.H., Castren, K., Welsh, J.A., Bowman, E.D., Khan, M.A., Flieder, D.B., and Harris, C.C., 1999. Environmental tobacco smoke, genetic susceptibility, and risk of lung cancer in never-smoking women. J. Natl. Cancer Inst., 91, 2009-2014.

Berenblum, I., and 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.

Besarati, N., 2001. Molecular dosimetry studies of smoking - induced carcinogenesis in target and surrogate tissues of humans. PhD Dissertation, University of Maastricht, pp. 160.

Bhat, R., Wagner, C., and Bresnick, E., 1997. The homodimeric form of glycine N-methyltransferase acts as a polycyclic aromatic hydrocarbon-binding receptor. Biochemistry, 36, 9906-9910.

Bjørseth, A., Biørseth, O., and Fjeldstad, P.E., 1978. Polycyclic aromatic hydrocarbons in the work atmosphere. II. Determination in a coke plant. Scand. J. Work Environ. and Health, 4, 224-236.

Bláha, L., Kapplová, P., Vondrácek, J., Upham, B., and Machala, M., 2002. Inhibition of Gap-Junctional intercellular communication by environmentally occurring polycyclic aromatic hydrocarbons. Toxicol. Sci., 65, 43-51.

Bock, F.G., and King, D.W., 1959. A study of the sensitivity of the mouse forestomach toward certain polycyclic hydrocarbons. J. Natl. Cancer Inst., 23, 833-838.

Bommel, H., Li-Weber, M., Serfling, E., and Duschl, A., 2000. The environmental pollutant pyrene induces the production of IL-4. J. Allergy Clin. Immunol., 105, 796-802.

Booth, E.D., Brandt, H.C., Loose, R.W., and Watson, W.P., 1998. Correlation of ^{32}P-postlabelling-detection of DNA adducts in mouse skin in vivo with the polycyclic aromatic compound content and mutagenicity in *Salmonella typhimurium* of a range of oil products. Arch. Toxicol., 72, 505-513.

Brauze, D., Wielgosz, S.M., Pawlal, A.L., and Baer-Dubowska, W., 1997. Effect of the route of benzo[a]pyrene administration on sister chromatid exchange and DNA binding in bone marrow of mice differing with respect to cytochrome P450 1A1 induction. Toxicol. Letters., 91, 211-217.

Brookes, P., and Lawley, P.D., 1964. Evidence of the binding of polynuclear aromatic hydrocarbons to the nucleic acids of mouse skin: Relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid. Nature, 202, 781-784.

Brown, J.R., Field, R.A., Goldstone, M.E., Lester, J.N., and Perry, R., 1996. Polycyclic aromatic hydrocarbons in central London air during 1991 and 1992. Sci. Total Environ., 177, 73-84.

Brown, R. and Mittelman, A., 1993. Evaluation of existing methods to rank the relative carcinogenicity of polycyclic aromatic compounds (PAH), Draft. Technical Resources, Inc., Contract No. 68-01-0022, for Office of Emergency and Remedial Response, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Office of Pesticides, Pollution Prevention and Toxic Substances (OPPTS).

Brune, H., Deutsch-Wenzel, R. P., Habs, M., Ivankovic, S., and Schmähl, D., 1981. Investigation of the tumourigenic response to benzo[a]pyrene in aqueous caffeine solution applied orally to Sprague-Dawley rats. J.Cancer Res. Clin. Oncol., 102, 153-157.

Buchet, J.P., Gennart, J.P., Mercado-Calderon, F., Delavignette, J.P., Cupers, L., and Lauwerys, R., 1992. Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. Br. J. Ind. Med., 49, 761-768.

Bui, Q.Q., Tran, M.B., and West, W.L., 1986. A comparative study of the reproductive effects of methadone and benzo[a]pyrene in the pregnant and pseudopregnant rat. Toxicology, 42, 195-204.

Burchiel, S.W., and Luster, M.I., 2001. Signaling by environmental polycyclic aromatic hydrocarbons in human lymphocytes. Clin. Immunol., 98, 2-10.

Burczynski, M.E. and Penning, T.M., 2000. Genotoxic polycyclic aromatic hydrocarbon ortho-quinones generated by also-keto reductases induce CYP1A1 via nuclear translocation of the aryl hydrocarbon receptor. Cancer Res., 60, 908-915.

Busby, W.F.Jr, Smith, H., Crespi, C.L. and Penman, B.W., 1995. Mutagenicity of benzo[a]pyrene and dibenzopyrenes in the *Salmonella typhimurium* TM677 and the MCL-5 human cell forward mutation assays. Mutat. Res., 342, 9-16.

Busby, W.F.Jr., Stevens, E.K., Kellenbach, E.R., Cornelisse, J. and Lugtenburg, J., 1988. Dose-response relationships of the tumourigenicity of cyclopenta[cd]pyrene, benzo[a]pyrene and 6-nitrochrysene in a newborn mouse lung adenoma bioassay. Carcinogenesis, 9, 741-746.

Busby, W.F.Jr., E.K. Stevens, C.N. Martin, F.L. Chow, and R.C. Garner, 1989. Comparative lung tumourigenicity of parent and mononitro-polynuclear aromatic hydrocarbons in the BLU:Ha newborn mouse assay. Toxicol. Appl. Pharmacol., 99, 555-563.

Buss, D.H., and Lindsay, D.G., 1978. Reorganization of the UK total diet study for monitoring minor constituents of food. Fd Cosmet. Toxicol., 16, 597-600.

Butler, J.P., Post, G.B., Lioy, P.J., Waldman J.M., and Greenberg, A., 1993. Assessment of carcinogenic risk from personal exposure to benzo[a]pyrene in the total human environmental exposure study (THEES). J. Air Waste Manage. Assoc., 43, 970-977.

Carretero, M.V., Latasa, M.U., Garcia-Trevijano, E.R., Corrales, F.J., Wagner, C., Mato, J.M., and Avila, M.A., 2001. Inhibition of liver methionine adenosyltransferase gene expression by 3-methylcholanthrene: protective effect of S-adenosylmethionine. Biochem Pharmacol, 61, 1119-1128.

Cauchi, S., Stücker, I., Solas, C., Laurent-Puig, P., Cénée, S., Hémon, D., Jacquet, M., Kremers, P., Baune, P., and Massaad-Massade, L., 2001. Polymorphisms of human aryl hydrocarbon receptor (AhR) gene in a French population: relationship with CYP1A1 inducibility and lung cancer. Carcinogenesis, 22, 1819-1824.

Charles, G.D,, Bartels, M.J., Zacharewski, T.R., Gollapudi, B.B., Freshour, N.L., and Carney, E.W., 2000. Activity of benzo[a]pyrene and its hydroxylated metabolites in an estrogen receptor-α reporter gene assay. Toxicol. Sci., 55, 320-326.

Chen, Y.M., Chen, L.Y., Wong, F.H., Lee, C.M., Chang, T.J., and Yang-Feng, T.L., 2000. Genomic structure, expression, and chromosomal localization of the human glycine N-methyltransferase gene. Genomics, 66, 43-47.

Chen, B.H., and Lin, Y.S., 1997. Formation of Polycyclic Aromatic Hydrocarbons during processing of duck meat. J. Agric. Food Chem., 45, 1394-1403.

Cherng, S.-H., Lin, P., Yang, J.-L., Hsu, S.-L., and Lee, H., 2001. Benzo[g,h,I]pyrelene synergistically transactivates benzo[a]pyrene-induced CYP1A1 gene expression by Aryl hydrocarbon receptor pathway. Toxicol. Appl. Pharmacol., 170, 63-68.

Cheung, Y.L., Gray, T.J., and Ioannides, C., 1993. Mutagenicity of chrysene, its methyl and benzo derivatives, and their interactions with cytochromes P-450 and the Ah-receptor; relevance to their carcinogenic potency. Toxicology, 81,69-86.

Chu, E.W., and Malmgren, R.A., 1965. An inhibitory effect of vitamin A on the induction of tumours of forestomach and cervix in the Syrian hamster by carcinogenic polycyclic hydrocarbons. Cancer Res., 25, 884-895.

Coleman, P., Conolly, C., Donovan, B., Jenkin, M., Jones, K., Lee, R., Peters, A., and Watterson, J., 1999. Toxic Organic Micropollutant Monitoring 1996 to 1999. Report AEAT-1970/Issue 1. NETCEN National Environmental Technology Centre, Culham, United Kingdom.

Coles, B., Nowell, S.A., MacLeod, S.L., Sweeney, C., Lang, N.P., and Kadlubar, F.F., 2001. The role of human glutathione S-transferases (hGSTs) in the detoxification of the food-derived carcinogen metabolite N-acetoxy-PhIP, and the effect of a polymorphism in hGSTA1 on colorectal cancer risk. Mutat. Res., 482, 3-10.

Corradetti, E., Mazzanti, L., Poli, G., and Zucchetti, G., 1990. Sulla contaminazione delle colture esposte agli idrocarburi policiclici aromatici (IPA) di origine industriale ed autoveicolare. Boll. Chim. Igien. 41, 441-479.

Cortessis, V., Siegmund, K., Chen, Q., Zhou, N., Diep, A., Frankl, H., Lee, E., Zhu, Q.-S., Haile, R., and Levy, D., 2001. A case-control study of microsomal epoxide hydrolase, smoking, meat consumption, GSTM3 and Risk of colorectal cancer. Cancer Res., 61, 2381-2385.

COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment), 2002. Polycyclic Aromatic Hydrocarbons in the 2000 Total Diet Study. Reports TOX/2002/26, TOX/2002/26 Annex A (Draft) and TOX/2002/26 Annex B. United Kingdom. http://www.food.gov.uk/multimedia/pdfs/TOX-2002-26.PDF and http://www.food.gov.uk/science/ouradvisors/toxicity/meetings/COT2002/COT18Jun02/minutes

Crofton-Sleigh, C., Doherty, A., Ellard, S., Parry, E.M., and Venitt. S., 1993. Micronucleus assays using cytochalasin-blocked MCL-5 cells, a proprietary human cell line expressing five human cytochromes P-450 and microsomal epoxide hydrolase. Mutagenesis, 8, 363-372.

Csaba, G., and Inczefy-Gonda, A., 1992. Benzpyrene exposure at 15 days of prenatal life reduces the binding capacity of thymic glucocorticoid receptors in adulthood. Gen. Pharmacol., 23, 123-124.

Csaba, G., Inczefi-Gonda, A., and Szeberenyi, S., 1991. Lasting impact of a single benzpyrene treatment in prenatal and growing age on the thymic glucocorticoid receptors of rats. Gen. Pharmacol., 22, 815-818.

CSTEE (Scientific Committee for Toxicity, Ecotoxicity and the Environment), 2001. Scientific Questions to the CSTEE on PAHs. Opinion on: Position Paper on Ambient Air Pollution by Polycyclic Aromatic Hydrocarbons (PAH) – Version 4, February 2001. Opinion expressed at the 24th CSTEE plenary meeting, Brussels, 21 June 2001. http:/europa.eu.int/comm/food/fs/sc/sct/out108_en.htm

Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S., and Beland, F.A., 1996. DNA adduct measurements in relation to small intestine and forestomach tumour incidence during the chronic feeding of coal tar or benzo[a]pyrene to mice. Polycyclic Aromatic Hydrocarbons, 11, 161-168.

Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S., and Beland, F.A., 1998. A comparison of the tumours induced by coal tar and benzo[a]pyrene in a 2-year bioassay. Carcinogenesis, 19, 117-124.

Daniel, F.B., Reddy, T.V., Stober, J.A., and Olson, G.R., 1991. Site-specific modulation of carcinogen-induced gastrointestinal tract nuclear anomalies in B6C3F1 mice by chloroform. Anticancer Res., 11, 665-670.

Davies, D.P., Gomez, M.P., Barton, S.L., Montano, R.M., and Burchiel, S.W., 1991. Suppression of local gut-associated and splenic mitogen responsiveness of lymphoid cells following oral exposure of B6C3F1 mice to 7,12-dimethylbenz[a]anthracene. Fund. Appl. Toxicol., 17, 429-431.

Davila, D.R., Davis, D.P., Campbell, K., Cambier, J.C., Zigmond, L.A., and Burchiel, S.W., 1995. Role of alterations in Ca++ associated signaling pathways in the immunotoxicity of polycyclic aromatic hydrocarbons. J. Toxicol. Environ. Health, 45, 101-126.

Davila, D.R., Romero, D.L., and Burchiel, S.W., 1996. Human T-cells are highly sensitive to suppression of mitogenesis by polycyclic aromatic hydrocarbons and this effect is differentially reversed by alpha-naphtoflavone. Toxicol. Appl. Pharmacol., 139, 333-341.

De Jong, W.H., Kroese, E.D., Vos, J.G., and Van Loveren, H., 1999. Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol. Sci., 50, 214-220.

De Vos, R.H., Van Dokkum, W., Schouten, A., and De Jong-Berkhout, P., 1990. Polycyclic aromatic hydrocarbons in Dutch total diet samples (1984-1986). Food Chem. Toxicol. 28, 263-268.

Dell'Omo, M. and Lauwerys, R.R., 1993. Adducts to macromolecules in the biological monitoring of workers exposed to polycyclic aromatic hydrocarbons. Crit. Rev. Toxicol., 23, 111-126.

Dejmek, J., Solanský, I., Beneš, I., Leniček, J., and Šrám, R.J., 2001. Air pollution and pregnancy outcome. In: Teplice Program: Impact of Air Pollution on Human Health (Šrám, R.J., Ed.), Academia, Prague, pp. 127-137.

Delgado-Rodriguez, A., Ortiz-Marttelo, R., Graf, U., Villalobos-Pietrini, R., and Gomez-Arroyo, S.,1995.Genotoxic activity of environmentally important polycyclic aromatic hydrocarbons and their nitro derivatives in the wing spot test of Drosophila melanogaster. Mutat Res., 341,235-247.

Deml, E., Oesterle, D., and Wiebel, F. J. 1983, "Benzo[a]pyrene initiates enzyme-altered islands in the liver of adult rats following single pretreatment and promotion with polychlorinated biphenyls", Cancer Lett., vol. 19, pp. 301-304.

Denison, M.S., and Phelan, D., 1998. The Ah receptor signal transduction pathway. In: Toxicant-Receptor Interactions (Denison, M.S., and Helferich, W.G., Eds.), Taylor and Francis, London, pp. 3-33.

Dennis, M.J., Massey, R.C., McWeeny, D.J., Knowles, M.E., and Watson, D., 1983. Analysis of polycyclic aromatic hydrocarbons in UK total diets. Food Chem. Toxicol., 21, 569-574.

Dennis, M. J., Massey, R. C., McWeeny, D.J., Larsson, B., Eriksson, A., and Sahlberg, G., 1984. Comparison of a capillary gas chromatographic and a high-performance liquid chromatographic method of analysis for polycyclic aromatic hydrocarbons in food. J. Chromatogr., 285, 127-133.

Dennis, M.J., Cripps, G., Venn, I., Howarth, N., and Lee, G., 1991. Factors affecting the polycyclic aromatic hydrocarbon content of cereals, fats, and other food products. Food Add. Contam., 8, 517-530.

Dertinger, S.D., Nazarenko, D.A., Silverstone, A.E., and Gasiewicz, T.A., 2001. Aryl hydrocarbon receptor signalling plays a significant role in mediating benzo[a]pyrene- and cigarette smoke condensate-induced cytogenic damage in vivo. Carcinogenesis, 22, 171-177.

Deutsch Wenzel, R.P., Brune, H., Grimmer, G., Dettbarn, G., Misfeld, J. and Timm, J., 1984. Investigation on the carcinogenicity of emission condensate from brown coal-fired residential furnaces applied to mouse skin. Cancer Lett., 25, 103-111.

Donnelly K.C., Brown, K.W., Markiewicz, K.V., Anderson, C.S., Manek, D.J., Thomas, J.C., and Giam, C.S., 1993. The use of short-term bioassays to evaluate the health and environmental risk posed by an abandoned coal-gasification site. Hazard. Waste Hazard. Mater., 10, 59-70.

Donnelly, K.C., Phillips, T.D., Onufrock, A.M., Collie, S.L., Huebner, H.J., and Washburn, K.S., 1996. Genotoxicity of model and complex mixtures of polycyclic aromatic hydrocarbons. In: Environmental toxicology and risk asessment: biomarkers and risk assessment (Bengtson, D.A., and Henshel, D.S., Eds.), 5th vol., ASTM STP 1306, American Association of Testing and Materials, pp. 138-148.

Dontenwill, W. and Mohr, U., 1962. Experimentelle Untersuchungen zum Problem der Carcinomentstehung im Respirationstrakt. I: Die unterschiedliche Wirkung des Benzpyrens auf die Epithelien der Haut, der Mundhöhle und der Trachea des Goldhamsters. Z. Krebsforsch., 65, 56-61.

Dörr, G., Hippelein, M., Kaupp, H., and Hutzinger, O., 1996. Baseline contamination assessment for a new resource recovery facility in Germany: Part VI: levels and profiles of polycyclic aromatic hydrocarbons (PAH) in ambient air. Chemosphere, 33, 1569-1578.

Duell, E.J., Wiencke, J.K., Cheng, T.J., Varkonyi, A., Zuo, Z.F., Ashok, T.D., Mark, E.J., Wain, J.C., Christiani, D.C. and Kelsey, K.T., 2000. Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis, 21 (5), 965-971.

Durant, J.L., Busby, W.F. Jr, Lafleur, A.L., Penman, B.W., and Crespi, C.L., 1996. Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. Mutat. Res., 371, 123-157.

EC (European Commission), 2001a. Commission Decision 2001/773/EC of 26 October 2001 amending Commission Decision 94/652/EC establishing the inventory and distribution of tasks to be undertaken within the framework of cooperation by Member States in the scientific examination of questions relating to food. Official Journal of the European Communities, 7.11.2001, L290/9-11.

EC (European Communities), 2001b. Ambient Air Pollution by Polycyclic Aromatic Hydrocarbons (PAH). Position Paper. July 27th 2001 (prepared by the Working Group On Polycyclic Aromatic Hydrocarbons). Office for Official Publications of the European Communities, Luxembourg, ISBN 92-894-2057-X
http://europa.eu.int/comm/environment/air/pp_pah.pdf

EC (European Communities), 2001c. Ambient Air Pollution by Polycyclic Aromatic Hydrocarbons (PAH) - Position Paper Annexes, July 27th 2001 (prepared by the Working Group On Polycyclic Aromatic Hydrocarbons).
http://europa.eu.int/comm/environment/air/annex_pah.pdf

EEC (European Economic Community), 1988. Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. Official Journal of the European Communities, 15.7.1988, L184/61-67.

Edwards, N.T., 1983. Polycyclic hydrocarbons (PAHs) in the terrestrial environment. A review. J. Environ. Qual., 12, 427-441.

Einolf, H.J., Amin, S., Yagi, H., Jerina, D.M., and Baird, W.M., 1996. Benzo[c]phenanthrene is activated to DNA-binding diol epoxides in the human mammary carcinoma cell line MCF-7 but only limited activation occurs in mouse skin. Carcinogenesis, 17,2237-2244.

Eisenstadt, E., Warren, A.J., Porter, J., Atkins, D., and Miller, J.H., 1982. Carcinogenic epoxides of benzo[a]pyrene and cyclopenta[cd]pyrene induce base substitutions via specific transversions. Proc. Natl. Acad. Sci. U.S.A., 79, 1945-1949.

Elferink, C.J., and Whitlock, J.P. Jr., 1994. Dioxin-dependent, DNA sequence-specific binding of a multiprotein complex containing the Ah receptor. Receptor, 4, 157-163.

Evans, W.H., Thomas, N.C., Boardman, M.C., and Nash, S.J., 1993. Relationships of polycyclic aromatic hydrocarbon yield with particulate matter (water and nicotine free) yields in mainstream and sidestream cigarette smoke. Sci. Total Environ., 136, 101-109.

Fahy, O., Tsicopoulos, A., Hammad, H., Pestel, J., Tonnel, A.B., and Wallaert, B., 1999. Effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells. J. Allergy Clin. Immunol., 103, 1115-1124.

Fernandez-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., Lee, S.S.T., Kimura, S., Nebert, D., Rudikoff, S., Ward, J.M., and Gonzalez, F.J., 1995. Immune system inpairment and hepatic fibrosis in mice lacking the dioxin-binding Ah-receptor. Science, 268, 722-726.

Flesher, J.W., Horn, J. and Lehner, A.F., 1998. 9-Sulfooxymethylanthracene is an ultimate electrophilic and carcinogenic form of 9-hydroxymethylanthracene. Biochem. Biophys. Res. Comm., 251, 239-243.

Fontana, R.J., Lown, K.S., Paine, M.F., Fortlage, L., Santella, R.M., Felton, J.S., Knize, M.G., Greenberg, A., and Watkins, P.B., 1999. Effects of a chargrilled meat diet on expression of CYP3A, CYP1A, and P-glycoprotein levels in healthy volunteers. Gastroenterology, 117, 89-98.

Fritsche, E., Brüning, T., Jonkmanns, C., Ko, Y., Bolt, H.M., and Abel, J., 1999. Detection of cytochrome P450 1B1 BfrI polymorphism: genotype distribution in healthy German individuals and in patients with colorectal cancer. Pharmacogenetics, 9, 405-408.

Fritzenschaf, H., Kohlpoth, M., Rusche, B., and Schiffmann, D.,1993. Testing of known carcinogens and noncarcinogens in the Syrian hamster embryo (SHE) micronucleus test in

vitro; correlations with in vivo micronucleus formation and cell transformation. Mutat. Res., 319, 47-53.

Gaines, T.B., 1969. Acute toxicity of pesticides. Toxicol. Appl. Pharmacol., 14, 515-534.

Garg, A., Beach, A.C., and Gupta, R.C., 1993. Interception of reactive, DNA adduct-forming metabolites present in rodent serum following carcinogen exposure: implications for use of body fluids in biomonitoring. Teratog. Carcinog. Mutagen., 13,151-166.

Garrison, P.M., Tullis, K., Aarts, J.M.M.J.G., Brouwer, A., Giesy, J.P., and Denison, M.S., 1996. Species-specific recombinanT-cell lines as bioassay systems for the detection of 2,3,7,8-tetrachlorodibenzo-p-dioxin-like chemicals. Fundam. Appl. Toxicol., 30, 194-203.

Garte, S., and Crosti, F., 1999. A nomenclature system for metabolic gene polymorphisms. In: Metabolic polymorphisms and suceptibility to cancer (Vineis, P., Malats, N., Laang, M., d'Erricco, A., Caporaso, N., Cuzick, J., and Bofetta, P., Eds). IARC Scientific publication, 148, 5-12.

Garte, S., Gaspari, L., Alexandrie, A.K., Ambrosone, C., Autrup, H., Autrup, J.L., Baranova, H., Bathum, L., Benhamou, S., Boffetta, P., Bouchardy, C., Breskvar, K., Brockmöller, J., Cascorbi, I., Clapper, M.L., Coutelle, C., Daly, A., Dell'Omo, M., Dolzan, V., Dresler, C.M., Fryer, A., Haugen, A., Hein, D.W., Hildesheim, A., Hirvonen, A., Hsieh, L.L., Ingelman-Sundberg, M., Kalina, I., Kang, D., Kihara, M., Kiyohara, C., Kremers, P., Lazarus, P., Le Marchand, L., Lechner, M.C,, van Lieshout, E.M., London, S., Manni, J.J., Maugard, C.M., Morita, S., Nazar-Stewart, V., Noda, K., Oda, Y., Parl, F.F., Pastorelli, R., Persson, I., Peters, W.H., Rannug, A., Rebbeck, T., Risch, A., Roelandt, L., Romkes, M., Ryberg, D., Salagovic, J., Schoket, B., Seidegard, J., Shields, P.G., Sim, E., Sinnet, D., Strange, R.C., Stucker, I., Sugimura, H., To-Figueras, J., Vineis, P., Yu, M.C., and Taioli, E., 2001. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol. Biomarkers Prev., 10, 1239-1248.

Gatehouse, D., 1980. Mutagenicity of 1,2 ring-fused acenaphthenes against *S. typhimurium* TA1537 and TA1538: structure-activity relationship. Mutat. Res., 78, 121-135.

Gerarde, H.W., 1960. Toxicology and biochemistry of aromatic hydrocarbons. In: Elsevier monographs on toxic agents (Browning, E., Ed.), Elsevier, Amsterdam, pp. 240-321.

Germany, Ministry of Environment, 1994. Cited in IPCS (1998).

Gertig, D.M., Stampfer, M., Haiman, C., Hennekens, C.H., Kelsy, K., and Hunter, D.J., 1998. Glutathione S-transferase GSTM1 and GSTT1 polymorphisms and colorectal cancer risk: A prospective study. Cancer Epidemiol. Biomarkers Prev., 7, 1001-1005.

Gibel, W., 1964. Experimenteller Beitrag zur Synkarzinogenese beim Speiseröhrenkarzinom. Der Krebsarzt, 19, 268-272.

Gladen, B.C., Zadorozhnaja, T.D., Chislovska, N., Hryhorczuk, D.O., Kennicutt, M.C., and Little, R.E., 2000. Polycyclic aromatic hydrocarbons in placenta. Hum. Exper. Toxicol., 19, 597-603.

Godschalk, R.W., Moonen, E.J., Schilderman, P.A., Broekmans, W.M., Kleinjans, J.C., Van Schooten, F.J., 2000. Exposure-route-dependent DNA adduct formation by polycyclic aromatic hydrocarbons. Carcinogenesis, 21, 87-92.

Goldstein, L.S., Weyand E.H., Safe, S., Steinberg, M., Culp, S.J., Gaylor, D.W., Beland, F.A., and Rodriguez, L.V., 1998. Tumours and DNA adducts in mice exposed to benzo[a]pyrene and coal tars: implications for risk assessment. Environ. Health Perspect., 106, Suppl 6, 1325-1330.

Gott, D., personal communication.

Grimmer, G., 1983. Cigarettes. In: Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons (Grimmer, G., Ed.), CRC Press, Boca Raton, pp. 77-84.

Grimmer, G., Brune, H., Deutsch Wenzel, R., Dettbarn, G. and Misfeld, J., 1984a. Contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of gasoline engine exhaust condensate evaluated by implantation into the lungs of rats. J. Natl. Cancer Inst., 72, 733-739.

Grimmer, G., Brune, H., Deutsch Wenzel, R., Dettbarn, G., Misfeld, J., Abel, U. and Timm, J., 1984b. The contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of emission condensate from coal-fired residential furnaces evaluated by topical application to the skin of mice. Cancer Lett., 23, 167-176.

Grimmer, G., Brune, H., Deutsch Wenzel, R., Dettbarn, G., Misfeld, J., Abel, U. and 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., Brune, H., Deutsch Wenzel, R., Dettbarn, G. and Misfeld, J., 1987a. Contribution of polycyclic aromatic hydrocarbons and polar polycyclic aromatic compounds to the carcinogenic impact of flue gas condensate from coal-fired residential furnaces evaluated by implantation into the rat lung. J. Natl. Cancer Inst., 78, 935-942.

Grimmer, G., Brune, H., Deutsch Wenzel, R., Dettbarn, G., Jacob, J., Naujack, K.W., Mohr, U. and Ernst, H., 1987b. Contribution of polycyclic aromatic hydrocarbons and nitro- derivatives to the carcinogenic impact of diesel engine exhaust condensate evaluated by implantation into the lungs of rats. Cancer Lett., 37, 173-180.

Grimmer, G., Brune, H., Dettbarn, G., Naujack, K.W., Mohr, U. and Wenzel Hartung, R., 1988. Contribution of polycyclic aromatic compounds to the carcinogenicity of sidestream

smoke of cigarettes evaluated by implantation into the lungs of rats. Cancer Lett., 43, 173-177.

Grimmer, G., Dettbarn, G., and Jacob, J., 1993. Biomonitoring of polycyclic aromatic hydrocarbons in higly exposed coke plant workers by measurement of urynary phenanthrene and pyrene metabolites (phenols and dihydrodiols). Int. Arch. Occup. Environ. Health, 65, 189-199.

Guillen, M.D., Sopelana, P., and Partearroyo, M.A., 1997. Food as a source of polycyclic aromatic carcinogens. Rev. Environ. Health, 12, 133-146.

Guillen, M.D., Sopelana, P., and Partearroyo, M.A., 2000. Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. J Agric Food Chem., 48, 5083-5087.

Gustavsson, P., Plato, N., Hallqvist, J., Hogstedt, C., Lewne, M., Reuterwall, C., Scheele, P., 2001. A population-based case-referent study of myocardial infarction and occupational exposure to motor exhaust, other combustion products, organic solvents, lead, and dynamite. Stockholm Heart Epidemiology Program (SHEEP) Study Group. Epidemiology, 12, 222-228.

Hainaut, P., and Pfeifer, G.P., 2001. Patterns of p53 G-->T transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. Carcinogenesis, 22, 367-374.

Hakura, A., Tsutsui, Y., Sonoda, J., Tsukidate, K., Mikami, T., and Sagami, F., 2000. Comparison of the mutational spectra of the lacZ transgene in four organs of the MutaMouse treated with benzo[a]pyrene: target organ specificity. Mutat. Res., 447, 239-247.

Hamers, T., van Schaardenburg, M.D., Felzel, E.C., Murk, A.J., and Koeman, J.H., 2000. The application of reporter gene assays for the determination of the toxic potency of diffuse air pollution. Sci. Total Environ., 262, 159-174.

Hansen, C., Asmussen, I., and Autrup, H., 1993. Detection of carcinogen-DNA adducts in human fetal tissues by the ^{32}P-postlabeling procedure. Environ.Health Perspect., 99, 229-231.

Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., and Niemeier, R.W., 1981. Testing of selected workplace chemical for teratogenic potential. Scand. J. Work Environ.Health, 7, 66-75.

Hardin, B.D., Schuler, R.L., Burg, J.R., Booth, J.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J., and Smith, K.N., 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog. Carcinog. Mutag., 7, 29-48.

Harper, N., Steinberg, M., and Safe, S., 1996. Immunotoxicity of a reconstituted polynuclear aromatic hydrocarbon mixture in B6C3F1 mice. Toxicology, 109, 31-38.

Hasegawa, R., Mutai, M., Imaida, K., Tsuda, H., Yamaguchi, S., and Ito, N. 1989. Synergistic effects of low-dose hepatocarcinogens in induction of glutathione S-transferase P-positive foci in the rat liver. Jpn. J. Cancer Res., 80, 945-951.

Haugen, A., Becher, G., Benestad, C., Vahakangas, K., Trivers, G.E., Newman, M.J., and Harris, C.C., 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.

Hayashi, S.-I., Watanabe, J., Nakachi, K., Eguchi, H., Gotoh, O., and Kawajiri, K., 1994. Interindividual differences in expression of human Ah receptor and related genes. Carcinogenesis, 15, 801-806.

Heidel, S.M., Holston, K., Buters, J.T., Gonzalez, F.J., Jefcoate, C.R., and Czupyrynski, C.J., 1999. Bone marrow stromal cell cytochrome P4501B1 is required for pre-B cell apoptosis induced by 7,12-dimethylbenz[a]anthracene. Mol. Pharmacol., 56, 1317-1323.

Heidel, S.M., MacWilliams, P.S., Baird, W.M., Dashwood, M., Buters, J.T., Gonzalez, F.J., Larsen, M.C., Czuprynski, C.J., and Jefcoate, C.R., 2000. Cytochrome P4501B1 mediates induction of bone marrow cytotoxicity and preleukemia cells in mice treated with 7,12-DMBA. Cancer Res., 60, 3454-3460.

Heinrich, U., Roller, M. and Pott, F., 1994. Estimation of a lifetime unit lung cancer risk for benzo(a)pyrene based on tumour rates in rats exposed to coal tar/pitch condensation aerosol. Toxicol. Lett., 72, 155-161.

Helleberg, H., Xu, H., Ehrenberg, L., Hemminki, K., Rannug, U., and Tornqvist, M., 2001. Studies of dose distribution, premutagenic events and mutation frequencies for benzo[a]pyrene aiming at low dose cancer risk estimation. Mutagenesis, 16,333-337.

Henderson, C.J., Smith, A.G., Ure, J., Brown, K., Bacon, E.J., and Wolf, C.R., 1998. Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. Proc. Natl. Acad. Sci. USA, 95, 5275-5280.

Heo, Y., Saxon, A., and Hankinson, O., 2001. Effect of diesel exhaust particles and their components on the allergen-specific IgE and IgG1 response in mice. Toxicology, 159, 143-158.

Hermann, M., 1981.Synergistic effects of individual polycyclic aromatic hydrocarbons on the mutagenicity of their mixtures. Mutat. Res., 90, 399-409.

Hietaniemi, V., Ovaskainen, M.L., and Hallikainen A., 1999. PAH compounds and their intake from foodstuffs on the market. National Food Administration, Research Notes 6.

Holladay, S.D., and Smith, B.J., 1995a. Alterations in murine fetal thymus and liver hematopoietic cell populations following developmental exposure to 7,12-dimethylbenz[a]anthracene. Environ. Res., 68, 106-113.

Holladay, S.D., and Smith, B.J., 1995b. Benzo[a]pyrene-induced alterations in total immune cell number and cell surface antigen expression in the thymus, spleen and bone marrow of B6C3F1 mice. Vet. Hum. Toxicol., 1995b, 37, 99-104.

Holladay, S.D., 2001. Perinatal immunotoxicant exposure and increased risk of autoimmune disease. Toxicologist, 60, 65.

Hood, D.B., Nayyar, T., Greenwood, M., Aramandla, R., Nunes, M., Inyang, F., Nyanda, A., and Archibong, A., 2001. Subacute exposure of timed-pregnant rats to benzo[a]pyrene:carbon black aerosol; effects on development in the F1 generation-I. Toxicologist, 60, 275. Abstract No.1307.

Hood, D.B., Nayyar, T., Ramesh, A., Greenwood, M., and Inyang, F., 2000. Modulation in the developmental expression profile of Sp1 subsequent to transplacental exposure of fetal rats to desorbed benzo[a]pyrene following maternal inhalation. Inhal.Toxicol., 12, 511-513.

Hoshino, K., Hayashi, Y., Takehira, Y., and Kameyama, Y., 1981. Influences 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.

Hu, X., Xia, H., Srivastava, S.K., Pal, A., Awasthi, Y.C., Zimniak, P., and Singh, S.V., 1998. Catalytic efficiencies of allelic variants of human glutathione S-transferase P1-1 towards carcinogenic anti-diol epoxides of benzo[c]phenanthrene og benzo[g]chrysene. Cancer Res., 58, 5340-5343.

Hu, X., Herzog, C., Zimniak, P., and Singh, S.V., 1999. Differential protection against benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide-induced DNA damage in HepG2 cells stably transfected with allelic variants of pi-class human glutathione S-transferase. Cancer Res., 59, 2358-2362.

Huggins, C., and Yang, N.C., 1962. Induction and extinction of mammary cancer. A striking effect of hydrocarbons permits analysis of mechanisms of causes and cure of breast cancer. Science, 137, 257-262.

Hughes, N.C., Pfau, W., Hewer, A., Jacob, J., Grimmer, G., and Phillips, D.H., 1993. Covalent binding of polycyclic aromatic hydrocarbon components of coal tar to DNA in mouse skin. Carcinogenesis, 14, 135-44.

Hughes, N.C., and Phillips, D.H., 1993. ^{32}P-postlabelling analysis of the covalent binding of benzo[ghi]perylene to DNA in vivo and in vitro. Carcinogenesis, 14, 127-133.

Husterts, E., Haberl, M., Burk, O., Wolbold, R., He, Y.-Q., Klein, K., Nuessler, A.C., Neuhaus, P., and Wojnowski, L., 2001. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics, 11, 773-779.

IARC, 1973. Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 3, International Agency for Research on Cancer, Lyon.

IARC, 1983. Polynuclear Aromatic Compounds. Part 1, Chemical, Environmental and Experimental Data. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 32, International Agency for Research on Cancer, World Health Organization, Lyon.

IARC, 1984. Polynuclear Aromatic Compounds: Part 3, Industrial Exposures to Aluminum Production, Coal Gasification, Coke Production, and Iron Steel Founding. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 34, International Agency for Research on Cancer, World Health Organization, Lyon.

IARC, 1985. Polynuclear Aromatic Compound: Part 4, Bitumens, Coal tars and Derived products, Shale-oils and Soots. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 35, International Agency for Research on Cancer,World Health Organization, Lyon.

IARC, 1986. Tobacco Smoking. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 38, International Agency for Research on Cancer, World Health Organization, Lyon.

IARC, 1987. Overall evaluation of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Supplement 7, International Agency for Research on Cancer, World Health Organization, Lyon.

IARC, 1999. Metabolic polymorphisms and susceptibility to cancer (Vineis, P., Malats, N., Lang, M., d'Ericco, A., Capaaso, N., Cuzick, J., and Boffetta, P., Eds.). IARC Scientific Publications no 148, International Agency for Research on Cancer, World Health Organization, Lyon, pp 505.

Ingelman-Sundberg, M., 2001. Genetic susceptibility to adverse effects of drugs and environmental toxicants. The role of the CYP family enzymes. Mutat. Res., 482, 11-19.

IPCS, 1998. Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria 202. International Programme on Chemical Safety, World Health Organization, Geneva.

IRIS, 2002. Integrated Risk Information System (IRIS): summaries for acenaphtene (dated 1994), anthracene (dated 1993), fluoranthene (dated 1993), fluorene (dated 1990),

naphtalene (dated 1998), pyrene (dated 1993), and benzo[a]-pyrene (dated 1994). US Environmental Protection Agency, Washington, DC.

Izzotti, A., Cartiglia, C., Lewtas, J., and De Flora, S., 2001a. Increased DNA alterations in atherosclerotic lesions of individuals lacking the GSTM1 genotype. FASEB J., 15, 752-757.

Izzotti, A., Camoirano, A., Cartiglia, C., Tampa, E., and De Flora, S., 2001b. Formation of DNA adducts in the aorta of smoke-exposed rats, and modulation by chemopreventive agents. Mutat. Res., 494, 97-106.

Jongeneelen, F.J., van Leeuwen, F.E., Oosterink, S., Anzion, R.B.M., van der Loop, F., Bos, R.P., and van Veen, H.G., 1990. Ambient and biological monitoring of cokeoven workers: determinants of the internal dose of polycyclic aromatic hydrocarbons. Br. J. Ind. Med., 47, 454-461.

Kang, D.H., Rothman, N., Poirier, M.C., Greenberg, A., Hsu, C.H., Schwartz, B.S., Baser, M.E., Groopman, J.D., Weston, A., and Strickland, P.T., 1995. Interindividual differences in the concentration of 1-hydroxypyrene-glucuronide in urine and polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells after charbroiled beef consumption. Carcinogenesis, 16, 1079-1085.

Karakaya, A., Yucesoy, B., Turhan, A., Erdem, O., Burgaz, S., and Karakaya, A.E., 1999. Investigation of some immunological functions in a group of asphalt workers exposed to polycyclic aromatic hydrocarbons. Toxicology, 135, 43-47.

Karl, H., and Leinemann, M., 1996. Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns. Z. Lebensm. Unters. Forsch., 202, 458-464.

Kawai, M., 1979. Review of toxicological and occupational aspects of naphthalene. Aromatics, 31, 168-181 (in Japanese, cited in IPCS, 1998).

Kawamura, Y., Kamata, E., Ogawa, Y., Kaneko, T., Uchiyama, S., and Saito, Y., 1988. The effect of various foods on the intestinal absorption of benzo[a]pyrene in rats. J. Food Hyg. Soc. Jpn., 29, 21-25.

Kayali, M.N., and Rubio-Barroso, S., 1995. Determination of benzo[a]pyrene in total particulate matter of Virginia and black tobacco smoke by HPLC with fluorimetric detection. J. Liquid Chromatogr., 18, 1617-1632.

Kayali-Sayadi, M.N., Rubio-Barroso, S., Cuesta-Jimenez, M.P., Polo-Diez, L.M., 1999. A new method for the determination of selected PAH in coffee brew samples by HPLC with fluorimetric detection and solid-phase extraction. J. Liquid Chromatogr. Relat. Technol., 22, 615-627.

Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A., and Rothman, N., 2001. Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. Food Chem. Toxicol., 39, 423-436.

Keohavong, P., and Thilly, W.G., 1992. Determination of point mutational spectra of benzo[a]pyrene-diol epoxide in human cells. Environ. Health Perspect., 98, 215-219.

Keohavong, P., Melacrinos, A., and Shukla, R., 1995. In vitro mutational spectrum of cyclopenta[cd]pyrene in the human HPRT gene. Carcinogenesis, 16,855-860.

Kier, L.D, Brusick, D.J., Auletta, A.E., Von Halle, E.S, Brown, M.M., Simmon, V.F., Dunkel, V, McCann, J., Mortelmans, K., Kier, L.E., *et al.*, 1986. The Salmonella typhimurium/mammalian microsomal assay. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res., 168, 69-240.

Klein, M., 1963. Susceptibility of strain B6AF1/J hybrid infant mice to tumourigenesis with 1,2-benzanthracene, deoxycholic acid, and 3-methylcholanthrene. II. Tumours called forth by painting the skin with dibenzpyrene. Cancer Res, 23, 1701-1707.

Knize, M.G., Salmon, C.P., Pais, P., and Felton, J.S., 1999. Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. Adv. Exp. Med. Biol., 459, 179-193.

Koganti, A., Singh, R., Rozett, K., Modi, N., Goldstein, L.S., Roy, T.A., Zhang, F., Harvey, R.G., Weyland, E.H., 2000. 7H-benzo[c]fluorene: a major DNA adduct-forming component of coal tar. Carcinogenesis 21, 1601-1609.

Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, W.O., Sorge, J.A., Putman, D.L., and Short, J.M., 1991. Spectra of spontaneous and mutagen-induced mutations in the lacI gene in transgenic mice. Proc. Natl. Acad. Sci. USA, 88, 7958-7962.

Krewski, D., Thorslund, T., and Withey, J., 1989. Carcinogenic risk assessment of complex mixtures. Toxicol. Ind. Health, 1989, 5, 851-867.

Krieger, J.A., Born, J.L., and Burchiel, S.W., 1994. Persistence of Calcium elevation in the HPB-ALL human T-cell line correlates with immunosuppressive properties of polycyclic aromatic hydrocarbons. Toxicol. Appl. Pharmacol., 127, 268-274.

Kroese, E.D., Muller, J.J.A., Mohn, G.R., Dortant, P.M. and Wester, P.W., 2001. Tumourigenic effects in Wistar rats orally administered benzo[a]pyrene for two years (gavage studies). Implications for human cancer risks associated with oral exposure to polycyclic aromatic hydrocarbons. National Institute of Public Health and the Environment, RIVM Report no. 658603 010, November 2001, Bilthoven.

Kyrtopoulos, S.A., Georgiadis, P., Autrup, H., Demopoulos, N.A., Farmer, P., Haugen, A., Katsouyanni, K., Lambert, B., Ovrebo, S., Sram, R., Stephanou, G., Topinka, J., Stefanou, G., Demopoulos, N., 2001. Biomarkers of genotoxicity of urban air pollution. Overview and descriptive data from a molecular epidemiology study on populations exposed to

moderate-to-low levels of polycyclic aromatic hydrocarbons: the AULIS project. Mutat. Res., 496, 207-228.

Könemann, W.H. and Pieters, M.N. (1996). Confusion of concepts in mixture toxicology. Fd Chem. Toxicol., 34, 1025-1031.

Lafleur, A.L., Longwell, J.P., Marr, J.A., Monchamp, P.A., Plummer, E.F., Thilly, W.G., Mulder, P.P., Boere, B.B., Cornelisse, J., and Lugtenburg, J., 1993. Bacterial and human cell mutagenicity study of some C18H10 cyclopenta-fused polycyclic aromatic hydrocarbons associated with fossil fuels combustion. Environ. Health Perspect., 101,146-153.

Lai, Z.-W,, Pineau, T., and Esser, C., 1996. Identification of dioxin-responsive elements (DREs) in the 5'regions of putative dioxin-inducible genes. Chem. Biol. Interact., 100, 97-112.

Larsen, J.C., and Larsen, P.B., 1998. Chemical Carcinogens. In: Air Pollution and Health (Hester, R.E., and Harrison, R.M., Eds.), Issues in Environmental Sciences and Technology, 10, The Royal Society of Chemical, Cambridge.

Larsson, B.K., 1982. Polycyclic Aromatic Hydrocarbons in Smoked Fish. Z. Lebensm. Unters. Forsch., 174, 101-107.

Larsson, B., and Sahlberg, G., 1982. Polycyclic aromatic hydrocarbons in lettuce. Influence of a highway and an aluminium smelter. In : Polynuclear Aromatic Hydrocarbons: Physical and biological chemistry (Cooke, M., Denis, A.J., and Fisher, G.L., Eds.). Battelle Press, Colombus, Ohio, pp 417-426.

Larsson, B.K., Eriksson, A.T., and Cervenka, M., 1987. Polycyclic Aromatic Hydrocarbons in Crude and Deodorized Vegetable Oils. J. Am. Oil Chem. Soc., 64, 365-370.

Laurent, C., Feidt, C., Lichtfouse, E., Grova, N., Laurent, F., and Rychen, G., 2001. Milk-blood transfer of -1-4C-tagged polycyclic aromatic hydrocarbons (PAH) in pigs. J. Agric. Food Chem., 49, 2493-2496.

Lavoie, E.J., Braley J., Rice J.E., and Rivenson A., 1987. Tumourigenic activity of non-alternant polynuclear aromatic hydrocarbons in newborn mice. Cancer Lett., 1987, 34, 15-20.

Lechner, W., Huber, M., Bonn, G.K., and Daxenbichler, G., 1991. Analysis of polycyclic aromatic hydrocarbons in lipid-containing biological matrices. Study of contamination of breast milk by benzo(a)pyrene along transit routes through Tyrol. Wien. klin. Wochenschr., 103, 88-90 [in German]. Abstract available from Pubmed database.

Lee, J., Kang, D., Lee, K.H., Ichiba, M., Zhang, J., Tomokuni, K., Hwang, E.S., Park, C.G., Ha, M., Kim, S., Han, S.B., Choi, J.W., Lee, E., Jang, J.Y., Strickland, P.T., Hirvonen, A.,

and Cho, S.H., 2002. Influence of GSTM1 genotype on association between aromatic DNA adducts and urinary PAH metabolites in incineration workers. Mutat. Res., 514, 213-221.

Legraverend, C., Harrison, D.E., Ruscetti, F.W., and Nebert, D.W., 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., Guenthner, T.M., and Nebert, D.W., 1984. Importance of the route of administration for genetic differences in benzo[a]pyrene-induced in utero toxicity and teratogenicity. Teratology, 29, 35-47.

Lewtas, J., 1988. Genotoxicity of complex mixtures: strategies for the identification and comparative assessment of airborne mutagens and carcinogens from combustion sources. Fundam. Appl. Toxicol., 10, 571-589.

Lijinsky, W., 1991. The formation and occurrence of Polynuclear Aromatic Hydrocarbons associated with food. Mutat. Res., 259, 251-261.

Lijinsky, W., and Ross, A.E., 1967. Production of carcinogenic polynuclear hydrocarbons in the cooking of food. Food Cosmet. Toxicol., 5, 343-347.

Lijinsky, W., and Shubik, P., 1965a. Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. Science, 145, 53-55.

Lijinsky, W., and Shubik, P., 1965b. Polynuclear hydrocarbon carcinogens in cooked meat and smoked foods. Ind. Med. Surg., 34, 152-154.

Lin, H.J., Probst-Hensch, N.M., Louie, A.D., Kau, I.H., Witte, J.S., Ingles, S.A., Frankl, H.D., Lee, E.R., and Haile, R.W., 1998. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. Cancer Epidemiol. Biomarkers Prev., 7, 647-652.

Lintas, C., De Matthaeis, M.C., and Merli, F., 1979. Determination of benzo[a]pyrene in smoked, cooked and toasted food products. Food Cosmet. Toxicol., 17, 324-328.

London, S.J., Yuan, J.-M., Chung, F.-L., Gao, Y.-T., Coetzee, G.A., Ross, R.K., and Yu, M.C., 2000. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet, 356, 724-729

Long, D.J. 2nd, Waikel, R.L., Wang, X.-J., Perlaky, L., Roop, D.R., and Jaiswal, A.K., 2000. NAD(P)H:Quinone oxidoreductase 1 deficiency increases susceptibility to BP-induced mouse skin carcinogenesis. Cancer Res., 60, 5913-5915.

Longuemaux, S., Delomenie, C., Gallou, C., Mejean, A., Vincent-Viry, M., Bouvier, R., Droz, D., Krishnamoorthy, R., Galteau, M.M., Junien, C., Beroud, C., and Dupret, J.M.m 1999. Candidate genetic modifiers of individual susceptibility to renal cell carcinoma: a

study of polymorphic human xenobiotic-metabolizing enzymes. Cancer Res., 59 (12), 2903-2908.

Lopez-Abente, G., Sanz-Anquela, J.M., and Gonzalez, C.A., 2001. Consumption of wine stored in leather wine bottles and incidence of gastric cancer. Arch Environ Health, 56, 559-561.

Machala, M., Vondracek, J., Bláha, L., Ciganek, M., and Neca, J., 2001a. Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. Mutat. Res., 497, 49-62.

Machala, M., Neča, J., Bláha, L., Dejmek, D., and Šrám, R.J., 2001b. Aromatase (CYP19) and 7-ethoxyresorufin O-deeethylase (CYP1A1) activities in human placental samples collected in two Czech areas differing in pollution rates. In: Teplice Program: Impact of Air Pollution on Human Health (Šrám, R.J., Ed.), Academia, Prague, pp. 139-144.

MacKenzie, K.M., and Angevine, D.M., 1981. Infertility in mice exposed in utero to benzo[a]pyrene. Biol. Reprod., 24, 183-191.

Madhavan, N.D., and Naidu, K.A., 1995. Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. Hum. Exp. Toxicol., 14, 503-506.

Maier, H.G., 1991. Carcinogenic compounds content in coffee beans. In : Café, cacao, the (Paris), vol XXXV, n°2, pp 133-142.

Malmgren, R.A., Bennison, B.E., and McKinley, T.W. Jr., 1952. Reduced antibody titers in mice treated with carcinogenic and cancer chemotherapeutic agents. Proc. Soc. Exp. Biol. Med., 72 : 484-488.

Mann, K.K., Matulka, R.A., Hahn, M.E., Trombino, A.F., Lawrence, B.P., Kerkvliet, N.I., and Sherr, D.H., 1999. The role of polycyclic aromatic hydrocarbon metabolism in DMBA-induced pre-B-lymphocyte apoptosis. Toxicol Appl. Pharmacol., 161, 10-22.

Marsch, G.A., Jankowiak, R., Small, G.J., Hughes, N.C., and Phillips, D.H., 1992. Evidence of involvement of multiple sites of metabolism in the in vivo covalent binding of dibenzo[a,h]pyrene to DNA. Chem. Res. Toxicol., 5, 765-772.

Mass, M.J., Abu-Shakra, A., Roop, B.C., Nelson, G., Galati, A.J., Stoner, G.D., Nesnow, S., and Ross, A., 1996. Benzo[b]fluoranthene: tumourigenicity in strain A/J mouse lungs, DNA adducts and mutations in the Ki-ras oncogene. Carcinogenesis, 17,1701-1704.

Matikainen, T., Perz, G.I., Jurisicova, A., Pru, J.K., Schlezinger, J.J., Ryu, H.-Y., Laine, J., Sakai, T., Korsmeyer, S.J., Casper, R.F., Sherr, D.H., and Tilly, J.L., 2001. Aromatic hydrocarbon receptor driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat. Genet., 28, 355-359.

Mattison, D.R., and Nightingale, M.S., 1980. 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, D.R., Singh, H., Takizawa, K., and Thomford, P.J., 1989. Ovarian toxicity of benzo[a]pyrene and metabolites in mice. Reprod.Toxicol., 3, 115-125.

Mattison, D.R., White, N.B., Nightingale, M.S., 1980. 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.

McCormick, D.L., Burns, F.J., and Albert, R.E., 1981. Inhibition of benzo[a]pyrene-induced mammary carcinogenesis by retinyl acetate. J. Natl. Cancer Inst., 66, 559-564.

McLemore, T.L., Martin, R.R., Busbee, D.L., Richie, R.C., Springer, R.R., Toppell, K.L., and Cantrell, E.T., 1978. Dissociation between aryl hydrocarbon hydroxylase activity in cultured pulmonary macrophages and blood lymphocytes from lung cancer patients. Cancer Res., 38, 3805-3811.

Meehan, T., Straub, K., and Calvin, M., 1977. Benzo[alpha]pyrene diol epoxide covalently binds to deoxyguanosine and deoxyadenosine in DNA. Nature, 269, 725-727.

Menichini, E., Monfredini, F., and Merli, F., 1999. The temporal variability of the profile of carcinogenic polycyclic aromatic hydrocarbons in urban air: a study in a medium traffic area in Rome, 1993-1998. Atmos. Environ., 33, 3739-3750.

Menzie, C.A., Potocki, B.B., and Santodonato, J., 1992. Exposure to carcinogenic PAH in the environment. Environ. Sci. Technol., 1992, 26, 1278-1284.

Mersch-Sundermann, V., Mochayedi, S., and Kevekordes, S., 1992. Genotoxicity of polycyclic aromatic hydrocarbons in Escherichia coli PQ37. Mutat. Res.,278,1-9.

Mersch-Sundermann, V., Wintermann, F., Kern, S., and Hof, H., 1993. Influence of S9 mix composition on the SOS response in Escherichia coli PQ37 by polycyclic aromatic hydrocarbons. Mutat Res., 291, 53-60.

Miller, M.L., Vasunia, K., Talaska, G., Andringa, A., de Boer, J., and Dixon, K., 2000. The tumour promoter TPA enhances benzo[a]pyrene and benzo[a]pyrene diolepoxide mutagenesis in Big Blue mouse skin. Environ. Mol. Mutagen., 35, 19-27.

Miller, M.M., Plowchalk, D.R., Weitzman, G.A., London, S.N., and Mattison, D.R., 1992. The effect of benzo[a]pyrene on murine ovarian and corporoa lutea volumes. Am. J. Obs. Gyn., 166, 1535-1541.

Misra, B., Amin, S., and Hecht, S.S., 1992. Metabolism and DNA binding of 5,6-dimethylchrysene in mouse skin. Chem. Res. Toxicol., 5, 242-247.

Miyata, M., Furukawa, M., Takahashi, K., Gonzalez, F.J., and Yamazoe, Y., 2001. Mechanism of 7,12-dimethylbenz[a]anthracene-induced immunotoxicity : role of metabolic activation at the target organ. Jpn. J. Pharmacol., 86, 302-309.

Montizaan, G.K., Kramers, P.G.N., Janus, J.A., and Posthumus, R, 1989. Integrated Criteria Document Polynuclear aromatic hydrocarbons (PAH): Effects of 10 selected compounds. Appendix to RIVM Report no. 758474007, National Institute of Public Health and Environmental Protection, RIVM, Bilthoven.

Moret, S., Amici, S., Bortolomeazzi, R., and Lercker, G., 1995. Determination of polycyclic aromatic hydrocarbons in water and water-based alcoholic beverages. Z. Lebensm. Unters. Forsch., 201, 322-326.

Morita, T., Asano, N., Awogi, T., Sasaki, Y.F., Sato, S., Shimada, H., Sutou, S., Suzuki, T., Wakata, A., Sofuni, T., Hayashi, M., 1997. Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test. Mammalian Mutagenicity Study Group. Mutat. Res., 389, 3-122.

Morris, H.P., Velat, C.A., Wagner, B.P., Dahlgard, M., and Ray, F.E., 1960. Studies of carcinogenicity in the rat of derivatives of aromatic amines related to N-2-fluorenylacetamide. J. Natl. Cancer Inst., 24, 149-180.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E.,1986. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ Mutagen., 8, Suppl. 7,1-119.

Mottier P., Parisod V., and Turesky R.J., 2000. Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. J. Agric. Food Chem., 48, 1160-1166.

Mounho, B.J., Davila, D.R., and Burchiel, S.W., 1997. Characterization of intracellular calcium responses produced by polycyclic aromatic hydrocarbons in surface marker-defined human peripheral blood mononuclear cells. Toxicol. Appl. Pharmacol., 145, 323-330.

Nagy, S.R., Sanborn, J.R., Hammock, B.D., and Denison, M.S., 2002. Development of a green fluorescent protein-based cell bioassay for the rapid and inexpensive detection and characterization of the Ah receptor agonists. Toxicol. Sci., 65, 200-210.

National Toxicology Program, 1991. Final study report and appendix:Developmental toxicity evaluation of naphthalene (CAS No 91-20-3) administered by gavage to Sprague-Dawley [cd] rats on gestational days 6 through 15. Research Triangle Park, North Carolina. US Department of Health and Human Services. (Report TER-91006).

National Toxicology Program, 1992. Final study report and appendix: Developmental toxicity evaluation of naphthalene (CAS No 91-20-3) administered by gavage to New Zealand White (NZW) rabbits on gestational days 6 through 19. Research Triangle Park, North Carolina. US Department of Health and Human Services. (Report TER-91021).

Nayyar, T., Greenwood, M., Nunes, M., and Hood, D.B., 2001. Developmental effects of mid-gestational transplacental exposures to dioxin and a benzo[a]pyrene:carbon black aerosol in timed-pregnant rats: effects on the F1 generation-II. Toxicologist, 60, 274. Abstract No 1306.

Neal, J. and Rigdon, R.H., 1967. Gastric tumours in mice fed benzo[a]pyrene: A quantitative study. Tex. Rep. Biol. Med., 25, 553-557.

Near, R., Matulka, R.A., Mann, K.K. , Schneider, A.M., Golgate, S.U., Trombino, A.F., and Sherr, D.H., 1999. Regulation of pre-B cell apoptosis by AhR/transcription factor expressing stromal/adherenT-cells. Proc. Soc. Exp. Biol. Med., 221, 242-252.

Nebert, D.W., and Jensen, N.M., 1979a. Benzo[a]pyrene-initiated leukemia in mice. Association with allelic differences at the Ah locus. Biochem. Pharmacol., 27, 149-151.

Nebert, D.W., and Jensen, N.M., 1979b. The Ah locus. Genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. CRC Crit. Rev. Biochem., 6, 401-437.

Nebert, D.W., Jensen, N.M., Levitt, R.C., and Felton, J.S., 1980. Toxic chemical depression of the bone marrow and possible aplastic anemia explainable on a genetic basis. Clin. Toxicol., 16, 99-122.

Nesnow, S., Ross, J.A., Stoner, G.D., and Mass, M.J., 1995. Mechanistic linkage between DNA adducts, mutations in oncogenes and tumourigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice. Toxicology, 105, 403-413.

Nesnow, S., Ross, J.A., Mass, M.J., and Stoner, G.D., 1998. Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumourigenesis in strain A mice. Exp Lung Res., 24, 395-405.

Nesnow, S., Davis, C., Nelson, G., Ross, J.A., Allison, J., Adams, L., and King, L.C., 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.

Nicol, C.J., Harrison, M.L., Laposa, R.R., Gimelshtein, I.L., and Wells, P.G., 1995. A teratogenic suppressor role for p53 in benzo[a]pyrene-treated transgenic p53-deficient mice. Nat. Genet., 10, 181-187.

Nielsen, T., Jørgensen, H.E., Poulsen, M., Palmgren Jensen, F., Larsen, J.C., Poulsen, M. Jensen, A.B., Schramm, J., and Tønnesen, J., 1995. Traffic PAH and other mutagens in air in Denmark. Miljøprojekt 285, Danish Environmental Protection Agency, Copenhagen.

Nielsen, T., Jørgensen, H.E., Larsen, J.C., and Poulsen, M., 1996. City air pollution of polycyclic aromatic hydrocarbons and other mutagens: occurrence, sources and health effects. Sci. Tot. Environ., 189/190, 41-49.

Nisbet, I.C., and LaGoy, P.K., 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAH). Regul. Toxicol. Pharmacol., 16, 290-300.

Nousiainen, U., Törrönen, R., and Hänninen, O., 1984. Differential induction of various carboxylesterases by certain polycyclic aromatic hydrocarbons in the rat. Toxicology, 32, 243-251.

Obana, H., Hori, S., Kashimoto, T. and Kunita, N., 1981. Polycyclic aromatic hydrocarbons in human fat and liver. Bull. Environ. Contam. Toxicol., 27, 23-27.

Oberly, T.J., Bewsey, B.J., and Probst, G.S., 1984. An evaluation of the L5178Y TK+/- mouse lymphoma forward mutation assay using 42 chemicals. Mutat. Res., 125,291-306.

Oberly, T.J., Michaelis, K.C., Rexroat, M.A., Bewsey, B.J., and Garriott, M.L., 1993. A comparison of the CHO/HGPRT+ and the L5178Y/TK+/- mutation assays using suspension treatment and soft agar cloning: results for 10 chemicals. Cell Biol. Toxicol., 9, 243-257.

O'Neill, I. K., Goldberg, M. T., el Ghissassi, F., and Rojas Moreno, M., 1991. Dietary fibre, fat and beef modulation of colonic nuclear aberrations and microcapsule-trapped gastrointestinal metabolites of benzo[a]pyrene-treated C57/B6 mice consuming human diets. Carcinogenesis, 12, 175-180.

Otto, D., Skalik, I., Hudnell, K., House, D., and Šrám, R.J., 2001. Neurobehavioural effects of exposure to environmental pollutants in Czech children. In: Teplice Program: Impact of Air Pollution on Human Health (Šrám, R.J., Ed.), Academia, Prague, 217-241.

Paigen, B., Holmes, P.A., Morrow, A., and Mitchell, D., 1986. Effect of 3-methylcholanthrene on atherosclerosis in two congenic strains of mice with different susceptibilities to methylcholanthrene-induced tumors. Cancer Res., 46, 3321-3324.

Pallardy, M.J., House, R.V., and Dean, J.H., 1989. Molecular mechanism of 7-12-dimethylbenz[a]anthracene-induced immunosuppression: evidence for action via the interleukin-2 pathway. Mol. Pharmacol., 36, 128-133.

Palli, D., Russo, A., Masala, G., Saieva, C., Guarrera, S., Carturan, S., Munnia, A., Matullo, G., and Peluso, M., 2001. DNA adduct levels and DNA repair polymorphisms in traffic-exposed workers and a general population sample. Int. J. Cancer, 94, 121-127.

Palli, D., Vineis, P., Russo, A., Berrino, F., Krogh, V., Masala, G., Munnia, A., Panico, S., Taioli, E., Tumino, R., Garte, S., and Peluso, M., 2000. Diet, metabolic polymorphisms and DNA adducts: the EPIC-Italy cross-sectional study. Int. J. Cancer, 87, 444-451.

Pauluhn, J., Thyssen, J., Althoff, J., Kimmerle, G., and Mohr, U., 1985. Long-term inhalation study with benzo[a]pyrene and SO2 in Syrian golden hamsters. Exp. Pathol., 28, 31.

Peirce, W.E.H., 1961. Tumour-promotion by lime oil in the mouse forestomach. Nature, 189, 497-498.

Penman, B.W., Chen, L., Gelboin, H.V., Gonzalez, F.J., and Crespi, C.L., 1994. Development of a human lymphoblastoid cell line constitutively expressing human CYP1A1 cDNA: substrate specificity with model substrates and promutagens. Carcinogenesis, 15,1931-1937.

Penn, A., and Snyder, C, 1988. Arteriosclerotic plaque development is 'promoted' by polynuclear aromatic hydrocarbons. Carcinogenesis, 9, 2185-2189.

Perera, F.P., Whyatt, R.M., Jedrychowski, W., Rauh, V., Manchester, D., Santella, R.M., and Ottoman, R., 1998. Recent developments in molecular epidemiology: a study of the effects of environmental polycyclic aromatic hydrocarbons on birth outcomes in Poland. Am. J. Epidemiol., 147, 309-314.

Perera, F.P., Jedrychowski, W., Rauh, V., and Whyatt, R.M., 1999. Molecular epidemiologic research on the effects of environmental pollutants on the fetus. Environ. Health Perspect., 107 (Suppl.3), 451-460.

Perez-Chiesa, Y., and Rodriguez, A., 1993. Absence of mutagenicity of benzo[c]phenanthridine alkaloids in somatic cells of Drosophila melanogaster: comparison with 7,12-dimethylbenz[a]anthracene and chrysene. Mutat Res., 298,277-283.

Perreault, S., Selevan, S., Zudová, D., Zudová, Z., Evenson, D.P., and Rubeš, J., 2001. Male reproductive health studies in the Teplice Program: air pollution and semen quality in young Czech men. In: Teplice Program: Impact of Air Pollution on Human Health (Šrám, R.J., Ed.), Academia, Prague, 145-156.

Pessah, I.N., Beltzner, C., Burchiel, S.W., Sridhar, G., Penning, T., and Feng, W., 2001. A bioactive metabolite of benzo[a]pyrene, benzo[a]pyrene-7,8-dione, selectively alters microsomal Ca++ transport and ryanodine receptor function. Mol. Pharmacol., 59, 506-513.

Pessah-Rasmussen, H., Stavenow, L., Seidegard, J., and Berglund, A., 1992. Human fibroblasts lacking trans-stilbene oxide active glutathione transferase exhibit increased cell death when exposed to polycyclic aromatic hydrocarbons. Pharmacol. Toxicol., 70, 361-365.

Pessah-Rasmussen, H., Stavenow, L., Xu, C.B., and Berglund, A., 1991. Increased smooth muscle cell proliferation by dimethylbenzanthracene is correlated to variations in activity of ornithine decarboxylase but not arylhydrocarbonhydroxylase. Artery, 18, 240-255.

Pfannhauser, W., 1991. Polyzyklische aromatische Kohlenwasserstoffe (PAK) in der Nahrung und auf ausgewählten Gemüseproben in Österreich [Polycyclic Aromatic Hydrocarbons (PAH) in Food and on Selected Samples of Vegetables in Austria]. Mitt. Gebiete Lebensm. Hyg., 82, 66-79.

Phillips, D.H., 1999. Polycyclic Aromatic Hydrocarbons in the Diet. Mutat. Res., 443, 139-147.

Phillips, D.H., Hewer, A., Seidel, A., Steinbrecher, T., Schrode, R., Oesch, F., and Glatt, H., 1991. Relationship between mutagenicity and DNA adduct formation in mammalian cells for fjord- and bay-region diol-epoxides of polycyclic aromatic hydrocarbons. Chem. Biol. Interact., 80,177-186.

Piskorska –Pliszczynska, J., Keys, B., Safe, S., and Newman, M.S., 1986. The cytosolic receptor binding affinities and the AHH induction potencies of 29 polynuclear aromatic hydrocarbons. Toxicol. Letters, 34, 67-74.

Plasterer, M.R., Bradshaw, W.S., Booth, G.M., Carter, M.W., Schuler, R.L., Hardin, B.D., 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, K.L., Pfeiffer, E., Petrovic, P., Friesel, H., Beermann, D., Hecker, E., and Oesch, F., 1990. Comparative tumourigenicity of picene and dibenz[a,h]anthracene in the mouse. Carcinogenesis, 1990, 11, 1721-1726.

Pott, F. and Stober, W., 1983. Carcinogenicity of airborne combustion products observed in subcutaneous tissue and lungs of laboratory rodents. Environ. Health Perspect., 47, 293-303.

Prahalad, A.K., Ross, J.A., Nelson, G.B., Roop, B.C., King, L.C., Nesnow, S., and Mass, M.J., 1997. Dibenzo[a,l]pyrene-induced DNA adduction, tumourigenicity, and Ki-ras oncogene mutations in strain A/J mouse lung. Carcinogenesis, 18, 1955-1963.

Probst-Hensch, N.M., Tannembaum, S.R., Chan, K.K., Coetzee, G.A., Ross, R.K., and Yu, M.C., 1998. Absence of the Glutathione S-transferase M1 gene increases cytochrome P4501A2 activity among frequent consumers of cruciferous vegetables in a Caucasian population. Cancer Epidemiol. Biomarkers Prev., 7, 635-638.

Quadri, S.A., Quadri, A.N., Hahn, M.E., Mann, K.K., and Sherr, D.H., 2000. The bioflovonoid galangin blacks AhR activation and PAH-induced pre-B cell apoptosis. Mol. Pharmacol., 58, 515-525.

Ramdahl, T, and Moller, M., 1983. Chemical and Biological Characterization of Emissions from a Cereal Straw Burning Furnace. Chemosphere, Vol. 12, 23-34.

Randerath, K., Randerath, E., Zhou, G.D., Supunpong, N., He, L.Y., McDonald, T.J., and Donnelly, K.C., 1999. Genotoxicity of complex PAH mixtures recovered from contaminated lake sediments as assessed by three different methods. Environ. Molec. Mutagen., 33, 303-312.

Rannug, U., Sjögren, M., Rannug, A., Toftgård, R., Gustafsson, J.-Å., Rosenkranz, H., and Klopman, G., 1991. Use of artificial intelligence in structure-affinity correlations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) receptor ligands. Carcinogenesis, 12, 2007-2015.

Reddy, T.V., Stober, J.A., Olson, G.R., and Daniel, F.B., 1991. Induction of nuclear anomalies in the gastrointestinal tract by polycyclic aromatic hydrocarbons. Cancer Lett., 56, 215-224.

Revis, N.W., Bull, R., Laurie, D., and Schiller, C.A., 1984. The effectiveness of chemical carcinogens to induce atherosclerosis in the white Carneau pigeon. Toxicology, 14, 32, 215-227

Rigdon, R.H., and Neal, J., 1965. Effects of feeding benzo[a]pyrene on fertility, embryos, and young mice. J. Natl Cancer Inst., 34, 297-305.

Rigdon, R.H., and Neal, J., 1966. Gastric adenomas and pulmonary adenomas in mice fed benzo[a]pyrene. Texas Rep. Biol. Med., 24, 195-207.

Rigdon, R.H. and Neal, J., 1969. Relationship of leukemia to lung and stomach tumours in mice fed benzo[a]pyrene. Proc Soc Exp Biol Med, 130, 146-148.

Rigdon, R.H., and Rennels, E.G., 1964. Effect of feeding benz[a]pyrene on reproduction in the rat. Experientia, 20, 224-226.

Roberts-Thomson, S.J., McManus, M.E., Tukey, R.H., Gonzalez, F.F., and Holder, G.M., 1993. The catalytic activities of four expressed human cytochrome p450s towards benzo[a]pyrene and the isomers of its proximate carcinogen. Biochem. BioPhys. Res. Commun., 192, 1373-1379.

Robinson, J.R., Felton, J.S., Levitt, R.C., Thorgeirsson, S.S., and Nebert, D.W., 1975. Relationship between 'aromatic hydrocarbon responsiveness' and the survival times in mice treated with various drugs and environmental compounds. Mol. Pharmacol., 11, 850-865.

Rodriguez, J.W., Kirlin, W.G., Wirsiy, Y.G., Matheravidathu, S., Hodge, T.W., and Urso, P., 1999. Maternal exposure to benzo[a]pyrene alters development of T lymphocytes in offspring. Immunopharmacol. Immunotoxicol., 21, 379-396.

Rodriguez, J.W., King, L.J., Kohan, M.J., Wirsiy, Y.G., and Kirlin, W.G., 2000. Maternal exposure to benzo[a]pyrene alters T lymphocyte development in mouse offspring and forms DNA-adducts in fetal immune cells. FASEB J., 14, A1356.

Rodriguez-Arnaiz, R., Vogel, E.W., and Szakmary, A., 1993. Strong intra-species variability in the metabolic conversion of six procarcinogens to somatic cell recombinagens in Drosophila. Mutagenesis, 8,543-51.

Rojas, M., Cascorbi, I., Alexandrov, K., Kriek, E., Auburtin, G., Mayer, L., Kopp-Schneider, A., Roots, I., and Bartsch, H., 2000. Modulation of benzo[a]pyrene-diol-epoxide-DNA adduc levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis, 21, 35-41.

Rojas, M., Godschalk, R., Alexandrov, K., Cascorbi, I., Kriek, E., Ostertag, J., Van Schooten, F.-J., and Bartsch, H, 2001. Myleoperoxidase – 463A variant reduces benzo[a]pyrene diol epoxide DNA adducts in skin of coal tar treated patients. Carcinogenesis, 22, 1015-1018.

Ross, J.A,. Nelson, G.B., Holden, K.L., Kligerman, A.D., Erexson, G.L., Bryant, M.F., Earley, K., Beach, A.C., Gupta, R.C., and Nesnow, S., 1992. DNA adducts and induction of sister chromatid exchanges in the rat following benzo[b]fluoranthene administration. Carcinogenesis., 13, 1731-1734.

Ross, J.A., Nelson, G.B.,Wilson, K.H., Rabinowitz, J.R., Galati, A., Stoner, G.D., Nesnow, S., and Mass, M.J., 1995. Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. Cancer Res., 55, 1039-1044.

Rosvold, E.A., McGlynn, K.A., Lustbader, E.D., and Buetow, K.H., 1995. Identification of an NAD(P)H:quinone oxidoreductase polymorphism and its association with lung cancer and smoking. Pharmacogenetics, 5, 199-206.

Rothman, N., Poirier, M.C., Haas, R.A., Correa-Villasenor, A., Ford, P., Hansen, J.A., O'Toole, T., and Strickland, P.T., 1993. Association of PAH-DNA adducts in peripheral white blood cells with dietary exposure to polyaromatic hydrocarbons. Environ. Health Perspect., 99, 265-267.

Rugen, P.J., Stern, C.D., and Lamm, S.H., 1989. Comparative carcinogenicity of the PAH as a basis for acceptable exposure levels (AELs) in drinking water. Regul. Toxicol. Pharmacol., 1989, 9, 273.

Ryberg, D., Skaug, V., Hewer, A., Phillips, D.H., Harries, L.W., Wolf, C.R., Øgreid, D., Ulvik, A., Vu, P., and Haugen, A. (1997). Genotypes of glutation transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. Carcinogenesis, 18, 1285-1289.

Safa, B., Lee, C., and Riddick, D.S., 1997. Role of the aromatic hydrocarbon receptor in the suppression of cytochrome P450 2C11 by polycyclic aromatic hydrocarbons. Toxicol. Letters, 90, 163-175.

Saint-Aubert, B., Cooper J.F., Astre C., Spiliotis J., and Joyeux H., 1992. Evaluation of the induction of polycyclic aromatic hydrocarbons (PAH) by cooking on two geometrically different types of barbecue. J. Food Compos. Anal., 5, 257-263.

Salama, S.A., Sierra-Torres, C.H., Oh, H.Y., Hamada, F.A., and Au, W.W., 2001. Variant metabolising gene alleles determine the genotoxicity of benzo[a]pyrene. Environ. Mol. Mutagen., 37, 17-26.

Salamone, M.F., Heddle, J.A., and Katz, M., 1979. The use of the Salmonella/microsomal assay to determine mutagenicity in paired chemical mixtures. Can. J. Genet. Cytol., 21, 101-107.

Sax, N.I., and Lewis, J.R. Sr., 1984. Dangerous properties of industrial materials, 7th Ed., Van Nostrand Reinhold Co, New York, 2451-2452.

Schabath, M.B., Spitz, M.R., Zhang, X., Delclos, G.L., and Wu, X., 2000. Genetic variants of myeloperoxidase and lung cancer risk. Carcinogenesis, 21, 1163-1166.

Schmähl, D., 1955. Testing of naphthalene and anthracene for carcinogenic effects in rats. Z Krebsforsch, 60, 697-710 (in German).

Schneider, K., Roller, R., Kalberlah, F., and Schuhmacher-Wolz, U., 2002. Cancer risk assessment for oral exposure to PAH mixtures. J. Appl. Toxicol., 22, 73-83.

Schocket, B., 1999. DNA damage in humans exposed to environmental and dietary polycyclic aromatic hydrocarbons. Mutat. Res., 8, 143-153.

Seidel, A., Friedberg, T., Löllmann, B., Schwierzok, A., Funk, M., Frank, H., Holler, R., Oesch, F., and Glatt, H., 1998. Detoxification of the optically active bay- and fjord-region polycyclic aromatic hydrocarbon dihydrodiol epoxides by human glutathione transferase P1-1 expressed in Chinese hamster V79 cells. Carcinogenesis, 19, 1975-1981.

Setiawan, V.W., Zhang, Z.-F., Yu, G.-P., Li, M.-L., Tsai, C.-J., Cordova, D., Wang, M.-R., Guo, C.H., Yu, S.-Z., and Kurtz, R.C., 2000. GSTT1 and GSTM1 null genotypes and risk of gastric cancer: A case-control study in a Chinese population. CEBP, 9, 73-80.

Shimada, T., Watanabe, J., Kawajiri, K., Sutter, T.R., Guengerich, F.P., Gillan, E.M.J., and Inoue, K., 1999. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis, 20, 1607-1613.

Shimizu, Y., Nakatsuru, Y., Ichinose, M., Takahashi, Y., Kume, H., Mimura, J., Fujii-Kuriyama, Y., and Ishikawa, T., 2000. Benzo[a]pyrene carcinogenicity is lsot in mice lacking the aryl hydrocarbon receptor. Proc. Natl. Acad. Sci., 97, 779-782.

Shopp, G.M., White, K.L. Jr., Holsapple, M.P., Barnes, D.W., Duke, S.S., Anderson, A.C., Condie, L.W. Jr., Hayes, J.R., and Borzelleca, J.F., 1984. Naphthalene toxicity in CD-1 mice: General toxicology and immunotoxicology. Fundam. Appl. Toxicol., 4, 406-419.

Shum, P., Jensen, N.M., and Nebert, D.W., 1972. The murine Ah locus: In utero toxicity and teratogenesis with genetic differences in benzo[a]pyrene metabolism. Teratology, 20, 365-376.

Silkworth, J.B., Antrim, L., and Kaminsky, L.S., 1984. Correlation between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57BL/6 and DBA/2 mice. Toxicol. Appl. Pharmacol., 75, 156-165.

Silkworth, J.B., Lipinskas, T., and Stoner, C.R., 1995. Immunosuppressive potential of several Polycyclic Aromatic Hydrocarbons found at a superfund site : new model used to evaluate additive interactions between benzo[a]pyrene and TCDD. Toxicology, 105, 375-386.

Sinha, R., Rothman, N., Brown, E.D., Mark, S.D., Hoover, R.N., Caporaso, N.E., Levander, O.A., Knize, M.G., Lang, N.P., and Kadlubar, F.F., 1994. Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycyclic aromatic hydrocarbons induces cytochrome P4501A2 activity in humans. Cancer Res., 54, 6154-6159.

Sinha, R., Kulldorff, M., Wong, H.C., Strickland, P., Kazerouni, N., Rothman, N., 2001. Dietary benzo[a]pyrene (BaP) intake and risk of colorectal adenomas. Proc. Am. Assoc. Cancer Res., 42, March 2001, Abstract no. 4106.

Sjögren, M., Ehrenberg, L., and Rannug, U., 1996. Relevance of different biological assays in assessing initiating and promoting properties of polycyclic aromatic hydrocarbons with respect carcinogenic potency. Mutat. Res., 358, 97-112.

Smyth, H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C., and Striegel, J.A., 1962. Range-finding toxicity data: List VI. Ind. Hyg. J., March-April, 95-107.

Snell, K.C., and Stewart, H.L., 1962. Pulmonary adenomatosis induced in DBA/2 mice by oral administration of dibenz [a,h]anthracene. J. Natl. Cancer Inst., 28, 1043-1049.

Somogyi, A., and Beck, H., 1993. Nurturing and Breast-Feeding: Exposure to Chemicals in Breast Milk. Environmental Health Perspectives, Vol. 101, Supplement 2, 45-52.

Song, K., and Milner, J.A., 2001. The influence of heating on the anticancer properties of garlic. J. Nutr., 131, 1055S-1057S.

Sparnins, V.L., Mott, A.W., Baraney, G., and Wattenberg, L.W., 1986. Effects of allyl methyl trisulfide on glutathione-S-transferase activity and BP-induced neoplasia in the mouse. Nutr. Cancer, 8, 211-215.

Speer, K., Steeg, E., Horstmann, P., Kühn, Th., and Montag, A., 1990. Determination and distribution of polycyclic aromatic hydrocarbons in native vegetable oils, smoked fish products, mussels and oysters, and bream from the river Elbe. J. High Res. Chrom., 13, 104-111.

Speer, K., and Montag, A., 1988. Polycyclische aromatische kohlenwasserstoffe in nativen pflanzlichen olen. Fat Sci. Technol., 90, 163-167.

Spitz, M.R., Duphorne, C.M., Detry, M.A., Pillow, P.C., Amos, C.I., Lei, L., de Andrade, M., Gu, X., Hong, W.K., and Wu, X., 2000. Dietary intake of isothiocyanates: Evidence of a joint effect with gluathione S-transferase polymorphisms in lung cancer risk. Cancer Epidemiol. Biomarkers Prev., 9, 1017-1020.

Šrám, R.J., 2001. Teplice Program: Impact of Air Pollution on Human Health. Academia, Prague.

Šrám, R.J., Binkova, B., Rossner, P., Rubes, J, Topinka, J., and Dejmek, J., 1999. Adverse reproductive outcomes from exposure to environmental mutagens. Mutat.Res., 428, 203-215.

Stall, W., and Eisenbrand, G., 1988. Determination of Polynuclear Aromatic Hydrocarbons and Nitrosamines. In: HPLC in Food Analysis (Macrae, R., Ed.), Academic Press, New York, 377-408.

Standing Committee on Foodstuffs, 2001. Outcome of the expert group meeting on 3 October on ways to prevent contamination of olive residue oil and other oils with polycyclic aromatic hydrocarbons (PAH). Summary record of the 85th meeting of the Standing Committee on Foodstuffs, 25th October 2001, agenda item 9. http://europa.eu.int/comm/food/fs/rc/scfs/rap09_en.pdf

Stavenow, L., and Pessah-Rasmussen, H., 1988. Effects of polycyclic aromatic hydrocarbons on proliferation, collagen secretion and viability of arterial smooth muscle cells in culture. Artery, 15, 94-108

Stijve, T., and Hischenhuber, C., 1987. Simplified determination of Benzo[a]pyrene and other polycyclic aromatic hydrocarbons in various food materials by HPLC and TLC. Deutsche Lebensmittel-Rundschau, 83, 276-282.

Stocker, K.J., Howard, W.R., Statham, J., and Proudlock, R.J., 1996. Assessment of the potential in vivo genotoxicity of fluoranthene. Mutagenesis, 11, 493-496.

Ström, J., Alfredsson, L., Gustavsson, P., and Malmfors, T., 1993. Polycyclic aromatic hydrocarbons and cardivascular disease – a review of the epidemiologic and toxicologic evidence for an etiological relationship. J. Occupat. Med. Toxicol., 1993, 2, 311-334.

Suzuki, T., Kanoh, T, Kanbayashi, M., Todome, Y, and Ohkuni, H., 1993. The adjuvant activity of pyrene in diesel exhaust on Ig E antibody production in mice. Jpn. J. Allergol., 42, 963-968.

Swartz, W.J., and Mattison, D.R., 1985. Benzo[a]pyrene inhibits ovulation in C57Bl/6N mice. Anat. Rec., 221, 268-276.

Szczeklik, A., Szczeklik, J., Galuszka, Z., Musial, J., Kolarzyk, E., and Targosz, D., 1994. Humoral immunosuppression in men exposed to polycyclic aromatic hydrocarbons and related carcinogens in polluted environments. Environ. Health Perspect., 102, 302-304.

Takaneka, H., Zhang, K., Diaz-Sanchez, D., Tsien, A., and Saxon, A., 1995. Enhanced IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: direct effects on B-cell IgE production. J. Allergy Clin. Immunol., 95, 103-115.

Tang, D., Phillips, D.H., Stampfer, M., Mooney, L.A., Hsu, Y., Cho, S., Tsai, W.Y., Ma, J., Cole, K.J., She, M.N., and Perera, F.P., 2001. Association between carcinogen-DNA adducts in white blood cells and lung cancer risk in the physicians health study. Cancer Res., 61, 6708-6712.

Tatematsu, M., Hasegawa, R., Imaida, K., Tsuda, H., and Ito, N., 1983. Survey of various chemicals for initiating and promoting activities in a short-term in vivo system based on generation of hyperplastic liver nodules in rats. Carcinogenesis, 4, 381-386.

Terada, N., Maesako, K., Hiruma, K., Hamano, N., Houki, G., Konno, A., Ikeda, T., and Sai, M., 1997. Diesel exhaust particulates enhance eosinophil adhesion to nasal epithelial cells and cause degranulation. Int. Arch. Allergy Immunol., 114, 167-174.

Thirman, M.J., Albrecht, J.H., Krueger, M.A., Erickson, R.R., Cherwitz, D.L., Park, S.S., Gelboin, H.V., Holtzman, J.L., 1994. Induction of cytochrome CYPIA1 and formation of toxic metabolites of benzo[a]pyrene by rat aorta: a possible role in atherogenesis. Proc. Natl. Acad. Sci. USA, 91, 5397-5401

Thorslund, T.W. and Farrar, D., 1990. Development of relative potency estimates for PAH and hydrocarbon combustion product fractions compared to benzo[a]pyrene and their use in carcinogenic risk assessment. EPA/600/R-92/134, Dept. Commerce, NTIS.

Thyssen, J., Althoff, J., Kimmerie, G., and Mohr, U., 1981. Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. J. Natl. Cancer Inst., 66, 575.

Tian, Y., Ke, S., Denison, M.S., Rabson, A.B., and Gallo, M.A., 1999. Ah receptor and NF-□B interactions, a potential mechanism for dioxin toxicity. J. Biol. Chem., 274, 510-515.

Tomingas, R., Pott, F. and Dehnen, W., 1976. Polycyclic aromatic hydrocarbons in human bronchial carcinoma. Cancer Lett., 1, 189-195.

Tomkins, B.A., Jenkins, R.A., Griest, W.H., Reagan, R.R., and Holladay, S.K., 1985. Liquid chromatographic determination of benzo[a]pyrene in total particulate matter of cigarette smoke. J. Assoc. Off. Anal. Chem., 68, 935-940.

Topinka, J., Schwarz, L.R., Kiefer, F., Wiebel, F.J., Gajdos, O., Vidova, P., Dobias ,L., Fried, M., Sram, R.J., and Wolff, T., 1998. DNA adduct formation in mammalian cell cultures by polycyclic aromatic hydrocarbons (PAH) and nitro-PAH in coke oven emission extract. Mutat. Res., 419, 91-105.

Törrönen, R., Nousianen, U., and Hänninen, O., 1981. Induction of aldehyde dehydrogenase by polycyclic aromatic hydrocarbons in rats. Chem. Biol. Interact., 36, 33-44.

Tucker, J.D., Auletta, A., Cimino, M.C., Dearfield, K.L., Jacobson-Kram, D., Tice, R.R., and Carrano, A.V., 1993. Sister-chromatid exchange: second report of the Gene-Tox Program. Mutat. Res., 297,101-180.

Tudek, B., Bird, R.P., and Bruce, W.R., 1989. Foci of aberrant crypts in the colons of mice and rats exposed to carcinogens associated with foods. Cancer Res., 49, 1236-1240.

Tuominen, J.P., Pyysalo, H.S., and Sauri, M., 1988. Cereal products as a source of polycyclic hydrocarbons. J. Agric. Chem., 36, 118-120.

Turrini, A., Saba, A., and Lintas, C., 1991. Study of the Italian reference diet for monitoring food constituents and contaminants. Nutr. Res., 11, 861-873.

Turrio-Baldassarri, L., di Domenico, A., La Rocca, C., Iacovella, N., and Rodriguez, F., 1996. Polycyclic aromatic hydrocarbons in Italian national and regional diets. Polycyclic Aromatic Compunds, 10, 343-349.

Ulrich, C.M., Bigler, J., Whitton, J.A., Bostick, R., Fosdick, L., and Potter, J.D., 2001. Epoxide hydrolase Tyr113His polymorphism is associated with elevated risk of colorectal polyps in the presence of smoking and high meat intake. Cancer Epidemiol. Biomarkers Prev., 10, 875-882.

Urso, P. and Gengozian, N. 1980. Depressed humoral immunity and increased tumour incidence in mice following in utero exposure to benzo[a]pyrene. J.Toxicol.Environ.Health, 6, 569-576.

Urso, P. and Gengozian, N. 1982. Alterations in the humoral immune response and tumour frequencies in mice exposed to benzo[a]pyrene and x-rays before or after birth. J.Toxicol.Environ.Health, vol. 10, pp. 817-835.

Urso, P. and Gengozian, N. 1984. Subnormal expression of cell-mediated and humoral immune responses in progeny disposed toward a high incidence of tumours after in utero exposure to benzo[a]pyrene. J.Toxicol.Environ.Health, vol. 14, pp. 569-584.

Urso, P., and Johnson, R.A., 1987. Early changes in T lymphocytes and subsets of mouseprogeny defective as adults in controlling growth of a syngeneis tumour after in utero insult with benzo[a]pyrene. Immunopharmacol., 14, 1-10.

Urso, P., and Johnson, R.A., 1988. Quantitative and functional change in T-cells of primiparous mice following injection of benzo[a]pyrene at the second trimester of pregnancy. Immunopharmacol. Immunotoxicol., 10, 195-217.

Urso, P., Zhang, W., and Cobb, J. R. 1992. Immunological consequences from exposure to benzo[a]pyrene during pregnancy. Scand.J.Immunol., vol. 36 Suppl 11, pp. 203-206.

Urso, P., Wirsiy, Y., Zhang, W., Rodriguez, J., and Moolenaar, P., 1994. Persistent defective T-cell differentiation in mice exposed to benzo[a]pyrene in utero. FASEB J., 8, A482.

US EPA, 1984a. Review and evaluation of the evidence for cancer associated with air pollution. EPA-450/5-83-006R, U.S. Environmental Protection Agency, Arlington.

US EPA, 1984b. Carcinogen Assessment of Coke Oven Emissions. EPA-600/6-82-003F, U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Washington, DC.

US EPA (1986). Guidance for health risk from exposure to chemical mixtures. U.S. Environmental Protection Agency, Fed Reg, 51, 34014.

US EPA, 1988. 13-Week mouse oral subchronic toxicity study (fluoranthene). TRL Study No. 042-008, U.S. Environmental Protection Agency, Washington DC, 104 pp.

US EPA, 1989a. Mouse oral subchronic toxicity study with acenaphthene. U.S. Environmental Protection Agency, Washington, DC, 89 pp.

US EPA, 1989b. Subchronic toxicity in mice with anthracene: Final report. HLA Study No. 2399-131, U.S. Environmental Protection Agency, Washington, DC, 465 pp.

US EPA, 1989c. Mouse oral subchronic toxicity study (fluorene). TRL Study No. 042010, U.S. Environmental Protection Agency, Washington, DC, 38 pp.

US EPA, 1989d. Mouse oral subchronic toxicity of pyrene. TRL Study No. 042-012, U.S. Environmental Protection Agency, Washington, DC, 102 pp.

US EPA, 1993. Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons. EPA/600/R-93/089, U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnatti, Ohio.

US EPA, 1999. Guidelines for Carcinogen Risk Assessment. Draft. U.S. Environmental Protection Agency, Washington, DC.

Vaca, C., Tornqvist, M., Rannug, U., Lindahl-Kiessling, K., Ahnstrom, G., and Ehrenberg, L., 1992. On the bioactivation and genotoxic action of fluoranthene. Arch. Toxicol., 66, 538-545.

Vaessen, H.A.M.G., Jekel A.A., and Wilbers A.A.M.M., 1988. Dietary intake of Polycyclic Aromatic hydrocarbons. Toxicol. Environ. Chem., 16, 281-294.

Vaessen, H.A.M.G., Schuller, P.L., Jekel, A.A., and Wilbers, A.A.M.M., 1984. Polycyclic aromatic hydrocarbons in selected foods: Analysis and occurrence. Toxicol. Environ. Chem., 7, 297-324.

Valerio, F., Ciccarelli, F., and Roggi, C., 1996. Esposizione personale a benzo[a]pirene in aree urbane e rurali (dati preliminari) [Personal exposure to benzo[a]pyrene in urban and rural areas (preliminary data)]. In: I valori di riferimento e i valori limite nella prevenzione ambientale e occupazionale (Aprea, C., Sciarra, G., Fiorentino, M.L., and Minoia, C., Eds.), Morgan Edizioni Tecniche, Milano, Italy, 337-341.

Vassilev, Z.P., Robson, M.G., and Klotz, J.B., 2001a. Associations of polycyclic organic matter in outdoor air with decreased birth weight: a pilot cross-sectional analysis. J. Toxicol. Environ. Health, 64, 595-605.

Vassilev, Z.P., Robson, M.G., and Klotz, J.B., 2001b. Outdoor exposure to airborne polycyclic organic matter and adverse reproductive outcomes: a pilot study. Am. J. Ind. Med., 40, 255-262.

Verschaeve, L., Van Gompel, J., Thilemans, L., Regniers, L., Vanparys, P., and van der Lelie, D., 1999. VITOTOX bacterial genotoxicity and toxicity test for the rapid screening of chemicals. Environ. Mol. Mutagen., 33, 240-248.

Vineis, P., Bartsch, H., Caporaso, N., Harrington, A.M., Kadlubar, F.F., Landi, M.T., Malaveille, C., Shields, P.G., Talaska, G., and Tannenbaum, S.R., 1994. Genetically based N-acetyltransferase metabolic polymorphism and low level environmental exposure to carcinogens. Nature, 369, 154-156.

Vogel, E.W., and Nivard, M.J., 1993. Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis, 8, 57-81.

Vondrácek, J., Machala, M., Minksová, K., Bláha, L., Murk, A.J., Kozubík, A., Neca, J., Svrckova, D., and Holoubek, I., 2001. Monitoring river sediments contaminated predominantly with polyaromatic hydrocarbons by chemical and in vitro bioassay techniques. Environ. Toxicol. Chem., 20, 1499-1506.

Vu Duc, T., and Hujnh, C.K., 1981. Les micropolluants organiques dans l'eau. Résultats préliminaires sur les haloformes et les hydrocarbures aromatiques polycycliques. Méd. Soc. Prév., 26, 315-316.

Wakabayashi, K., 1990. International Commission for Protection Against Environmental Mutagens and Carcinogens. ICPEMC Working Paper 7/1/3. Animal studies suggesting involvement of mutagen/carcinogen exposure in atherosclerosis. Mutat. Res., 239, 181-187.

Wang, C.X., Watanabe, K., Weisburger, J.H., and Williams, G. M., 1985. Induction of colon cancer in inbred Syrian hamsters by intrarectal administration of benzo[alpha]pyrene, 3- methylcholanthrene and N-methyl-N-nitrosourea. Cancer Lett., 27, 309-314.

Wang, J.S., Busby, W.F. Jr, and Wogan, G.N., 1995. Formation and persistence of DNA adducts in organs of CD-1 mice treated with a tumourigenic dose of fluoranthene. Carcinogenesis, 16,2609-2616.

Warshawsky, D., Livingston, G.K., Fonouni-Fard, M., and LaDow, K.,1995 Induction of micronuclei and sister chromatid exchanges by polycyclic and N-heterocyclic aromatic hydrocarbons in cultured human lymphocytes. Environ. Mol. Mutagen., 26,109-118.

Wattenberg, L.W., and Leong, J.L., 1970. Inhibition of the carcinogenic action of benzo[a]pyrene by flavones. Cancer Res., 30, 1922-1925.

Wei, S.J., Chang, R.L., Merkler, K.A., Gwynne, M., Cui, X.X., Murthy, B., Huang, M.T., Xie, J.G., Lu, Y.P., Lou, Y.R., Jerina, D.M., and Conney, A.H., 1999. Dose-dependent mutation profile in the c-Ha-ras proto-oncogene of skin tumours in mice initiated with benzo[a]pyrene. Carcinogenesis, 20, 1689-1696.

Welch, R.M., Gommi, B., Alvares, A.P., and Conney, A.H., 1972. Effect of enzyme induction on the metabolism of benzo[a]pyrene and 3-methyl-4-monomethylamino-azobenzene in the pregnant and fetal rat. Cancer Res., 32, 973-978.

Welling, P., and Kaandorp, B., 1986. Determination of polycyclic aromatic hydrocarbons (PAH) in edible vegetable oils by liquid chromatography and programmed fluorescence detection. Z. Lebensm. Unters. Forsch., 183, 111-115.

Wells, P.G., Wilson, B., and Lubek, B.M., 1989. In vivo murine studies on the biochemical mechanism of naphthalene cataractogenesis. Toxicol. Appl. Pharmacol., 99, 466-473.

WHO, 1988. Evaluation of short-term tests for carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vivo Assays. Cambridge University Press, Cambridge, WHO, 1988.

WHO, 2001. Polynuclear aromatic hydrocarbons (PAH). In: WHO Air Quality Guidelines for Europe, 2nd Edition, World Health Organization, Regional Office for Europe, Copenhagen.

White, K.L., Lysy, H.H., and Holsapple, M.P., 1985. Immunosuppression by polycyclic aromatic hydrocarbons: A structure-activity relationship in B6C3F1 and DBA/2 mice. Immunopharmacology, 9, 155-164.

Whong, W.Z., Stewart, J.D., Cutler, D., and 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.

Whyatt, R.M., Santella, R.M., Jedrychowski, W., Garte, S.J., Bell, D.A., Ottman, R., Gladek-Yarborough, A., Cosma, G., Young, T.L., Cooper, T.B., Randall, M.C., Manchester, D.K., and Perera, F.P., 1998. Relationship between ambient air pollution and DNA damage in Polish mothers and newborns. Environ.Health Perspect., 106 (Suppl.3), 821-826.

Whyatt, R.M., Perera, F.P., Jedrychowski, W., Santella, R.M., Garte, S., and Bell, D.A., 2000. Association between polycyclic aromatic hydrocarbon-DNA adduct levels in maternal and newborn white blood cells and glutathione S-transferase P1 and CYP1A1 polymorphisms. Cancer Epidemiol. Biomarkers Prev., 9, 207-212.

Whyatt, R.M., Jedrychowski, W., Hemminki, K., Santella, R.M., Tsai, W.Y., Yang, K., and Perera, F.P., 2001. Biomarkers of polycyclic aromatic hydrocarbon-DNA damage and cigarette smoke exposures in paired maternal and newborn blood samples as a measure of differential susceptibility. Cancer Epidemiol. Biomarkers Prev., 10, 581-588.

Wickström, K., Pyysalo, H., Plaami-Heikkilä, S., and Tuominen, J., 1986. Polycyclic aromatic compounds (PAC) in leaf lettuce. Z. Lebensm. Unters. Forsch., 183, 182-185.

Wilson, C.L., and Safe, S., 1998. Mechanisms of ligand iduced aryl hydrocarbon receptor mediated biochemical and toxicological responses. Toxicol. Pathol., 26, 657-671.

Winn, L.M., and Wells, P.G., 1997. Evidence for embryonic prostaglandin H synthase-catalyzed bioactivation and reactive oxygen species-mediated oxidation of cellular macromolecules in phenytoin and benzo[a]pyrene teratogenesis. Free Radic. Biol. Med., 22, 607-621.

Winker, N., Tuschl, H., Kovac, R., and Weber, E., 1997. Immunological investigations in a group of workers exposed to various levels of polycyclic aromatic hydrocarbons. J. Appl. Toxicol., 17, 23-29.

Wolfe, J.M., and Bryan, W.R., 1939. Effects induced in pregnant rats by injection of chemically pure carcinogenic agents. Am. J. Cancer, 36, 359-368.

Wong, M.Y., Harper, P.A., Meyer, U.A., Bock, K.W., Mörike, K., Lagueux, J., Ayotte, P., Tyndale, R.F., Sellers, E.M., Manchester, D.K., and Okey, A.B., 2001. Ethnic variability in the allelic distribution of human aryl hydrocarbon receptor codon 554 and assessment of variant receptor function in vitro. Pharmacogenetics, 11, 85-94.

Yabiku, H.Y., Martins, M.S., and Takahashi, M.Y., 1993. Levels of benzo[a]pyrene and other Polycyclic Aromatic Hydrocarbons in liquid smoke flavour and some smoke foods. Food Addit. Contam., 10, 399-405.

Yamaguchi, K., Matulka, R.A., Shneider, A.M., Toselli, P., Trombino, A.F., Yang, S., Hafer, L.J., Mann, K.K., Tao, X.-J., Tilly, J.L., Near, R.I., Sherr, D.H., 1997. Induction of preB cell apoptosis by 7,12-DMBA in long term primary murine bone marrow cultures. Toxicol. Appl. Pharmacol., 147, 190-203.

Yamaguchi, K., Near, R., Shneider, A., Cui, H., Ju, S.-T., and Sherr, D.H., 1996. Fluoranthene-induced apoptosis in murine T-cell hybridomas is independent of the AhR. Toxicol. Appl. Pharmacol., 139, 144-152.

Yang, H., Mazur-Melnyk, M., de Boer, J.G., and Glickman, B.W., 1999. A comparison of mutational specificity of mutations induced by S9-activated B[a]P and benzo[a]pyrene-7,8-diol-9,10-epoxide at the endogenous aprt gene in CHO cells. Mutat. Res., 423, 23-32.

You, L., Wang, D., Galati, A.J., Ross, J.A., Mass, M.J., Nelson, G.B., Wilson, K.H., Amin, S., Stoner, J.C., Nesnow, S., *et al.*, 1994. Tumour multiplicity, DNA adducts and K-ras mutation pattern of 5-methylchrysene in strain A/J mouse lung. Carcinogenesis, 15, 2613-2618.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K., 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutagen., 19 (Suppl. 21), 2-141.

Zhang, Y.J., Weksler, B.B., Wang, L., Schwartz, J., Santella, R.M., 1998. Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. Atherosclerosis, 140, 325-331

Zuelzer, W.W., and Apt, L., 1949. Acute hemolytic anemia due to naphthalene poisoning. J. Am. Med. Assoc., 141, 185-190.

APPENDIX 1 Further examples of PAH metabolism

Specific examples of PAH metabolism to describe differences

Chrysene

The metabolism of chrysene illustrates the production of different DNA binding metabolites by independent routes. Chrysene-1,2-dihydrodiol is the precursor for bay region diol epoxides which bind to DNA. A second pathway also produces DNA-binding metabolites of chrysene in vivo. This metabolite has been suggested to be a diol-epoxide containing a phenolic hydroxyl group situated on a ring remote from the diol-epoxide termed a triol epoxide, believed formed from phenolic dihydrodiols. Two adducts were isolated following treatment of mouse skin with ^{3}H-chrysene, one with chromatographic properties similar to chrysene anti-1,2-diol-3,4-epoxide adducts whilst the second was more polar and resembled products of microsomal metabolism of chrysene-1,2-dihydrodiol or 3-hydroxychrysene, it was postulated to be the result of further metabolism of 9-OH-chrysene-1,2-diol. The metabolism of chrysene has been studied in a number of biological systems.

Benzo[b]fluoranthene

Initial studies on benzo[*b*]fluoranthene indicated that none of the identified metabolites accounted for the potent tumourigenic activity of benzo[*b*]fluoranthene. The bay region metabolite anti- benzo[*b*]fluoranthene -9,10-diol-11,12-epoxide is not primarily responsible for the genotoxicity and the adducts formed in vivo are more polar than those from benzo[*b*]fluoranthene-9,10-diol. Metabolism studies have shown that the principal metabolites following in vitro metabolism of benzo[*b*]fluoranthene-9,10-diol are trans-9,10-dihydro-5,9,10-trihydoxy-benzo[*b*]fluoranthene or trans-9,10-dihydro-6,9,10-trihydoxy-benzo[*b*]fluoranthene rather than benzo[*b*]fluoranthene-9,10,11,12-tetraol. Further studies showed the involvement in tumourigenesis in mouse skin of phenolic dihydrodiols and triolepoxides such as 5-OH- benzo[*b*]fluoranthene-9,10-diol and 5-OH-benzo[*b*]fluoranthene-9,10-diol-11,12-epoxide. Studies with benzo[*b*]fluoranthene-9,10-diol epoxide and dGUO resulted in similar adducts to those seen in mouse skin with 5-OH-benzo[*b*]fluoranthene-9,10-diol suggesting this is further metabolised to the diol epoxide and guanosine is the principal base involved in adduct formation.

Dibenzanthracenes.

Dibenzanthracenes illustrates the effect that conformational and spatial arrangements can have on the metabolism and reactivity of PAH. There are differences in mutagenic and carcinogenic potencies of the three dibenzanthracene isomers; dibenzo[*a,c*]anthracene, dibenz[*a,h*]anthraceneand DBdibenz[*a,j*]anthracene. These isomers each have two bay regions but differ in the location of the two aromatic angular rings on the anthracene spine.

Studies on initiating activity in mouse skin of the three possible dihydrodiols of dibenz[*a,c*]anthracene showed that the 1,2- and 10,11-dihydrodiols were more potent than dibenz[*a,c*]anthracene itself whilst the 3.4-dihydrodiol was not (the latter is a putative

precursor of bay region diol epoxide). Adducts were not detected in mouse skin following treatment with dibenz[*a,c*]anthracene in vivo. However dibenz[*a,c*]anthracene produced adducts in vitro in both microsomal metabolism in the presence of DNA and primary cultures of hamster embryo cells. These had chromatographic characteristics of anti-10,11-diol-12,13-epoxide DNA adducts. Although the 10,11-dihydrodiol was shown to be metabolised to its non-bay-region diol epoxide by rat liver microsomes, there were a number of other adducts observed in this test system. The 10,11-dihydrodiol is the intermediate in the activation of dibenz[*a,c*]anthracene which may be activated to a vicinal non-bay-region diol epoxide either with or without additional metabolism elsewhere in the molecule.

Both in vitro and in vivo studies on the metabolism of dibenz[*a,h*]anthracene have identified more than 20 metabolites. Although dibenz[*a,h*]anthracene-3,4-diol (pecursor of the bay region diol epoxides) is formed extensively and is a potent initiator in mouse skin, it has not been shown to be converted to the vicinal diol epoxides by Aroclor 1254 induced rat hepatic microsome preparations. Both dibenz[*a,h*]anthracene and its 3,4-diol can form a range of polyhydroxylated products including bis-dihydrodiols, it was hypothesised that an epoxide formed through further metabolism of a bis-diol. Subsequent studies demonstrated that dibenz[*a,h*]anthracene was activated to a minor extent via the bay-region diol-epoxide anti-dibenz[*a,h*]angthracene-3,4-diol-1,2-epoxide but the major adduct resulted from treatment with dibenz[*a,h*]anthracene-3,4,10,11-bis-diol, which co-chromatographed with the major adduct following treatment of mouse skin with dibenz[*a,h*]anthracene or dibenz[*a,h*]anthracene-3,4-diol.

Studies showed that (±)anti-dibenz[*a,j*]anthracene-diol-epoxide was a more potent than its parent compound as a tumour initiator in mouse skin. This together with pure enantiomers of both syn- and anti-dibenz[*a,j*]anthracene diol epoxides bound to both guanosine and adenosine residues in calf thymus DNA. Tritiated dibenz[*a,j*]anthracene in mouse keratinocytes formed a variety of bay region diol epoxide DNA adducts to both guanosine and adenosine residues but much of the radioactivity was associated with more polar compounds. In mouse epidermis 11 radioactive peaks were identified. The principal adduct was formed from (+)anti-dibenz[*a,j*]anthracene-diol-epoxide bound to a guanosine residue, the isomer having an absolute configuration of (4R,3S)-diol-(2S,1R)-epoxide. This configuration represents the most tumourigenic configuration of the four configurational isomers of bay region diol epoxides observed with benzo[*a*]pyrene, chrysene, benzo[*a*]anthracene and benzo[*c*]phenanthrene. The more polar products were shown to include three adducts tentatively identified as arising from the 3,4-10,11-bis-diol of dibenz[*a,j*]anthracene.

This effect of structure on the microsomal metabolism, DNA binding and tumourigenicity of PAH can also be seen with the nitro-substituted PAH. For example studies on 7-nitrodibenz[*a,h*]anthracene demonstrated that nitro-substitution perpendicular or nearly perpendicular to the aromatic moiety resulted in weak or non-direct acting mutagenicity and lower tumourigenicity. This was due to the generation of reactive metabolites of the

ring structure and nitroreduction was not involved in the metabolic activation leading to tumour formation.

Anthracene

Data are available on the metabolism of anthracene in vitro and in rats in vivo, suggesting that the major route followed is based on 1,2-epoxidation followed by epoxide hydrolysis and conjugation of the resulting dihydrodiol. There is also some evidence that, in the rat, methylated metabolites (including the weakly tumourigenic 9,10-dimethylanthracene) may also form by a different metabolic route. Information on anthracene metabolites other than those found in the urine is almost completely lacking. Given that significant metabolism of PAH occurs in the gastrointestinal tract, the absence of data on bile and faeces metabolites is notable.

Information regarding toxicokinetics and metabolism in man are completely lacking. However, the pattern of anthracene metabolism in the rat broadly follows that of other polycyclic aromatic hydrocarbons, for which human metabolism is known to be qualitatively similar to that of the rat. This suggests that, on a qualitative level, human metabolism of anthracene may also be comparable to that seen in the rat. On the other hand, it is clear that no evaluation of the relative quantitative importance of competing metabolic pathways in man can be made. In the absence of other information, it will be assumed that the metabolic pathways of anthracene in man are not different from those observed in the experimental animal systems investigated.

Anthracene illustrates the role of metabolic interactions in determining the risk from a specific compound. It further demonstrates how metabolic data can be used to support or refute evidence from other studies such as genotoxicity in the overall risk assessment. The in vivo formation of 9-methylanthracene occurs in rat subcutaneous tissue but neither anthracene nor 9-methylanthracene appear to act as tumour initiators on mouse skin (in contrast to 9,10-dimethylbenz[*a*]anthracene). This may be due to the favoured route of 9-methylanthracene metabolism being demethylation to anthraquinone rather than hydroxylation and sulphate formation. In contrast 9-hydroxymethylanthracene cannot be metabolised to vicinal bay-region dihydrodiol-epoxides but is a potent electrophilic mutagen in the presence of hepatic cytosolic sulphotransferase and 3'-phosphoadenosine-5'-phosphosulphate. The S-adenosyl-L-methionine-dependent methyltransferase activity of rat hepatic cytosol catalyses mesoregion methylation of anthracene, benz[*a*]anthracene, dibenz[*a,h*] anthracene and benzo[*a*]pyrene.

Benzo[c]phenanthrene

Baum and co-workers (2001) studied the metabolic activation of benzo[*c*]phenanthrene by cytochrome P450 enzymes in human liver and lung. These studies illustrate that for dietary exposure hepatic metabolism may be a more appropriate consideration in risk analysis of benzo[*c*]phenanthrene and care is necessary extrapolating the results of inhalation studies.

Fjord region benzo[*c*]phenanthrene-3,4-dihydrodiol-1,2-epoxides are potent ultimate carcinogens whilst benzo[*c*]phenanthrene is a weak carcinogen in rodents. The first step is production of two enantiomeric benzo[*c*]phenanthrene-3,4-dihydrodiols which are converted to the four epoxide enantiomers. All 4 epoxides can form DNA adducts in mouse epidermis after skin application with (-)-anti-3,4-DH-1,2-epoxide being the most potent. However in rodent specific metabolism may account for the discrepancy in potency. In rat liver microsomes only small amounts of 3,4-DH are formed. The 3,4-DH results in six-fold higher adduct formation than benzo[*c*]phenanthrene in mouse skin in vivo. Thus formation of 3,4-DH is the critical step in formation of the ultimate carcinogen.

There are limited data on human metabolism of benzo[*c*]phenanthrene. Human mammary carcinoma cells (MCF-7) activates both benzo[c]phenanthrene and benzo[*c*]phenanthrene -3,4-dihydrodiol mainly to (-)-anti- and (+)-syn- benzo[*c*]phenanthrene-3,4-DH-1,2-epoxide which form predominantly deoxyadenosine adducts. Using V79 cells genetically engineered to express human P450 enzymes, CYP1A1, CYP 1A2, CYP 1B1, CYP 2AA6 and CYP 2E1 were all able to activate benzo[*c*]phenanthrene to the dihydrodiol.

The study used microsomes from 18 human liver and 11 human lung tissue samples at doses of 5 and 30 μM benzo[*c*]phenanthrene. Similar metabolic profiles were obtained but levels of metabolites varied 2-10 fold, with benzo[*c*]phenanthrene-3,4-dihydrodiol as the main metabolite and benzo[*c*]phenanthrene-5,6-dihydrodiol as a minor metabolite in all samples, up to 4 more polar peaks were seen in samples, three of which correlated with dihydrodiol formation and were suggested to be triols and tetraols. Total P450 levels in the microsomes varied 2-3 fold and specific enzyme activities were determined using specific substrates. These activities were correlated with metabolite formation using a Spearman correlation analysis; significant correlations were found for EROD activity (1A1 and 1A2) and formation of benzo[*c*]phenanthrene-3,4-dihydrodiol, 4-OH- benzo[*c*]phenanthrene and (to a lesser degree) benzo[*c*]phenanthrene -5,6-didydrodiol, 3A4 activity and benzo[*c*]phenanthrene-5,6-dihydrodiol formation. No evidence was found for involvement of CYP2C19, CYP2D6, CYP2E1 or CYP2A6.

Rat and pig hepatic microsomes produced benzo[*c*]phenanthrene-5,6-dihydrodiol as the main metabolite and benzo[*c*]phenanthrene-3,4-dihydrodiol as a minor metabolite whilst bovine hepatic microsomes formed both in similar amounts. In human lung microsomes benzo[*c*]phenanthrene-5,6-dihydrodiol was the main metabolite and benzo[*c*]phenanthrene-3,4-dihydrodiol was a minor component. Using E coli with recombinant human CYP1A2 and CYP1B1 similar metabolic profiles were obtained although more of the secondary polar metabolites were formed. The CYP1B1 is widely distributed in extrahepatic tissues and may be of greater importance in these tissues.

APPENDIX 2 TOXIC EQUIVALENCY FACTORS FOR PAH

The application of the concept of response addition has been suggested by the US EPA (1986) to determine the cancer risk from mixtures containing carcinogenic compounds. The assumption was that such compounds show simple dissimilar action with a complete negative correlation of tolerance. By definition, there is a complete negative correlation between the effects of two chemicals if the individuals that are most susceptible to one toxicant are least susceptible to the other. This is the simplest form of response additivity. The proportion (P) of individuals responding to the mixture is equal to the sum of the responses to each of the components. However, as pointed out by Könemann and Pieters (1996) for compounds with presumed linear dose-response curves, such as genotoxic and carcinogenic compounds for which it is assumed that a no-effect-level does not exist and for which the mechanism of action may be regarded as similar, response addition and dose addition will provide identical results. Therefore, various authors have applied dose addition and used different terminology in the assessment of PAH, such as relative response factors, relative potency factors or toxicity equivalency factors (TEF).

Several attempts have been made to derive relative potency factors, expressed as toxic equivalency factors (TEF) for individual PAH (relative to benzo[*a*]pyrene) with the purpose of summarising the contributions from individual PAH in a mixture into a total benzo[*a*]pyrene equivalent dose, assuming additivity in their carcinogenic effects (Nisbeth and Lagoy 1992; Rugen *et al.*, 1989; Thorslund and Farrar 1990; Krewski, 1989; Larsen and Larsen, 1998). Because there is a total lack of adequate data from oral carcinogenicity studies on PAH others than benzo[*a*]pyrene, TEF values for PAH have been suggested based on studies using skin application, pulmonary instillation and subcutaneous or intraperitoneal injections.

One of the main points to consider when assessing the combined action of chemicals in mixtures is whether there will be either no interaction or interaction in the form of synergism or antagonism. The TEF approach relies on dose addition, in which case there is no interaction between the components of the mixture. The TEF approach is used to normalise exposures to chemicals with the same mechanism of action (common mechanism chemicals) with different potencies to yield a total equivalent exposure (TEQ) to one of the chemicals, the “index compound”.

The TEF approach was initially developed to estimate the potential toxicity of mixtures of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). Use of the TEF approach, and thus dose addition, to the risk assessment of chemical mixtures is only scientifically justifiable when all the chemicals in the mixture act in the same way, by the same mechanism, and thus differ only in their potencies. Application of the dose addition model should not be applied to mixtures of chemicals that act by mechanisms for which the additivity assumptions are invalid. It should be realised that with the exception of a few groups of chemicals, such as some organophosphorous and carbamate pesticides and some

polychlorinated dibenzo-*p*-dioxins, - dibenzofurans and - biphenyls, precise mechanistic information on their toxic effects are scarce.

There are several problems in using the TEF approach in the risk assessment of PAH in food. The use of the TEF approach requires that the compounds in question excert the toxicological effect by the same mechanism of action, such as is the case for the polychlorinated dibenzo-p-dioxins and – dibenzofurans, which act through binding to the Ah-receptor. Although a number of PAH bind to the Ah receptor, this effect is not the only effect that determines the carcinogenic potency of PAH. DNA binding and induction of mutations are other significant effects in the carcinogenesis of PAH, and there is no indication that different PAH are activated via the same metabolic route, binds DNA in the same positions, and induce the same types of mutations in the same organs or tissues. In fact, the study by Culp *et al.* (1998) showed that the coal-tar mixture of PAH also produced tumours in other tissues and organs than those affected by benzo[*a*]pyrene alone, and that the additional PAH in the mixture did not significantly contribute to the incidence of stomach tumours observed after benz[*a*]pyrene alone.

Futhermore, studies on mixtures of individual PAH have shown that they may interact metabolically in a number of ways resulting in not only additive but also synergistic and/or antagonistic effects (Montizaan *et al.*, 1989).

The limitations in using the TEF approach for the assessment of PAH carcinogenicity following oral administration was illustrated when it was used on the carcinogenicity data and the analytical data on the PAH composition in the coal tars used in the study by Culp *et al.* (1998). When the TEF values derived by Larsen and Larsen (1998) (Table A.2.1) were used the carcinogenic potency of both coal tar mixtures was predicted to be only approximately 1.5 times that of the benzo[*a*]pyrene content. However, the observed potencies of the coal tar mixtures were up to 5 times that accounted for by the benzo[*a*]pyrene content. In this case, the use of the TEF approach for PAH carcinogenicity would underestimate it.

Schneider *et al.* (2002) also examined the use of the TEF approach on the data from the Culp *et al.* (1998) study and from several other studies using dermal or lung application of PAH mixtures of known composition. They used the TEF derived by Brown and Mittelsman (1993) (Table A.2.1) and concluded that the benzo[*a*]pyrene equivalency factors do not adequately describe the potency of PAH mixtures and lead to underestimation of the carcinogenic potencies in most cases.

Table A.2.1 Estimates of carcinogenic potencies of various PAH, relative to benzo[*a*]pyrene (BaP)

Compound	Studies using rat lung installation		Studies using mouse skin painting			Combined estimates from different types of studies				
	Calc. A)	Publ. B)	Calc. C)	Calc. D)	Publ. E)	Publ. F)	Publ. G)	Publ. H)	Publ. I)	Publ. J)
Anthracene				<0.0046		0.32		0.01	0.0005	0.01
Fluorene								0.001	0.0005	0
Phenanthrene	0.0004							0.001	0.0005	0
Benz[*a*]anthracene					0.0039-0.0055	0.145		0.1	0.005	0.1
Chrysene	0.030			0.013		0.0044	0.0044	0.01	0.03	0.01
Cyclopenteno[*cd*]pyrene			0.0084				0.023		0.02	
Fluoranthene				<0.105				0.001	0.05	0.01
Pyrene				<0.0046		0.081	0.081	0.001	0.001	0
Benzo[*b*]fluoranthene	0.089	0.123	0.18	0.037	0.023	0.140	0.140	0.1	0.1	1
Benzo[*j*]fluoranthene	0.053	0.052	0.022	0.040	0.075		0.061		0.05	0.1
Benzo[*k*]fluoranthene	0.052	0.053	$4x10^{-8}$	0.0004		0.066	0.066	0.1	0.05	0.1
Benzo[*ghi*]fluoranthene	0.012	0.021				0.022			0.01	0.01

Compound	**Studies using rat lung installation**		**Studies using mouse skin painting**			**Combined estimates from different types of studies**				
	Calc. A)	Publ. B)	Calc. C)	Calc. D)	Publ. E)	Publ. F)	Publ. G)	Publ. H)	Publ. I)	Publ. J)
Benzo[*a*]pyrene	1	1	1	1	1	1	1	1	1	1
Benzo[*e*]pyrene	0.0019	0.007		0.0039			0.004		0.002	
Dibenz[*a,h*]anthracene	1.23			0.65	0.59	1.1	1.1	5.0	1.1	1
Anthanthrene	0.340	0.316					0.320		0.3	
Benzo[*ghi*]perylene							0.022	0.01	0.02	0.01
Dibenzo[*a,e*]pyrene				0.221					0.2	0.1
Dibenzo[*a,h*]pyrene				0.843					1	1
Dibenzo[*a,i*]pyrene				0.082					0.1	1
Dibenzo[*a,l*]pyrene				1.27					1	1
Indeno[1,2,3-*cd*]pyrene	0.102	0.278	$4x10^{-8}$	0.035	0.0059	0.232	0.234	0.1	0.1	0.1
Coronene			0.007						0.01	

Notes to table A.2.1

a) Calculated by Nielsen *et al.*, 1995 based on studies by Deutsch Wenzel *et al.*, 1983a and b; Grimmer *et al.*, 1984a, 1987a and b, and 1988; Wenzel Hartung *et al.*, 1990: Maeda *et al.*, 1986; Horikawa *et al.*, 1991.

b) Thorslund and Farrar, 1990.

c) Calculated by Nielsen *et al.*, 1995 based on studies by Grimmer *et al.*, 1983, 1984b and 1985.

d) Calculated by Nielsen *et al.*, 1995 based on studies by Habs *et al.*, 1980; Grimmer *et al.*, 1982; Wynder and Hoffman, 1959a and b.

e) Rugen *et al.*, 1989.

f) Clement, 1988, as cited by Nielsen *et al*., 1995.

g) Krewski *et al.*, 1989.

h) Nisbet and Lagoy, 1992.

i) Larsen and Larsen, 1998.

j) Brown and Mittelsman 1993 (US EPA OPPTS)