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Scientific Committee on Food **SCF/CS/CNTM/PAH/29 Final**

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Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food

(expressed on 4 December 2002)

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TERMS OF REFERENCE AND BACKGROUND FROM THE COMMISSION

Terms of reference

The Scientific Committee on Food (SCF) is requested by the Commission to assess the health risks to consumers associated with exposure to PAH in foodstuffs.

The SCF is invited in particular to indicate, on the basis of current knowledge, which PAH are of most concern for public health, and for which there may be an urgent need for further studies.

In considering these issues the Committee is asked to take note of the work of other scientific committees, particularly where exposure to similar PAH compounds arises from foods as well as from other sources.

Background

The Commission is considering proposing the establishment of maximum levels for polycyclic aromatic hydrocarbons (PAH) in food at Community level, based on the legal framework of Council Regulation EEC 315/93 of 8 February 1993 (EEC, 1993). It therefore seeks the advice of the SCF on the risks to human health from exposure to these compounds.

PAH form a class of diverse organic compounds, each of them containing two or more aromatic rings. Compounds that are relevant in this context are in particular benzo[*a*]pyrene (BaP), the 15 other PAH considered by the United States Environmental Protection Agency (US EPA) in connection with the analysis of municipal and industrial waste waters (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3,*c,d*]-pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene) (US EPA, 1984a) and the additional PAH considered by IPCS in 1998 (IPCS, 1998) (see table 1.1 for compounds included and abbreviations used in this assessment).

For the general population, the major routes of exposure to PAH are from food and inhaled air. PAH enter the environment via the atmosphere from a variety of combustion processes and pyrolysis sources. PAH have been detected in a variety of foods, notably vegetables, as a result of the deposition of airborne PAH, and in fish and mussels from contaminated

waters. They have also been found in vegetable oils and margarine. PAH are also formed as a result of certain food preparation methods, such as grilling, roasting and smoking.

Benzo[*a*]pyrene was last considered by IARC in 1987 (IARC, 1987), which concluded that it is a probable human carcinogen. Some other PAH have also been identified as being carcinogens, with possible genotoxic properties.

The Commission has started a Scientific Co-operation task (SCOOP task 3.2.12: 'Collection of occurrence data on polynuclear aromatic hydrocarbons in food') to collect and collate recent data on the occurrence in foodstuffs of benzo[*a*]pyrene and any other PAH with data (EC, 2001a).

Acknowledgements

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1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) constitute a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. Hundreds of individual PAH may be formed and released during incomplete combustion or pyrolysis of organic matter, during industrial processes and other human activities. PAH are also formed in natural processes, such as carbonisation.

For non-smokers, exposure to PAH occurs mainly by inhalation of air and by ingestion of food. Other, possible minor, exposure routes are ingestion of dust and soil, ingestion of drinking water, dermal absorption from soil and water, and the use of PAH-contaminated products (such as preparations containing coal-tar).

Exposure to airborne PAH occurs both indoors and outdoors. Human exposure to PAH from inhalation of ambient air varies according to the degree of urbanization, traffic and industrialization. In the 1990's, typical annual mean levels of benzo[*a*]pyrene in rural background areas varied between 0.1 and 1 ng/m^3; for urban areas levels were typically between 0.5 and 3 ng/m^3 (with kerbside sites at the upper boundary of this range). In terms of daily benzo[*a*]pyrene intake this concentration range would cover an intake from 2 to 60 ng assuming an inhalation rate of 20 m^3 of air per day. Levels up to 30 ng/m^3 have been measured within the immediate vicinity of a cokery (EC, 2001c). Indoor exposure to PAH includes exposure to tobacco smoke, cooking fumes and open fireplaces. Additional contributions from the use of unvented heating sources can increase PAH concentrations in indoor air, in certain cases to very high levels. Occupational activities, such as working in coke oven batteries, retort-houses of coal-gas works, and in the metal smelting industry and the processing and use of mineral oil products, may result in elevated exposures through both air inhalation and dermal absorption. Such occupational exposures are normally limited to small subgroups of the population.

In food, PAH may be formed during processing and domestic food preparation, such as smoking, drying, roasting, baking, frying or grilling. Vegetables may be contaminated by the deposition of airborne particles or by growth in contaminated soil. Meat, milk, poultry and eggs will normally not contain high levels of PAH due to rapid metabolism of these compounds in the species of origin. However, some marine organisms, such as mussels and lobsters are known to adsorb and accumulate PAH from water, which may be contaminated, for example by oil spills.

Of the many hundreds of PAH, the most studied is benzo[*a*]pyrene, which is often used as a marker for PAH in ambient air and food.

Although studies in experimental animals on individual PAH, most notably benzo[*a*]pyrene, have shown various toxicological effects, such as haematological effects, reproductive and developmental toxicity and immunotoxicity, it is the carcinogenic and genotoxic potential of these compounds that has attracted most attention. A number of PAH as well as coal-tar and some occupational exposures to combustion emissions containing these compounds have shown carcinogenicity in experimental animals and genotoxicity and mutagenicity *in vitro* and *in vivo* (IARC, 1973, 1983, 1984, 1985, 1987, 1989; US EPA, 1984b; Montizaan *et al.*, 1989; ATSDR, 1994; Hughes *et al.*, 1997; IPCS, 1998; WHO, 2001). In its assessments of PAH in food the Committee took note of the evaluations performed by these various international expert groups. These assessments have mostly focused on the lung cancer risk from exposure by inhalation of airborne PAH mixtures, whereas the Committee focused its assessment on studies using oral administration of PAH. However, studies using other routes of application were included when they were considered of value for the risk assessment of PAH in food, for example in clarifying mode of action.

The individual PAH included in the assessment were the 33 compounds that were also included in the recent IPCS Environmental Health Criteria document on PAH (IPCS, 1998). These compounds were selected on the basis of available information on their occurrence and toxic effects. They are listed in Table 1.1.

The Committee is aware that in addition to the "classical" PAH, a number of heterocyclic aromatic compounds (e.g. carbazole, acridine), as well as derivatives of PAH, such as nitro-PAH and oxygenated PAH can be generated by incomplete combustion and from chemical reactions in ambient air. These compounds occur together with PAH in air (and presumably food) in complex mixtures and the total mixture is often referred to as polycyclic aromatic compounds (PAC). The Committee did not include these other individual compounds in the assessment. However, results from studies using complex mixtures, such as coal-tar or combustion products, may reflect an additional contribution from such compounds to the overall effects recorded.

Unless otherwise stated, the information used in this assessment is taken from the above-mentioned recent IPCS Environmental Health Criteria document on PAH (IPCS, 1998). A more extensive description of the studies cited and a more detailed overview of available information on levels and profiles of PAH in foods and dietary exposures are presented in the Annex.

Table 1.1 Polycyclic aromatic hydrocarbons included in the opinion.

Common name	CAS name	CAS Registry No.	Abbreviation
Acenaphthene	Acenaphthylene	83-32-9	AC
Acenaphthylene	Acenaphthylene, 1,2-dihydro-	208-96-8	ACL
Anthanthrene	Dibenzo[*def,mno*]chrysene	191-26-4	ATR
Anthracene	Anthracene	120-12-7	AN
Benz[*a*]anthracene	Benz[*a*]anthracene	56-55-3	BaA
Benzo[*a*]fluorene	11 H-Benzo[*a*]fluorene	238-84-6	BaFL
Benzo[*b*]fluorene	11 H-Benzo[*b*]fluorene	243-17-4	BbFL
Benzo[*b*]fluoranthene	Benz[*e*]acephenanthrylene	205-99-2	BbFA
Benzo[*ghi*]fluoranthene	Benzo[*ghi*]fluoranthene	203-12-3	BghiF
Benzo[*j*]fluoranthene	Benzo[*j*]fluoranthene	205-82-3	BjFA
Benzo[*k*]fluoranthene	Benzo[*k*]fluoranthene	207-08-9	BkFA
Benzo[*ghi*]perylene	Benzo[*ghi*]perylene	191-24-2	BghiP
Benzo[*c*]phenanthrene	Benzo[*c*]phenanthrene	195-19-7	BcPH
Benzo[*a*]pyrene	Benzo[*a*]pyrene	50-32-8	BaP
Benzo[*e*]pyrene	Benzo[*e*]pyrene	192-97-2	BeP
Chrysene	Chrysene	218-01-9	CHR
Coronene	Coronene	191-07-1	COR
Cyclopenta[*cd*]pyrene	Cyclopenta[*cd*]pyrene	27208-37-3	CPP
Dibenz[*a,h*]anthracene	Dibenz[*a,h*]anthracene	53-70-3	DBahA
Dibenzo[*a,e*]pyrene	Naphtho[1,2,3,4-*def*]chrysene	192-65-4	DBaeP
Dibenzo[*a,h*]pyrene	Dibenzo[*b,def*]chrysene	189-64-0	DBahP
Dibenzo[*a,i*]pyrene	Benzo[*rst*]pentaphene	189-55-9	DBaiP
Dibenzo[*a,l*]pyrene	Dibenzo[*def,p*]chrysene	191-30-0	DBalP
Fluoranthene	Fluoranthene	206-44-0	FA
Fluorene	9H-Fluorene	86-73-7	FL
Indeno[1,2,3-*cd*]pyrene	Indeno[1,2,3-*cd*]-pyrene	193-39-5	IP
5-Methylchrysene	Chrysene, 5-methyl-	3697-24-3	5-MCH
1-Methylphenanthrene	Phenanthrene, 1-methyl-	832-69-9	1-MPH
Naphthalene	Naphthalene	91-20-3	NA
Perylene	Perylene	198-55-0	PE
Phenanthrene	Phenanthrene	85-01-8	PHE
Pyrene	Pyrene	129-00-0	PY
Triphenylene	Triphenylene	217-59-4	TRI

2 EXPOSURE ASSESSMENT

2.1 Sources and occurrence of PAH in foods

Food can be contaminated by environmental PAH that are present in air (by deposition), soil (by transfer) or water (by deposition and transfer), or during processing and cooking. In areas remote from urban or industrial activities, the levels of PAH found in unprocessed foods reflects 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 (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 of 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 the Annex, table 1.1.1.

2.1.1 Contamination of food by environmental PAH

The natural and anthropogenic sources of PAH in the environment are numerous. PAH are formed and released during incomplete combustion or pyrolysis of organic matter, during industrial processes and other human activities. PAH are also formed in natural processes, such as carbonisation. PAH compounds are emitted from a number of environmental sources, such as processing of coal, crude oil, petroleum, and natural gas, production of aluminium, iron and steel, heating in power plants and residences (oil, gas, charcoal-fired stoves, wood stoves), combustion of refuse, fires including wood fires, motor vehicle exhaust and used motor lubricating oil. PAH, especially those of higher molecular mass, entering the environment via the atmosphere are adsorbed onto particulate matter. The hydrosphere and geosphere are affected secondarily by wet and dry deposition. Soils, surface waters, precipitations and sediments may be contaminated by PAH due to atmospheric fallout, urban runoff, deposition from sewage, and certain wastes, such as oil or gasoline spills.

The contamination of food with environmental PAH depends on a number of physical and chemical properties of the PAH such as their relative solubility in water and organic solvents, volatility, chemical reactivity, and biotic and abiotic degradability.

PAH are lipophilic compounds having poor water solubility. Water solubility generally decreases with increasing molecular mass (the octanol-water partition coefficient (K_{ow}) increases). They will not accumulate in plant tissues with high water content and transfer from contaminated soil to root vegetables will be limited. Because adsorption of PAH to the organic fraction of soil is strong they do not penetrate deeply in soils, others than sandy soils, and therefore leaching to groundwater and uptake by plants is low.

Most PAH are of low volatility and have a high tendency to adsorb on organic particulate matter. In the atmosphere, PAH containing 5 or more aromatic rings are found predominantly in association with 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 vapour phase, whereas those with 4 rings are in an intermediate position. The association of the heavier PAH with particulate matter makes atmospheric fall out a principal route of contamination (Edwards, 1983; Nielsen *et al.*, 1996). Consequently, vegetables with large leaves and browsing cattle and pecking poultry, which may ingest particulate matter from contaminated grass and 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. The concentrations of PAH are generally greater on the plant surface (peel, outer leaves) than in internal tissue.

When PAH-containing particulates fall out into surface water, they are transported in suspension and finally may end up in the fresh water or marine sediments to which they are strongly bound. These sediments constitute a pollution reservoir from which PAH may be released. Filter-feeding bivalves such as mussels and oysters may accumulate particles contaminated by high molecular mass PAH because they filter large volumes of water and they are not capable of efficiently metabolising all PAH. Depuration of contaminated mussels in clean water will not reduce their level of PAH significantly. The contamination of sediment dwelling organisms may potentially cause contamination higher up in the aquatic food chain. However, because most of these organisms have a high biotransformation potential for PAH, a significant bio-magnification has not been reported in aquatic systems.

PAH are chemically stable and very poorly degraded by hydrolysis (Howard *et al.*, 1991). In the presence of light, they are susceptible to oxidation and photo-degradation. Depending on various parameters (type of adsorption onto particles, molecular mass, etc.) the half lives in air range from a few hours to days. In soil, PAH may also be degraded by microbial activity. The estimated half lives in soils vary for individual PAH, from several months to

several years. Degradation of PAH may lead to the formation of oxidized reaction compounds, which have a great tendency to react with biological components such as those, found in foods. Also nitro derivatives can be formed in the atmosphere following reaction with nitrogen oxides or nitric acid and finally they may contaminate foods. Thus although parent compounds cannot always be detected in PAH contaminated foods, degradation products or derivatives may be present.

2.1.2 Generation of PAH during processing and cooking of food

Processing procedures, such as smoking and drying, and cooking of food is commonly thought to be the major source of contamination by 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 source and pyrolysis of the food due to high temperatures, above 200°C (Lijinsky and Shubik, 1965a,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) (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.

Grilled food

PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, the duration of cooking and the temperature used. For example a heavily barbecued lamb sausage contained 14 µg/kg of the sum of six PAH, considered by IARC (1987) to be carcinogenic (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 barbecue prevented fat from dripping onto the heat source, and the PAH levels were 10-30 times lower than with the horizontal system (Saint-Aubert *et al.*, 1992).

Smoked foods

Data reported in the literature on PAH in smoked foods are highly variable. The main reason for such discrepancies is the differences in the procedures used for smoking. Such variables include: the type and composition of wood, type of generator (internal or external), oxygen accessibility, temperature of smoke generation, and smoking time. The

PAH content in smoked fishery products from modern smoking kilns with external smoke generation and procedures that remove high-boiling compounds such as PAH and particles potentially containing PAH have been compared with products from traditional smoking kilns where the smoke is generated in direct contact with the product. The average benzo[*a*]pyrene concentration determined for the traditional kilns was 1.2 µg/kg and 0.1 µg/kg for the modern kilns (Karl and Leinemann, 1996).

PAH intake may also originate from the use of smoke flavourings in food. 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.

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 tea leaves increase the PAH content (Stall and Eisenbrand, 1988). A Finnish study showed that roasted ground coffee and dried tea leaves 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; limits of detection not available). In other studies it has been shown that the PAH contents in the coffee brew were only a few ng/L (Kayali-Sayadi *et al.*, 1999).

Human milk

In a study conducted in the Federal Republic of Germany in 1984 a number of individual PAH compounds were found to be present 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).

2.1.3 Measures to reduce PAH contamination of foods

The amount of PAH formed during cooking or processing of food depends markedly on the conditions used. Direct contact of oil seeds or cereals with combustion products during drying processes has been found to result in formation of PAH and should therefore be avoided. Simple practices such as selecting preferentially lean meat and fish and avoiding contact of foods with flames for barbecuing, using less fat for grilling, and cooking at lower temperature for a longer time, results in a significantly reduced contamination of foods by PAH (Lijinsky and Ross, 1967; Knize *et al.*, 1999). Broiling (heat source above) can significantly reduce PAH levels. 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 colour of grilled foods. It is therefore not necessary to overcook the food to get the flavour. However, cooking must always remain effective as regards inactivation of any possible contaminating bacteria or endogenous toxins.

The PAH contamination of smoked foods can be significantly reduced by replacing direct smoking (with smoke developed in the smoking chamber, traditionally in smokehouses) with indirect smoking. The latter is obtained by an external smoke generator which, in modern industrialised kilns, is operated automatically under properly controlled conditions (Karl and Leinemann, 1996). Also the use of smoke flavourings is generally considered to be of less health concern than the traditional smoking process, as it may minimise PAH contamination (Chen and Lin, 1997). A smoke flavouring (also known as 'liquid smoke') is produced from condensed smoke, which is then fractionated and purified to remove most PAH. A maximum allowed content of benzo[*a*]pyrene and benz[*a*]anthracene in smoke flavouring is being set in the EU (CEC, 2002).

The waxy surface of vegetables and fruits can concentrate low molecular mass PAH mainly through surface adsorption. The concentrations of PAH are generally greater on plant surface (peel, outer leaves) than on internal tissue. Consequently, washing or peeling may remove a significant proportion 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 vapour phase can penetrate the waxy layer of fruits and vegetables and are less efficiently removed by washing.

2.2 Profiles of PAH in food

Because different investigators analyse different sets of PAH in foods the Committee examined the possibility of using benzo[*a*]pyrene, which almost always is included in any analysis, as a marker for PAH in foods. Therefore, the patterns of PAH distributions (profiles) relative to benzo[*a*]pyrene in various foods were examined. The PAH profiles were calculated from relevant and representative studies selected from the literature. The criteria used for this selection are outlined in the Annex together with a detailed tabulation of the results. The profiles were calculated as a set of ratios between the available concentrations of any individual PAH and benzo[*a*]pyrene ([PAH]/[BaP]) and, for any individual PAH, the ratio between the maximum and the minimum [PAH]/[BaP] was taken as an indicator of the maximum variability of [PAH]/[BaP] in different foods.

Profiles were also calculated for coke-oven fumes and urban air because it has been suggested to use benzo[*a*]pyrene as a marker (WHO, 2001; EC, 2001b) or as a semi-quantitative marker (CSTEE, 2001) for the carcinogenic risk of PAH in ambient air or other environmental matter. In addition, profiles were calculated for coal tar because new experimental studies, considered in this opinion, concerning PAH carcinogenicity by ingestion included a comparison of benzo[*a*]pyrene 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 expected to be contaminated only by atmospheric contamination and those where PAH are formed during processing.

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

- The lower-molecular mass, i.e. 3- and 4-ring PAH (fluorene, anthracene, phenanthrene, fluoranthene and pyrene) show markedly higher variability than higher-molecular mass PAH.
- The variability for almost all the higher-molecular mass PAH (from benzofluorenes upwards) was within a factor of ten when the raw data from all the selected studies were used. However, a significant part of the variability is likely due to the different analytical procedures used in different investigations and to any poor accuracy possibly present in some investigations and/or for some PAH.
- The variability markedly decreased after excluding outlying data (10% of all data), resulting within one order of magnitude for all these higher-molecular mass PAH. This holds especially for the carcinogenic PAH measured (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*j*]fluoranthene, indeno[1,2,3-

cd]pyrene, dibenz[*a,h*]anthracene), whose profiles in different foods were constantly within a factor of five.

- When the comparison of profiles in different foods was based on analyses performed in the same laboratory with the same procedure, as was the case of the investigation of Dennis *et al.* (1991), the variability for the higher-molecular mass PAH (from benz[*a*]anthracene upwards) resulted to be at most within a 5-fold factor.
- The median profile of measured carcinogenic PAH (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*j*]fluoranthene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene) in foods was very similar to those in coke-oven fumes and in urban air, within an approximate factor of two.
- The PAH median profile was also similar to that found in the coal tars used in a recent carcinogenicity study, well within one order of magnitude. In particular, for the measured carcinogenic PAH, the ratios between the values of [PAH]/[BaP] in coal tar and in foods (median values) were in the range 0.5-1.1.
- When processed foods, foods contaminated by deposition only and all foods were compared, similar profiles were observed except for anthracene and phenanthrene being more abundant in processed foods. As to the measured carcinogenic PAH, the profiles appeared to be similar, any difference being within an approximate factor of two.

It thus appears that benzo[*a*]pyrene can be used as an indicator of occurrence and concentration in food of the higher-molecular mass PAH (from benzofluorenes upwards), at least in terms of an order of magnitude. Benzo[*a*]pyrene can not be used as an indicator of lower-molecular mass PAH.

The Committee noted that these conclusions are in agreement with the results of a validation study performed by Kazerouni *et al.* (2001). To verify that benzo[*a*]pyrene is a good marker for other PAH, they analysed samples from each major food group for 15 PAH (2 to 5 rings) including seven PAH considered to be carcinogenic (4 and 5 rings). The Pearson correlation coefficient between concentrations of benzo[*a*]pyrene and the sum of the carcinogenic PAH was 0.98 (P=0.0001), while it decreased to 0.87 (P=0.0001) in the comparison of benzo[*a*]pyrene and the total of 15 PAH.

2.3 Intake estimates

2.3.1 Dietary intakes

Comparative intake data for individual PAH were collated from five total diet studies conducted in the United Kingdom (Dennis *et al.*, 1983; COT, 2002), Italy (Turrio-Baldassarri *et al.*, 1996), The Netherlands (De Vos *et al.*, 1990) and Austria (Pfannhauser, 1991). Benzo[*a*]pyrene intake estimates were 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). Details on the protocols used and the results of these studies are given in the Annex. The dietary intakes, as estimated by these national surveys, are shown in table 2.3.1. It is not possible to generate other intake estimates for the European population because: (i) food consumption surveys were carried out only in a 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. A scientific co-operation task to collect and collate data on the occurrence of PAH in foodstuffs in Europe is currently in progress (EC, 2001a). This will result in a more extensive database that could be used to generate intake estimates for more populations in Europe.

The results show that the PAH intakes are fairly uniform in the four EU countries where total diet studies are available. The personal intakes of individual PAH may span three orders of magnitude, up to some µg/day. The highest intakes were found to be of the lower-molecular mass (3- and 4-ring) PAH.

From the surveys conducted in the 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. This was consistent with the mean benzo[*a*]pyrene intakes estimated in two US surveys (0.05-0.14 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).

Table 2.3.1 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 2.3.1 (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[j]	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 2.3.1:

- 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.

PAH 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 methodology adopted for the adult population was also used for the children (UK/2 study in table 2.3.1). The intakes (per kg of body weight) are presented in table 2.3.2 together with those of adults for comparison purposes (the years of consumption surveys are given in the table, note b).

On a per kg body weight basis, the daily intake of benzo[*a*]pyrene as well as that of all PAH decreases with increasing age. The youngest age group (1.5-2.5 years) had the highest exposure, with an intake (of both benzo[*a*]pyrene and all PAH) about 2.4-fold higher than adults.

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

Temporal trend of intake

An evaluation of temporal trends in dietary intakes of PAH is only possible for the United Kingdom. The two total diet studies available from the UK (Dennis *et al.*, 1983; COT, 2002) involved foods collected at an interval of 21 years (1979 and 2000). Although a direct comparison is difficult because the food groupings and the sets of PAH determined were different, and the 1979 data were calculated only as 'lower-bound' values (i.e. 'not detected' data were treated as 'zero', see table 2.3.1) the COT, after re-elaborating the 1979 figures, made a comparison of the 1979 and 2000 data for benzo[*a*]pyrene and benz[*a*]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);
- 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.

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 2.3.3. 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 2.3.2 Dietary intakes of PAH for consumers of different age in UK (ng/kg bw/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 2.3.3 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 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.

2.3.2 The contribution from individual food groups to PAH intake

The contribution from individual food groups to the intake of each PAH was calculated for the UK diet (Dennis *et al.*, 1983). The major contributors were the oils and fats group (50% and 34% for benzo[*a*]pyrene and 11 PAH on average, respectively) and the cereals (30% and 31%, respectively). The former had the highest individual PAH levels, but the latter, although never showing high individual PAH levels, was a major contributor due to its relative weight to the total diet. The third major contributor were vegetables (8% and 12%, respectively), probably 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, at least in areas where barbecuing is an infrequent activity. These results were substantially confirmed in a 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 cereals (36%), followed by sugar and sweets (14%). 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 oils and fats was, at least partly, attributed to the well-known elevated PAH concentrations possibly present in vegetable oils.

The contributions from various foods to the intake of PAH estimated for the UK were 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 (identity not given), cereals were found to be the largest contributor (about 34%), followed by vegetables (about 18%) and by oils and fats (about 16%). Significant intakes were also found for fruit and smoked meat

products. Although smoked fish and grilled foods had the highest PAH levels, they made only a modest contribution since they are minor components of the usual diet.

However, it should be noted that smoked and grilled food may contribute significantly to the intake of PAH if such foods are part of the usual diet. Thus, for 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. In contrast to the available European surveys, grilled/barbecued meat contributed 21% to the mean daily intake of benzo[*a*]pyrene in the USA and was the food group contributing to the second intake of PAH after the 'bread, cereal and grain' group which contributed 29%.

In the most recent total diet study, performed in the UK (COT, 2002), cereals and vegetables were still major contributors to PAH intake. In fact, cereals contributed 24% and 35% for benzo[a]pyrene and total PAH (sum of 19 PAH), respectively, and vegetables contributed 12% and 13%, respectively. The contribution from oils and fats was far less than in the previous survey of Dennis *et al.* (1983): 6% and 3% for benzo[*a*]pyrene and total PAH, respectively. A significant contribution now was from beverages (28% and 8%) and from milk and dairy products group (12% and 9%).

2.3.3 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 were used. Details are given in the Annex. Table 2.3.4 shows the estimated contribution to the total personal PAH intake from the three routes of exposure.

The contribution from inhalation increases markedly for smokers and persons exposed to passive smoking. The additional benzo[*a*]pyrene intake for a person smoking 20 cigarettes/day was estimated to be 210 ng (see the Annex).

Table 2.3.4 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	2-200	100
Pyrene	600-1090	0.2-200	100
Benz[a]anthracene	<20-410	0.2-10	20
Chrysene	200-1530	200 [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 (BaP)	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 2.3.1).

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

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

[d] CHR + TRI.

3 HAZARD IDENTIFICATION

3.1 Absorption, distribution, metabolism and excretion of PAH

3.1.1 Absorption

Following intragastric administration in rats benzo(a)pyrene is rapidly absorbed with highest levels seen in the thoracic lymph nodes after 3-4 hours (Rees *et al.,* 1972 as cited in IPCS, 1998).

Laurent *et al.* (2001) studied the absorption of radiolabelled 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. Radioactivity was detectable within 1 hour in blood samples, peaked at 5-6 hours and reached background levels by 24 hours. The peak radioactivity was higher for phenanthrene despite the 3-fold lower dose.

The presence of bile increased the intestinal absorption of PAH in Sprague-Dawley rats. The absorption of benzo[*a*]pyrene was 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 *et al.* (1988) demonstrated that the composition of the diet influenced the absorption of ^{14}C-benzo[*a*]pyrene in rats. 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 results suggested 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.

3.1.2 Distribution

Distribution of PAH has been studied in rodents and detectable levels of PAH (probably more accurately PAH-derived material) are found in almost all organs. The organs rich in adipose tissue act as depots from which material is slowly released. High levels are found in the gastrointestinal tract irrespective of the route of administration.

Studies in pregnant mice and rats have shown that PAH (benzo[*a*]pyrene, 7,12-dimethylbenz[*a*]anthracene and 3-methylcholanthrene) were widely distributed in maternal tissues and was detected in fetuses showing that they crossed the placenta (Takahashi and

Yasuhira, 1973; Takahashi, 1978; 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 human study, 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 (Madhavan and Naidu, 1995 as cited in IPCS, 1998).

3.1.3 Excretion

PAH metabolites are excreted in urine, bile and faeces. The bile was the major route of excretion in bile-duct cannulated rats in the initial 6 hours after intravenous administration of ^{14}C-labelled benzo(a)pyrene, biliary excretion accounting for 60% of the dose whilst urinary excretion was 3%. The gastrointestinal microflora was shown to hydrolyse glucuronic acid conjugates of biliary PAH metabolites (Renwick and Drasar, 1976; Chipman *et al.*, 1981; Chipman, 1982; Boroujerdi *et al.*, 1981 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).

3.1.4 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 PAH has also been studied in a number of human cells and tissues. 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.

The general scheme of PAH metabolism, using benzo[*a*]pyrene as an example, 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 (figure 3.1.1).

Figure 3.1.1 Metabolism of benzo[*a*]pyrene (adapted from Besarati, 2001)

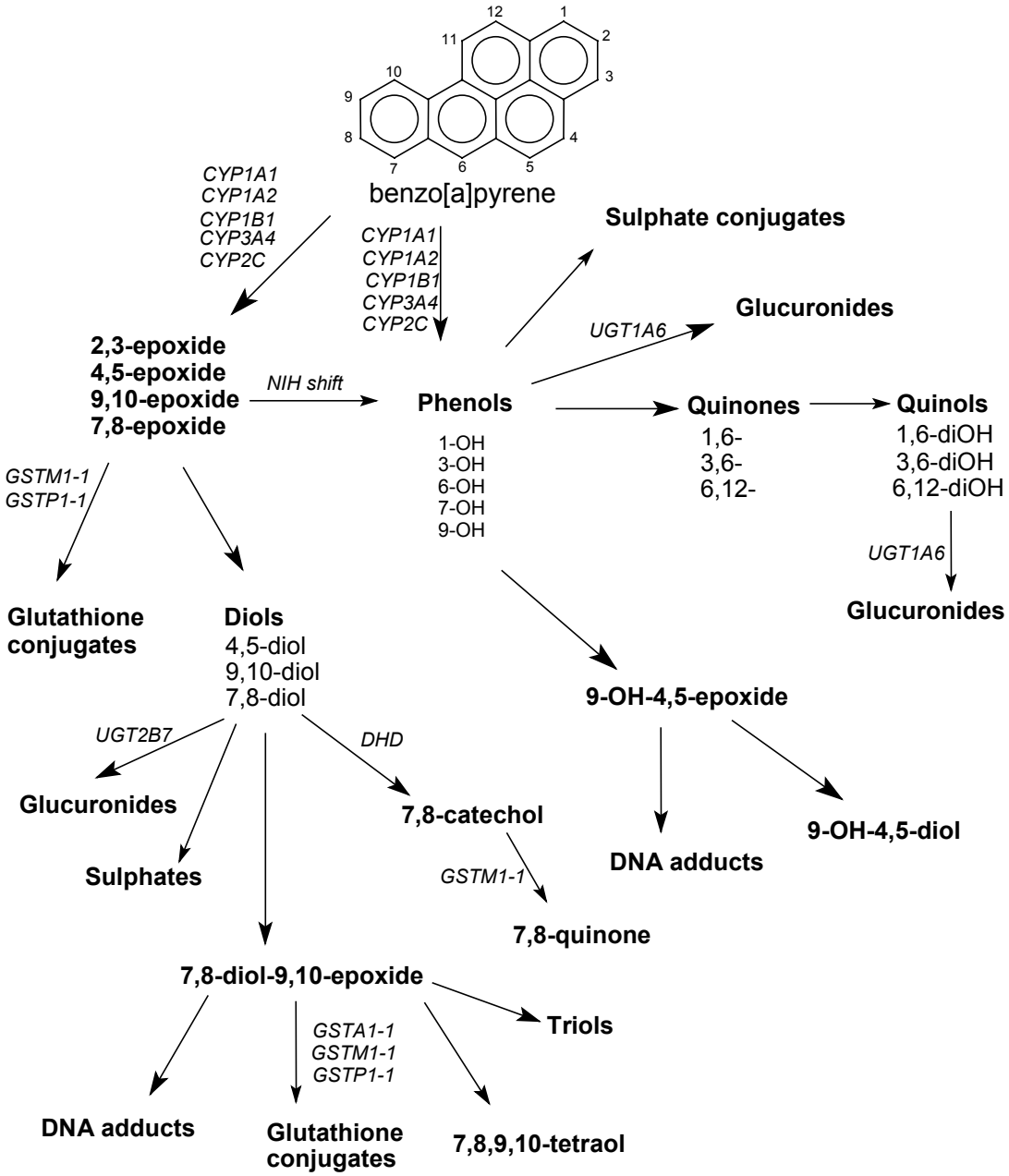

Table 3.1.1 Enzymes involved in the metabolism of PAH

Enzyme	Abbreviation
Cytochrome P450 1A1	CYP1A1
Cytochrome P450 1A2	CYP1A2
Cytochrome P450 1B1	CYP1B1
Cytochrome P450 3A4	CYP3A4
Cytochrome P450 3A5	CYP3A5
Cytochrome P450 2C	CYP2C
UDP Glucuronyl Transferase 1A6	UGT1A6
UDP Glucuronyl Transferase 2B7	UGT2B7
Glutathione S-Transferase A1-1	GSTA1-1
Glutathione S-Transferases M1-1	GSTM1-1
Glutathione S-Transferases P1-1	GSTP1-1
Dihydrodiol dehydrogenase	DHD
Microsomal epoxide hydrolase	mEH
Myeloperoxidase	MPO
NAD(P)H:quinone oxidoreductase	NQO1

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.

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 mutagenic and tumour inducing activity and that predominantly forming adducts by covalent binding to DNA, following benzo[a]pyrene exposure in various mammalian cells and organs.

The metabolism of other PAH covered in this opinion is equally complex. A number of dihydrodiols, diol-epoxides and other reactive metabolites have been identified, including some presumed reactive metabolites which can form adducts with DNA, thus potentially induce mutations. 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*.

The metabolism of chrysene and benzo[*b*]fluoranthene illustrate the production of different DNA binding metabolites by independent metabolic routes. The metabolism of the three dibenzanthracenes (dibenz[*a,c*]anthracene, dibenz[*a,h*]anthracene and dibenz[*a,j*]anthracene) illustrate the effect that conformational and spatial arrangements can have on the metabolism and reactivity of PAH and on their mutagenic and carcinogenic potencies. Fjord region benzo[*c*]phenanthrene-3,4-dihydrodiol-1,2-epoxides are potent ultimate carcinogens whilst the parent compound benzo[*c*]phenanthrene is a weak carcinogen in rodents. Studies on the metabolic activation of benzo[*c*]phenanthrene showed that the first step in the activation, the production of two enantiomeric benzo[*c*]phenanthrene-3,4-dihydrodiols, is the critical step in the formation of the ultimate carcinogens.

Anthracene illustrates the role of the route of metabolism in determining the risk from a specific compound. Thus reactive metabolites are not formed to a significant extent from anthracene *in vivo* whereas for benzo[*a*]pyrene their formation represents a significant route of metabolism and this is reflected in the data on the genotoxicity and carcinogenicity of these compounds.

The metabolism of these PAH is outlined in more details in the Annex.

3.1.5 Polymorphisms in PAH metabolising enzymes in humans

Many of the enzymes involved in the metabolism of PAH (see table 3.1.1) have a polymorphic distribution.

Genetic polymorphism has been defined as a genetic difference that can be seen in at least 1% of the population. Genetic polymorphism in xenobiotic metabolising enzymes may change the ratio of activation/deactivation of the PAH. An increased risk of certain cancers has been linked to these genetic polymorphisms (IARC, 1999).

The exact influence of genetic polymorphism on risk for PAH carcinogenesis has not been completely elucidated, because compensatory mechanisms or alternative enzymatic pathways may take over. Polymorphism in genes involved in the metabolism of PAH has many faces – sometimes beneficial, sometimes detrimental. The actual effect may depend on the number of gene-gene and gene-environment interactions including dose of the PAH, chemical nature of the PAH and interaction with nutritional status. The current information suggests that the effect of polymorphism depends on the level of exposure being more important at low doses of exposure.

Garte and Crosti (1999) have proposed a nomenclature system for gene polymorphisms in the CYP1A1 and GSTM1 enzymes (among others). 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. Letters A, B, etc. may follow the final number when allelic subtypes exist.

An increased risk of cancer and a higher level of PAH-DNA adducts was observed in subjects that have the CYP1A1*2/ or CYP1A1*3/ genotypes and do not express the detoxification enzyme GSTM1 (GSTM1*2/2) (Rojas *et al.*, 2000). CYP3A4, the major cytochrome P-450 in human liver is also involved in PAH metabolism, but the polymorphism detected in this gene has not been linked to altered cancer risk (Husterts *et al.,* 2001). Polymorphisms have also been described in several other enzymes involved in PAH metabolism, such as microsomal epoxide hydrolase (mEH), myeloperoxidase (MPO) and NAD(P)H:quinone oxidoreductase (NQO1).

The frequencies of high-risk genotypes in Caucasian populations are listed in table 3.1.2 (Garte *et al.*, 2001). Based upon the frequency of known genetic polymorphisms associated with an increased cancer risk, a high-risk population can be postulated, e.g. GSTM1*2/2, mEH “fast” phenotype and CYP1A1*2/ or CYP1A1*3/. The group having all three high-risk genotype will represent approximately 1% of the European population, as the genes are not linked.

Table 3.1.2 Frequency of genetic polymorphisms in genes involved in PAH metabolism – Caucasians

Gene	High risk genotypes	Frequency	Consequences
GSTM1	GSTM1*2/2 (null)	50%	Decreased detoxification
GSTP1	GSTP1*2/2	5%	Decreased detoxification
CYP1A1	CYP1A1*2/ and CYP1A1*3/	6%	Increased activation
CYP1B1	CYP1B1*2/	40%	Increased activation
mEH	"fast" phenotype	16%	Increased activation
MPO	G/G genotype (high activity)	56%	Increased activation
NQO1	"low" activity phenotype	38.6%	Decreased detoxification

3.1.6 DNA binding of PAH

The covalent binding of dibenzo[*a,h*]anthracene to DNA *in vivo* was first reported in 1961. Subsequently the levels of DNA binding of naphthalene, dibenzo[*a,c*]anthracene, dibenzo[*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 (Flesher *et al.*, 1998).

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 that the 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 with the N7 position of guanine. However, these adducts are labile and their detection is difficult.

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. The mutational spectrum induced by BaPDE in bacteria shows a prevalence of G>T transversions (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; Cooper, 2002), which may reflect 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, all changes, which may be of significance in carcinogenesis.

Godschalk *et al.* (2000) measured DNA adduct formation in stomach, lung, skin and white blood cells following administration to rats of 10 mg/kg bw benzo[*a*]pyrene by oral gavage, dermal and intratracheal routes. The results demonstrate that adduct formation occurs at both the site of contact and systemically. However, the adduct levels could not be readily correlated with peak or total systemic benzo[*a*]pyrene exposure.

3.2 Receptor mediated biochemical and toxicological effects

Several of the effects of PAH, such as enzyme induction, immunosuppression, teratogenicity, and tumour promotion are believed to be mediated by the sustained activation of the arylhydrocarbon receptor (AhR) and the subsequent disturbance of cellular homeostasis.

The AhR is a ligand-dependent transcriptional regulator of several genes including genes encoding enzymes involved in the metabolism of xenobiotics, e.g. the 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, which has subsequently been termed the dioxin receptor (Wilson and Safe, 1998). The AhR is located in the cytoplasm and following binding of the ligand (e.g. a PAH) the ligand-AhR complex enters the nucleus where it binds to specific DNA sequences flanking the genes regulated by the AhR. These sequences, termed AhR response elements (AHRE) are located in the promoter and enhancer regions of target genes and the binding results in an up-regulation of transcription and subsequent increases in mRNA and protein levels of these genes, e.g. CYP1A1.

In addition to CYP1A1, a large number of other genes and gene products (the Ah gene battery) are known to be up-regulated. 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 3.2.1 (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 3.2.1 Ah-receptor mediated increased gene expression

Xenobiotics 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

There are differences in the AhR-mediated toxicity by PAH and TCDD ligands. Exposure of mice to PAH induces the expression of the *Bax* gene and subsequent apoptosis in oocytes. This effect is AhR dependent but is only induced by PAH and not by TCDD. This difference is conveyed by a single base pair flanking the AHRE in the *Bax* promoter region (Matikainen *et al.*, 2001).

There are indications that the AhR is likely to interact with other signalling pathways. Thus, the activated AhR interacts with NF-κB, a key nuclear transcription factor, and this interaction is associated with mutual functional modulation of expression of genes involved in immunosuppression, carcinogenesis, cell growth and apoptosis. Therefore, some of the effects of PAH exposure related to carcinogenesis may also be mediated through this pathway.

The AhR has been detected in most cells and tissues. 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. 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. However, none of these polymorphisms appears to be associated with either inducibility of the marker enzyme, CYP1A1, or binding affinity of the receptor-ligand complex to the AhRE (Wong *et al.*, 2001; Cauchi *et al.*, 2001).

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 do not support transcriptional activation.

The AhR-inducing activity of many PAH has been demonstrated both *in vivo* and *in vitro*. Induction equivalency factors relative to the potency of benzo[*a*]pyrene have been established for a number of PAH using the CALUX assay in the rat hepatoma H4IIE cell

line. The activity was found significantly lower for PAH than for TCDD (Machala *et al.*, 2001).

3.2.1 AhR and immunotoxicity of PAH

A functional Ah-receptor is required for proper function of the immune system. This has been demonstrated using transgenic mice lacking the AhR (Fernandes-Salguero *et al.*, 1995). Furthermore, it has been shown that the AhR can influence PAH-mediated immunosupression (Near *et al.*, 1999). The spectrum of PAH immunotoxic outcomes suggests that PAH interfere with the lymphocyte programmed cell death/apoptosis machinery, e.g. induces pre-B cell apoptosis (Quadri *et al.*, 2000; Yamaguchi *et al.*, 1997). 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).

3.2.2 AhR and carcinogenicity of PAH

The AhR plays an important role in PAH-induced carcinogenesis. Early animal studies using parenteral administration showed a correlation between inducibility of arylhydrocarbon hydroxylase (AHH) activity and PAH-induced lung carcinogenesis (McLemore *et al.*, 1978). Recent studies have demonstrated that benzo[*a*]pyrene induces 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 BP-induced bone marrow cytotoxicity and genotoxicity which suggests that the AhR signalling has a net potentiation effect on PAH genetic toxicity (Dertinger *et al.*, 2001).

3.2.3 AhR and reproductive toxicity of PAH

Studies in Ah-inducible and non-inducible mice have demonstrated that the reproductive toxicity of PAH is mediated through the AhR. These effects are summarised in chapter 3.8.

3.2.4 Other receptor mediated effects of PAH

The steric resemblance of PAH to steroid molecules led to the postulation that they would be able to bind to the same receptors as steroid hormones, and both *in vivo* and *in vitro* studies have demonstrated that some PAH have both oestrogenic and anti-oestrogenic

activity. It has been shown that phenolic metabolites rather than the parent compound are responsible for these effects (Charles *et al.*, 2000). The potency of 3-hydroxy-benzo[*a*]pyrene and 9-hydroxy-benzo[*a*]pyrene was equivalent to that of oestradiol. The significance of this is not known.

3.2.5 Other PAH-induced biological effects

A strong correlation has been found between the tumour promoting activity of a compound in the two-stage skin carcinogenesis model and its ability to inhibit gap-junctional intercellular communication (GJIC). Using an *in vitro* test system, it was found that different PAH inhibit GJIC. Several PAH considered to possess low carcinogenic activity belong to the most potent inhibitors of GJIC, e.g. fluoranthene, 5-methylchrysene and picene. However, the PAH are of 1000-fold lower potency than TPA, that is the reference promoting compound (Bláha *et al.*, 2002).

3.3 Acute and short-term toxicity of PAH

Except for naphthalene, there are only a limited number of studies available on the acute oral toxicity of PAH. The LD_{50} values indicate that the acute oral toxicities of PAH are moderate to low. For naphthalene the acute oral LD_{50} values in mice and rats ranged from 350 – 9500 mg/kg bw (IPCS, 1998).

The results of available oral short-term toxicity studies on PAH are summarized in table 3.3.1.

Table 3.3.1 Summary of short-term toxicity tests following oral administration (gavage) for a number of polycyclic aromatic hydrocarbons

Compound	Species	Duration	Critical effect	NOAEL	Reference
Acenaphthene	mouse	90 days	liver toxicity	175 mg/kg bw	US EPA, 1989a
Anthracene	mouse	90 days	none	1000 mg/kg bw (highest dose)	US EPA 1989b
Benzo[*a*]pyrene	rat	90 days	liver weight	3 mg/kg bw	Kroese *et al.*, 2001
Fluoranthene	mouse	13 weeks	liver/kidney toxicity	125 mg/kg bw	US EPA, 1988
Fluorene	mouse	13 weeks	organ weight, haematology	125 mg/kg bw	US EPA, 1989c
Naphthalene	mouse	90 days	none	53 mg/kg bw	Shopp *et al.*, 1984
,,	rat	13 weeks	body weight, nephropathy	100 mg/kg bw (71 mg/kg bw adjusted)	BCL 1980 (cited in IRIS, 2002)
,,	rat	11 weeks	liver/kidney toxicity	150 mg/kg bw (LOAEL, only one dose level)	Kawai, 1979
,,	rabbit	4-13 weeks	cataract	500 mg/kg bw (LOAEL)	Wells *et al.*, 1989
Pyrene	mouse	13 weeks	kidney toxicity	75 mg/kg bw	US EPA, 1989d

3.4 Special short-term assays for induction of preneoplastic lesions

Benzo[*a*]pyrene induced aberrant crypt foci of the colon in female CD1 mice and female Sprague-Dawley rats in a dose-related fashion. The mouse was 15 times more sensitive than the rat (Tudek *et al.*, 1989). In the mouse colon a lesion termed nuclear anomalies (NA) was induced by benzo[*a*]pyrene and benzo[*b*]fluoranthene, whereas benzo[*a*]anthracene, pyrene and benzo[*e*]pyrene did not have any effect (Reddy *et al.*,1991).

Benzo[*a*]pyrene and benz[*a*]anthracene were effective as initiators of preneoplastic liver lesions in rats promoted by treatment with N-2-fluorenylacetamide and carbon tetrachloride. Administration by gavage was more effective than ingestion with the diet. Anthracene and pyrene had no effect (Tatematsu *et al.*, 1983).

3.5 Chronic toxicity/carcinogenicity of PAH

For most of the PAH it is the carcinogenic potential that constitutes the critical effect for the hazard- and risk characterisations. A number of PAH, as well as coal tar and various complex mixtures containing PAH from combustion emissions, have shown carcinogenicity in experimental animals and genotoxicity and mutagenicity *in vitro* and *in vivo* (IARC, 1973; IARC, 1983; IARC, 1989; Montizaan *et al.*, 1989; Hughes *et al.*, 1997; ATSDR, 1994; IPCS, 1998; US EPA, 1984a and 1984b).

3.5.1 Carcinogenicity of PAH following oral administration

Most studies to assess the carcinogenic potential of single PAH were carried out following dermal, subcutaneous, or inhalation exposure. Only a limited number of studies dealt with oral administration.

Benzo[*a*]pyrene, when administered by the oral route has produced tumours of the gastrointestinal tract, liver, lungs, and mammary glands of mice and rats. Of the few other PAH tested for carcinogenicity by the oral route, dibenz[*a,h*]anthracene and benz[*a*]anthracene have produced tumours of the gastrointestinal tract, lungs and liver in mice. No increases in tumour incidences were seen in rats after oral administration of benz[*a*]anthracene, phenanthrene, fluorene or naphthalene. No other PAH have been tested for carcinogenicity by oral administration (ATSDR, 1994; IARC, 1973, 1983; IPCS, 1998; Culp *et al.*, 1998; Kroese *et al.*, 2001).

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

In a study to compare the tumourigenicity of coal tar with that of benzo[*a*]pyrene female B6C3F1 mice were fed diets containing 0, 5, 25 or 100 mg/kg benzo[*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 papillomas 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).

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 High Pressure Liquid Chromatography (HPLC) (dose levels equivalent to 0.03, 0.09, 0.32, 0.96, 1.92 or 3.2 mg benzo[*a*]pyrene/kg bw/day), 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*]pyrene/kg bw/day). 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) and at 0.3% of mixture II (13/44). Also the incidences of liver tumours (0.3% mixture I), tumours of the small intestine (0.6 and 1.0% mixture I) and haemangiosarcomas (0.3 and 0.6% mixture I and 0.3% mixture II) were significantly increased (Culp *et al.*, 1998).

Wistar rats (52 per dose, and sex) were treated with benzo[*a*]pyrene (dissolved in soy oil) at 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 incidences of combined adenoma and carcinoma in the liver were 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 tissue 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).

3.5.2 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*]fluoranthene, 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; Kroese *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.

3.5.3 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 produced 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).

The 4-7 ring PAH fraction of condensates from car exhaust (gasoline, diesel), domestic coal stove emissions, and tobacco smoke contained almost all their carcinogenic potential. 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).

3.6 Genotoxicity of PAH

Fifteen out of the 33 PAH considered in this opinion (table 1.1) show clear evidence of mutagenicity/genotoxicity in somatic cells *in vivo* (table 3.6.1).

For six other 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 vitro*. 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[*e*]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.

In general, within this group of PAH the evidence of genotoxicity shows considerable overlapping with carcinogenicity (table 3.6.2), in agreement with the mechanistic link between DNA adducts formation, mutations, and cancer outcome following PAH exposure (You *et al.*, 1994; Nesnow *et al.*, 1995; 1998).

As regards induction of effects in germ cells, benzo[*a*]pyrene, benzo[*a*]anthracene and chrysene gave positive results in chromosome aberrations and/or dominant lethals tests in rodents. However, high doses were required.

Table 3.6.1 Genotoxicity of selected polycyclic aromatic hydrocarbons: overall evaluation of individual compounds

common name (CAS no.)	**conclusion of evaluation** [a]
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 genotoxicity (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 genotoxicity (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 genotoxicity (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)
cyclopenta[*cd*]pyrene (27208-37-3)	**genotoxic** (positive results in assays *in vitro* and for DNA binding *in vivo*)

common name (CAS no.)	**conclusion of evaluation** [a]
dibenz[*a,h*]anthracene (53-70-3)	**genotoxic** (positive results in assays *in vitro* and *in vivo* for multiple end-points)
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-methylphenanthrene (832-69-9)	**limited** evidence of genotoxicity (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 genotoxicity (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 genotoxicity (positive results from *in vitro* assays, no *in vivo* data available)

[a] mixed results, positive and negative results from studies using the same experimental system or addressing the same end-point

Table 3.6.2 Evaluations of genotoxicity and carcinogenicity of selected polycyclic aromatic hydrocarbons

common name (CAS no.)	**genotoxicity (this opinion)**	**carcinogenicity (IPCS, 1998)[a]**	**carcinogenicity (IARC, 1987)[b]**	**carcinogenicity (EC, 2001c)[c]**
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**

common name (CAS no.)	**genotoxicity (this opinion)**	**carcinogenicity (IPCS, 1998)[a]**	**carcinogenicity (IARC, 1987)[b]**	**carcinogenicity (EC, 2001c)[c]**
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**		
indeno[*1,2,3-cd*]pyrene (193-39-5)	**genotoxic**	**positive**	**2B**	
5-methylchrysene (3697-24-3)	**genotoxic**	**positive**	**2B**	
1-methylphenanthrene (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	

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

[b] IARC (1987)

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

3.7 Reproductive and developmental toxicity of PAH

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 foetus.

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).

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)

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 benzo[*a*]pyrene was given in the diet at a level of 1000 mg/kg diet (corresponding to an intake of 50 mg benzo[*a*]pyrene/kg bw/day), 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 intraperitoneal 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, subcutaneous 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 intrauterine growth retardation, 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.

3.8 Effects of PAH on the immune system

The immunotoxicity of PAH has been known for a number of years (Malmgren *et al.*, 1952). The immunotoxic effect most often reported following exposure to PAH is immunosuppression. A few reports also deal with immunopotentiation (stimulation) either *in vitro* or following inhalation or topical exposure. Immunosuppression is associated with an increased susceptibility of the exposed individuals to the development of cancers or infectious diseases, whereas immunopotentiation results in an increased secretion of cytokines by immune cells, thus leading to inflammation which in turn and under specific circumstances may facilitate tumour development or expression of hypersensitivity (allergy, contact hypersensitivity) or auto immunity (Burchiel and Luster, 2001). It should be noted that most studies on the immunotoxicity of PAH have used parenteral administration and that most of the available data consider only a few selected substances, benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene being most widely used.

Two main mechanisms have been suggested as promoting PAH-induced immunosuppression. One involves the reactivity of PAH with the Ah receptor and the other their capacity to increase the intracellular calcium concentration in immune cells possibly due to protein tyrosine kinase activation by PAH. In any case, antigen and mitogen receptor signaling pathways are altered leading to proliferation and/or death (apoptosis) of immune cells (Burchiel and Luster, 2001; Near *et al.*, 1999; Krieger *et al.*, 1994; Davila *et al.*, 1995; Mounho *et al.*, 1997).

3.8.1 Immunosuppressive effects of PAH

Benzo[*a*]pyrene treatment (50 or 100mg/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).

Ten PAH were evaluated for immunosuppression following both acute or 14 days of repeated exposure in female B6C3F1 mice using the antibody-forming cell response to sheep erythrocytes. Anthracene, chrysene, benzo[*e*]pyrene and perylene had no significant effect. Benz[*a*]anthracene, benzo[*a*]pyrene, dibenz[*a,c*]anthracene, and dibenz[*a,h*]anthracene suppressed the antibody-forming cell response by 55 to 91%. The greatest suppression was observed with the 3-methylcholanthrene and 7,12-dimethylbenz[*a*]anthracene. Studies using mice with different susceptibility to aryl hydrocarbon hydroxylase induction demonstrated that susceptible mice (B6C3F1) were not as immunosuppressed following exposure to PAH as were nonsusceptible mice (DBA/2) (White *et al.*, 1985).

From studies on the immunosuppressive effect in female B6C3F1 mice of a mixture of 17 congeners including PAH with 2 (indan, naphthalene, 1- and 2-methylnaphthalene), 3 (acenaphthylene, acenaphthene, dibenzofuran, fluorene, phenanthrene and anthracene), or 4 or more rings (pyrene, fluoranthene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene) it was concluded that the 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 and decreased numbers of red and white blood cells. Decreased serum IgM and IgA levels were noted after treatment of the animals with 30 and 90 mg/kg, respectively. Thymus weight and spleen B-cell populations were affected at a dose of 10 mg/kg, a level

where no overt toxicity was noted. The NOAEL for immunotoxicity of benzo[*a*]pyrene in the rat was 3 mg/kg bw/day (De Jong *et al.*, 1999).

3.8.2 Immunosuppressive effects observed in offspring after treatment *in utero*

Fetuses 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, respectively (Holladay and Smith, 1995b).

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).

3.8.3 Observations in humans

The status of humoral immunity has been evaluated in coke oven workers exposed chronically to a complex mixture of airborne chemicals including high amounts of PAH. Alterations found were marked depression of mean IgG and IgA together with a trend to decreased IgM and increased IgE values (Szceklik *et al.*, 1994). In another study of coke oven workers the effects found were decreased mitogenic reaction of T cells to phytohaemagglutinin, 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 lymphocyte subpopulations in peripheral blood and in immunoglobulin levels of serum (Winker *et al.*, 1997).

3.9 Special studies on cardiovascular effects

It has been hypothesised 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 (IPCS, 1998).

It is unequivocal that tobacco smoking is a major risk factor for cardiovascular disease and there is some evidence that occupational exposure to combustion products containing PAH might 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 reactive and toxic metabolites (Thirman *et al.*, 1994). 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).

DNA adducts have been detected in the endothelium of the internal mammary artery of smokers and in smooth muscle cells of human abdominal aorta affected by arteriosclerotic lesions. Individuals with the GSTM1*2/2 genotype (null) 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 but similar adducts in the aorta was observed in rats exposed to cigarette smoke (Izzotti *et al.*, 2001b).

7,12-Dimethylbenz[*a*]anthracene and benzo[*a*]pyrene, were shown to act as initiators and/or accelerators in atherosclerotic plaque formation in the chicken (Bond *et al.*, 1981), pigeon (Revis *et al.*, 1984) and Ah-responsive mice (Paigen *et al.*, 1986; Wakabayashi, 1990). Benzo[*a*]pyrene, benzo[*e*]pyrene, dibenz[*a,h*]anthracene, dibenz[*a,c*]anthracene, and 3-methylcholanthrene induced spontaneous aortic plaques in cockerels to grow to a larger size and at a faster rate than plaques in control animals (Penn and Snyder, 1988).

Although PAH related adducts have been observed in blood vessels in humans and some effects of PAH have been seen on vascular cells *in vitro*, no causal relationship has been established between increased cardiovascular risk and PAH exposure arising from tobacco smoking or occupational exposure to combustion products.

3.10 Further observations in humans

3.10.1 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. Genotoxic effects in lymphocytes have also been used as endpoints, but are not specific to PAH. 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 the reactive metabolite.

1-Hydroxypyrene, a metabolite of pyrene, has been widely used as urinary biomarker of PAH exposure. In contrast to other PAH metabolites, which are excreted mainly in faeces, 1-hydroxypyrene is excreted in urine. Its advantages are that pyrene is present in all PAH mixtures at relatively high concentrations (2-10%). However, the relationship between pyrene and benzo[*a*]pyrene may vary considerably between different exposures (IPCS, 1998). Five volunteers had a 100-250 fold higher benzo[*a*]pyrene intake when they consumed high-PAH meals than when they consumed low-PAH meals. However, intake of high-PAH meals only resulted in a 4-12 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-hydroxypyrene glucuronide excretion in urine above background returning to background within 24-72 hours (Kang *et al.*, 1995).

DNA adducts with reactive metabolites (mainly diol epoxides) of benzo[*a*]pyrene and other PAH have been identified in humans exposed to tobacco smoke or living in polluted areas in numerous studies (Kyrtopoulos *et al.*, 2001). PAH-DNA adducts have also been detected in peripheral white blood cells following intake of charbroiled meat (Kang *et al.*, 1995). 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. In a prospective study, 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).

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. Although the concentrations of PAH that occur under different exposure conditions differ by orders of magnitude, the differences in mean DNA adduct levels between populations are quite small, in contrast to the results on 1-hydroxypyrene excretion. However, in all populations studied, there is substantial interindividual 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).

In the general population the levels of PAH-DNA adduct in control subjects varied from 0.2 to about 10 adducts per 10^8 nucleotides in leukocytes (Dell'Omo and Lauwerys, 1993). For populations living in areas polluted by industry, adduct levels up to 13 adducts per 10^8 nucleotides were reported. 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 have elevated levels of adducts (5-70 adducts per 10^8 nucleotides) (IPCS, 1998).

In many cases exposure to PAH from food is a confounder when biomarkers are used 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, large changes in atmospheric levels of PAH are 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 of a lower intake of charbroiled meat (Phillips, 1999).

3.10.2 Epidemiological and other studies

There are almost no 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 also little information on human exposure to single, pure PAH except for accidental exposure to naphthalene.

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

Occupational exposure to PAH-containing emissions from coke production, coal gasification, aluminium production, iron and steel founding, coal tars and coal tar pitches, and soots have produced lung cancer in humans, and coal tars and coal tar pitches, non-refined mineral oils, shale oils, and soots have produced human skin and scrotal cancers

(IARC, 1984, 1985, 1987; IPCS, 1998). Although PAH are believed to be the main cause of cancer from these sources, a number of other compounds are present, probably also contributing to the effect. A particularly high rate of lung cancer mortality was found in coke-oven workers. The increases in lung cancer cases correlated closely with the time spent working on top of ovens where an average benzo[*a*]pyrene concentration of about 30 $\mu g/m^3$ has been detected (IARC, 1984).

A large volume of literature exists on the effects of tobacco smoke which contain PAH, on human lung cancer (see IARC, 1986). On the basis of a 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. Amongst the factors implicated are PAH with four or more aromatic rings (Grimmer *et al.*, 1988; IPCS, 1998).

Mortality from lung cancer in a rural county, Xuan Wei, located in the Yunnan Province, China is five times the Chinese national average, especially among the women. The mortality rate from lung cancer was correlated with domestic use of 'smoky' coal 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, including alkylated PAH, with a mean benzo[*a*]pyrene concentration of 15 $\mu g/m^3$, comparable to the exposure levels of coke-oven workers (Mumford *et al.*, 1995, cited in IPCS, 1998).

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 distillation of fir and pinewood, and which contain *inter alia* PAH. In order to assess this exposure with regard to risk of gastric cancer, 59 cases and 53 controls from the relevant area were selected from a multi-center case control study on gastric cancer. The exposure to wine stored in tar impregnated leather bottles was assessed by a self-administered questionnaire. Although an increased risk was reported, the study population was too small to achieve statistically significant increases. An exception was consumption of more than 2 litres of wine per week, which appeared to be associated with gastric cancer particularly in males (OR and 95% confidence interval = 10.5, 1.13-97.76).

In a published abstract Sinha and co-workers (2001) described an increased risk of colorectal adenomas associated with dietary benzo[*a*]pyrene intake in a case-control study of 146 newly diagnosed cases and 226 controls. Increased risk of colorectal adenomas was both associated with benzo[*a*]pyrene intake from meat as well as the total intake estimated

from all foods. Dietary intakes 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).

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 8.4 to 16 ng benzo[*a*]pyrene per g and also heterocyclic amines. 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 feeding study of pan-fried meat containing high levels of heterocyclic amines but unchanged and low levels of PAH, also found an induction of CYP1A2.

4 HAZARD CHARACTERISATION

4.1 Non-carcinogenic effects

Studies in experimental animals on individual PAH have shown various non-carcinogenic effects, such as haematological effects, liver toxicity, reproductive and developmental toxicity and immunotoxicity. NOAELs for effects on liver, kidney and haematology ranging from 70 to 1000 mg/kg bw/day have been reported for various PAH (acenaphthene, anthracene, flouranthene, fluorene, naphthalene, pyrene) in subchronic studies (see table 3.3.1). For benzo[*a*] pyrene a NOAEL for effects on the liver was reported at 3 mg/kg bw/day in a 90-day rat gavage study and a NOAEL of 3 mg benzo[*a*] pyrene/kg bw/day was also found for immunotoxicity in a 35 day rat gavage study. No NOAEL for reproductive and developmental toxicity has been identified for benzo[*a*] pyrene. For some of these compounds (e.g. anthracene, benzo[*a*]fluorene, fluorene, naphthalene, pyrene), an evaluation of the available carcinogenicity and genotoxicity data would conclude that they are not genotoxic and carcinogenic. For exposure to these compounds individually a threshold approach to risk assessment would be appropriate, and TDIs could be established where sufficient data exist. Based on the above-mentioned NOAELs the US EPA (IRIS, 2002) has derived Reference Doses (RfDs) for chronic oral exposure for acenaphthene, anthracene, fluoranthene, fluorene, naphthalene and pyrene. The US EPA uses the terminology "Reference Dose" instead of the Tolerable Daily Intake (TDI) used by this Committee, however the principles used in the derivation of the RfD and the TDI are similar. Large safety factors were used by the US EPA because of the limited databases available and the use of subchronic studies and not chronic studies for the derivation. However, it is the carcinogenic and genotoxic potentials of PAH that are critical for the risk assessment, because exposure to PAH in food is almost exclusively to a mixture of PAH which include genotoxic and carcinogenic PAH. The TDIs of individual components would therefore not be relevant to the assessment of the risk of such mixtures.

4.2 Carcinogenic PAH

Epidemiological data (of an older date) on lung cancer deaths in relation to inhalation of workplace air containing PAH, most notably exposure to coke-oven emissions, have been used in the hazard characterisation of PAH-exposure from ambient air (US EPA, 1984b; WHO, 2001). However, there are no human data available that can be used for the assessment of the health effects of PAH exposure from food. Therefore, the assessment must rely on results from studies using experimental animals.

4.2.1 Hazard characterisations performed by others for oral intake of benzo[*a*]pyrene alone and as part of a complex PAH mixture in food

Several authors and agencies have performed hazard characterisations for oral intake of benzo[*a*]pyrene as such or as a constituent of a coal tar mixture, based on carcinogenicity studies in experimental animals. Either quantitative risk estimates in the form of "slope factors" (human excess cancer risk from oral lifetime exposure to 1 mg benzo[*a*]pyrene/kg bw/day) or "virtually safe doses" (e.g. dose associated with an excess lifetime cancer risk of zero to $1x10^{-6}$) were calculated from the animal experiments. In several cases scaling factors were used to correct for differences in metabolic rates (caloric demands) between animals and man. In the following the Committee quote some of these hazard characterisations. However, it should be made clear that the Committee has not endorsed the use of these quantitative methods, nor has it made any comment on what might be considered a "virtually safe dose".

The US EPA made a quantitative risk estimate for oral exposure to benzo[*a*]pyrene that consisted of a range of "slope" factor values, from 4.5 to 11.7 per mg benzo[*a*]pyrene/kg bw/day with a geometric mean of 7.3 per mg benzo[*a*]pyrene/kg bw/day (IPCS, 1998; IRIS, 2002). Three methods were used to determine an upper bound (95%) on a linear low-dose term from data on the incidence of gastrointestinal tumours in mice exposed perorally to benzo[*a*]pyrene (Neal and Rigdon, 1967) and a linearised multistage procedure was used to derive an upper bound (95%) slope factor from the total numbers of gastrointestinal tumours in male and female rats exposed to benzo[*a*]pyrene in the diet (Brune *et al.*, 1981). In all cases scaling factors were used to correct for differences in metabolic rates between animals and man. Using the oral slope factor of 7.3 per mg benzo[*a*]pyrene/kg bw/day for the carcinogenic risk from benzo[*a*]pyrene exposure as developed by US EPA (IRIS, 2002) an oral "virtually safe dose" of 0.14 ng benzo[*a*]pyrene/kg bw/day can be calculated for a risk level of $1x10^{-6}$ via linear extrapolation.

The recent chronic oral (gavage) rat study by Kroese *et al.* (2001) with benzo[*a*]pyrene (dosed 3, 10 and 30 mg/kg bw/day, 5 days/week during 2 years) was used by the authors for the derivation of a "virtually safe dose" for benzo[*a*]pyrene calculated for a risk level of $1x10^{-6}$. The study resulted in dose-dependent tumour development in several organs and tissues, predominantly in liver and forestomach. Based on the incidences of treatment related tumours, as induced by benzo[*a*]pyrene, and applying a simple linear model, the authors calculated "virtually safe doses" for several individual tumour types and all tumours combined. The "virtually safe dose" ranged from 5 - 19 ng benzo[*a*]pyrene/kg bw per day for individual tumour types. For all tumours combined a "virtually safe dose" of 5 ng benzo[*a*]pyrene/kg bw per day was calculated. The value of 5 ng benzo[*a*]pyrene/kg bw per day is considerably higher than the value calculated and presented above from the "slope factors" given by the US EPA (IRIS, 2002), i.e. it implies a lower risk at any particular dose

level. This is most probably because 1) no scaling factor was used to correct for differences between species (rat and man), 2) a mean estimate was used instead of a 95% confidence limit, and 3) different animal data were used for extrapolation.

According to Kroese *et al.* (2001) the "virtually safe dose" calculated for a risk level of $1x10^{-6}$ from their rat study corresponded well with the results of the recent two-year carcinogenicity study of benzo[*a*]pyrene in mice (Culp *et al.*, 1998). When they applied the same extrapolation method as used for their own rat study to the results of Culp *et al.* (1998) a "virtually safe dose" of 5 ng benzo[*a*]pyrene/kg bw/day was calculated based on forestomach tumours and number of tumour-bearing animals. Kroese *et al.* (2001) also used benzo[*a*]pyrene as an indicator substance for the carcinogenic PAH compounds in the coal tars mixtures tested by Culp *et al.* (1998). By using the data from all treatment-related tumours for the two coal tar mixtures, a "virtually safe dose" of 1 ng benzo[*a*]pyrene/kg bw/day was calculated for mixture I (0.224% benzo[*a*]pyrene as determined by HPLC) and a "virtually safe dose" of 3 ng benzo[*a*]pyrene/kg bw/day was calculated for mixture II (0.367% benzo[*a*]pyrene as determined by HPLC).

The Norwegian Food Control Authority also used the study by Culp *et al.* (1998) to perform a hazard characterisation for benzo[*a*]pyrene in food (Alexander and Knutsen, 2001). They used a simple linear extrapolation from T_{25} (a chronic dose rate that will give 25% of the animals cancer at a specific tissue site after correction for spontaneous incidence, within the standard life-time of that species (Dybing *et al.*, 2000; Sanner *et al.*, 2001)). When this simple T_{25} model was used, and a dose scaling factor per kg body weight $W^{0.25}$ was used to take into account the comparative metabolic rates between species, they calculated that a daily intake of 5.7 ng benzo[*a*]pyrene/kg bw would be associated with an excess lifetime cancer risk of $1x10^{-5}$. This would correspond to a "virtually safe dose" of 0.57 ng benzo[*a*]pyrene/kg bw/day calculated for a risk level of $1x10^{-6}$. These authors also found that the coal tars used in the study by Culp *et al.* (1998) were approximately 2.5 times more potent in the development of lung cancer than in the development of forestomach tumours. For the development of forestomach tumours the potencies were approximately similar to that expected from the benzo[*a*]pyrene content.

Schneider *et al.* (2002) used the result from the study by Culp *et al.* (1998) in mice to calculate a combined cancer "slope factor" of 11.5 for human excess cancer risk for lifetime oral exposure to 1 mg benzo[*a*]pyrene/kg bw/day in a PAH mixture. They used the LED_{10} method (using the 95% lower confidence limit on the dose associated with 10% extra risk, adjusted for background, as a starting point for linear extrapolation) according to the US EPA revised cancer risk assessment guidelines (US EPA, 1999). A scaling factor per kg body weight $W^{0.25}$ was used to adjust for differences in metabolic rates between mouse and man. From the oral "slope factor" of 11.5 per mg benzo[*a*]pyrene/kg bw/day as part of a PAH mixture for the carcinogenic risk of PAH in food an oral "virtually safe dose" of 0.09

ng benzo[*a*]pyrene/kg bw/day in a PAH mixture can be calculated for a risk level of $1x10^{-6}$ via linear extrapolation.

Thus, comparison of the results of the carcinogenicity studies in rats and mice with benzo[*a*]pyrene and the study with two different coal tar mixtures in mice shows that oral administration of coal tar mixtures produced tumours in more tissues (lung, intestine, haemangiosarcomas, forestomach and liver) than benzo[*a*]pyrene alone (liver, forestomach, auditory canal). Furthermore, the carcinogenic potency of the coal tar mixtures in mice was up to 5 times higher than what would be expected from their benzo[*a*]pyrene content.

4.2.2 Hazard characterisation for other individual carcinogenic PAH

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. However, there are no data justifying extrapolation of relative potency data on carcinogenicity from other routes of exposure to the oral route.

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.

The use of the TEF approach requires that the compounds in question exert 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, bind to 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) 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) 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.

Therefore, the Committee did not find it appropriate to endorse the use of the TEF approach for the risk assessment of PAH in food.

4.2.3 Discussion and conclusions on the hazard characterisation for carcinogenic PAH in food

Instead of using the TEF approach in the risk assessment of PAH in food, a more suitable approach would be to use benzo[*a*]pyrene as a marker of the occurrence and effect of the carcinogenic PAH in food. In section 2.2, it was shown that the profiles (ratio relative to benzo[*a*]pyrene) of the measured carcinogenic PAH were surprisingly similar in various foods, irrespective of the supposed origin of the PAH contamination. Overall, these profiles seemed to vary within a factor of less than five. This suggests that benzo[*a*]pyrene can be used as a marker for occurrence of the carcinogenic PAH in foods. Furthermore, it was shown that the profile of the measured carcinogenic PAH in the coal tars used in the studies of carcinogenicity in mice varied within a factor of less than two from that seen in foods. However, not all carcinogenic PAH potentially present in the coal tars were measured. Given this less than 2-fold variation between the profiles of carcinogenic PAH in these coal tars and in various foods, and the finding that the carcinogenic potencies of the coal tar mixtures could be up to 5 times that predicted by their benzo[*a*]pyrene content, a conservative assessment would imply that the carcinogenic potency of total PAH in foods would be 10 times higher than expected on the basis of the benzo[*a*]pyrene content alone. This is also in agreement with results of studies in mice using skin painting which showed 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.

From the recent studies in rodents, several authors have estimated "virtually safe doses" of benzo[*a*]pyrene ranging from approximately 0.6 ng/kg bw/day to 5 ng/kg bw/day for a risk level of $1x10^{-6}$, when based on all tumours combined. Applying the above mentioned factor of 10, a "virtually safe dose" of benzo[*a*]pyrene as a marker of the mixture of carcinogenic PAH in food would be in the range 0.06 to 0.5 ng benzo[*a*]pyrene/kg bw/day. This is in accordance with the calculation by Schneider *et al.* (2002). Using the data from the study of coal tars in mice by Culp *et al.* (1998), they calculated a "slope factor" for humans of 11.5 (human excess risk per oral lifetime exposure with 1 mg benzo[*a*]pyrene/kg bw/day in a PAH mixture) for oral PAH exposure. From this oral "slope factor" an oral "virtually safe dose" of 0.09 ng benzo[*a*]pyrene/kg bw/day in a PAH mixture can be calculated for a risk level of $1x10^{-6}$ via linear extrapolation.

Experimental data show the importance of formation of specific DNA adducts and mutations in the carcinogenic process following PAH exposure (You *et al.*, 1994; Nesnow *et al.*, 1995, 1998), and our understanding is that genotoxicity is the most plausible mechanism for initiation of PAH carcinogenicity. However, the correlation between DNA adducts, mutagenesis and carcinogenesis of PAH is not straightforward. 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 orally with

benzo[*a*]pyrene. Similarly, in the chronic oral rat carcinogenicity assay on benzo[*a*]pyrene DNA adducts were found in all tissues, with remarkably high levels in organs devoid of any tumour development (i.e. lungs, kidneys) (Kroese *et al.*, 2001). Increased cell proliferation was seen in the organs where tumours developed (i.e. forestomach, liver). Culp *et al.* (1996; 1998) found that coal tar-induced cytotoxicity and cell proliferation were the critical factors rather than DNA binding in the tumour induction in the small intestine of mice. This shows that factors additional to DNA adduct formation are critical for tumour development by benzo[*a*]pyrene and other PAH and that genotoxic end-points alone may not adequately predict tumour outcome due to organ/tissue-specific mechanisms.

Thus, in addition to its effect on the initiation stage, the carcinogenicity of PAH shown at higher doses is enhanced by the promoting activity of the parent compound, such as inhibition of intracellular communication, resulting in clonal expansion of initiated cells. It is also modulated by the ability to induce Ah-receptor mediated responses, such as imunosuppression and inhibition of apoptosis (death of damaged cells), which may play a major role, at least at the high doses applied in cancer bioassays. These promotional effects are thought to be partly reversible and show thresholds. Extrapolation of the tumour risk observed as a result of high dose (promotional) administration to low dose (initiating and non-promotional) administration is likely to overestimate the actual risk associated with low dose exposures.

The Committee has reservations about the use of mathematical modelling to extrapolate from animal tumour data in order to estimate risks to humans at low exposures to substances that are both genotoxic and carcinogenic, such as PAH. For substances that are both genotoxic and carcinogenic the Committee uses a weight-of-evidence assessment of all the available scientific data in describing the hazard. However, characterisation of the risk has been more difficult and the Committee has generally recommended that exposures should be as low as reasonably achievable (ALARA). ALARA was found to be the appropriate recommendation for substances that are genotoxic and carcinogenic, because such substances are considered to be without a threshold in their action on DNA.

5 RISK CHARACTERISATION

5.1 Non-carcinogenic effects

In table 2.3.1 the estimated maximum dietary intake based on "overall European data" is given. For the most abundant PAH in food, such as anthracene, phenanthrene, fluoranthene and pyrene the figures are 5.6, 5.1, 4.3 and 4.0 μg/day per person, respectively. For a 70 kg person these values correspond to about 80, 70, 60 and 60 ng/kg bw/day for the four PAH. The NOAEL for anthracene in a short-term (90-day) mouse gavage study was 1000 mg/kg bw/day, the highest dose used. Thus, the estimated maximum intake of anthracene from food is about 12,500,000 times lower than this NOAEL. For fluoranthene, a NOAEL of 125 mg/kg bw/day was reported in a short-term (13-week) mouse study, based on liver and kidney toxicity seen at higher doses. The estimated maximum intake is about 2,100,000 times lower than the NOAEL. For pyrene a NOAEL was seen at 75 mg/kg bw/day in a short-term (13-week) mouse study, based on kidney toxicity at higher doses. This NOAEL is about 1,250,000 times higher than the estimated maximum dietaryintake 0.06 μg/kg bw/day. Studies are not available to identify a NOAEL for phenanthrene, however its toxicity is not expected to be markedly different from that of anthracene, pyrene and fluoranthene. For most of the other PAH included in the assessment dietary exposures are an order of magnitude lower and non-carcinogenic effects are not considered to be of concern.

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.

5.2 Carcinogenicity

A number of PAH have been demonstrated to be genotoxic and carcinogenic (see table 3.6.2). Therefore the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. It recommended that exposures to PAH should be as low as reasonably achievable.

The Committee considered that benzo[*a*]pyrene can be used as a marker of the occurrence and effect of the carcinogenic PAH in food. A conservative assessment would imply that

the carcinogenic potency of total PAH in food would be 10 times higher than expected from benzo[*a*]pyrene alone.

The estimated maximum daily intake of benzo[*a*]pyrene from food is approximately 420 ng benzo[*a*]pyrene per person (table 2.3.1), equivalent to approximately 6 ng/kg bw/day for a person weighing 70 kg. This is about 5 – 6 orders of magnitude lower than the daily doses observed to induce tumours in experimental animals.

6 CONCLUSION

Fifteen out of the 33 PAH considered in this opinion, 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 show clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals *in vivo*. With the exception of benzo[*ghi*]perylene they have also shown clear carcinogenic effects in various types of bioassays in experimental animals. Although only benzo[a]pyrene has been adequately tested using dietary administration, these compounds may be regarded as potentially genotoxic and carcinogenic to humans. They represent a priority group in the assessment of the risk of long-term adverse health effects following dietary intake of PAH.

For non-smoking humans, food is the main source of exposure to PAH. In cigarette smokers, the contributions from smoking and food may be of a similar magnitude. Data from EU surveys indicate that the estimated maximum dietary exposure of adults to each of the most abundant PAH such as anthracene, phenanthrene, fluoranthene and pyrene may be in the range of 60 – 80 ng/kg bw/day. The dietary exposure to the other PAH, including the 15 PAH considered to be potentially genotoxic and carcinogenic for humans, would be one order of magnitude lower. The Committee concluded that at these levels of intake non-carcinogenic effects are not to be expected and that the risk of heritable effects from dietary exposure to PAH is low. Concerning the carcinogenic action of PAH, the Committee noted that several authors and agencies used mathematical modelling to derive "virtually safe doses" for benzo[*a*]pyrene in order to estimate the health hazard of dietary exposure for humans. However, the Committee has reservations about the application of such models and for substances that are both genotoxic and carcinogenic such as PAH, the Committee recommends that exposures from food should be as low as reasonably achievable.

Attempts by others to derive relative potency factors, expressed as toxic equivalency factors (TEF) for individual PAH (relative to benzo[*a*]pyrene) were noted and discussed. However, the Committee could not advocate the use of the TEF approach for the risk assessment of PAH.

The Committee concluded that benzo[*a*]pyrene may be used as a marker of occurrence and effect of the carcinogenic PAH in food, based on examinations of PAH profiles in food and on evaluation of a recent carcinogenicity study of coal tars in mice. A conservative assessment would imply that the carcinogenic potency of total PAH in foods would be 10 times that contributed by benzo[*a*]pyrene alone. The Committee however stressed that though it considers benzo[*a*]pyrene as a marker of carcinogenic PAH in food, chemical

analyses should continue to collect data on the whole PAH profile in order to be able to evaluate the contamination of food commodities and any future change in the PAH profile.

In the context of the advice that exposures to PAH should be as low as reasonably achievable, it should be noted that the estimated maximum daily intake of benzo[*a*]pyrene from food is approximately 420 ng benzo[*a*]pyrene per person (table 2.3.1), equivalent to approximately 6 ng/kg bw/day for a person weighing 70 kg. This estimated maximum daily intake is about 5-6 orders of magnitude lower than the daily doses observed to induce tumours in experimental animals.

Food can be contaminated by environmental PAH that are present in air (by deposition), soil (by transfer) or water (by deposition and transfer), and during processing and cooking. The major contributors to PAH intake in the average diet are oils and fats, cereals,fruits and vegetables.

The natural and anthropogenic sources of PAH in the environment are numerous. PAH compounds are emitted from a number of environmental sources, such as processing of coal, crude oil, petroleum, natural gas, production of aluminium, iron and steel, heating in power plants and homes (oil, gas, charcoal-fired stoves, wood stoves), burning of refuse, wood fires, motor vehicle exhausts.

The waxy surface of vegetables and fruits can concentrate low molecular mass PAH through surface adsorption and particle-bound high-molecular-mass PAH can contaminate the surface due to atmospheric fallout. Crops from industrial areas or close to high-traffic roads may therefore contain elevated levels of PAH. PAH will not accumulate in plant tissues with high water content and transfer from contaminated soil to root vegetables will be limited, because adsorption of PAH to the organic fraction of soil is strong. Thus, the concentrations of PAH are generally greater on plant surface (peel, outer leaves) than on internal tissue. Consequently, washing or peeling may remove a significant proportion of the total PAH.

It should be noted that smoked and grilled food may contribute significantly to the intake of PAH if such foods are part of the usual diet. The highest PAH concentrations are usually found in charcoal grilled/barbecued foods (especially meat and meat products grilled under prolonged and severe conditions), foods smoked by traditional techniques (fish in particular), mussels and other seafood from polluted waters. Contamination of cereals and of vegetable oils (including seed oils and olive residue oils) with PAH usually occurs during technological processes like direct fire drying, where combustion products may come into contact with the grain, oil seeds or the oil.

The Committee noted that measures to reduce PAH intakes from food therefore depend both on the continuous control of emissions of PAH to the environment, and on instruction/education of both manufacturers and consumers. For example, avoiding the use of commercial smoking and drying processes where combustion products come into direct contact with the foods and giving appropriate advice to consumers on how to avoid contamination with high level of PAH when they prepare grilled and smoked food would help to reduce intake levels.

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