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Polyaromatic Hydrocarbons (PAH)

Evaluation of health hazards and estimation of a quality criterion in soil

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Polyaromatic Hydrocarbons (PAH). Evaluation of health hazards and estimation of a quality criterion in soil

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Content

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to Polyaromatic Hydrocarbons (PAH) and an estimation of a quality criterion in soil. This resulted in 2004 in the present report, which was prepared by J.C. Larsen, Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has finally been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Nature Agency,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
Danish Regions (former Amternes Videncenter for Jordforurening)
The Danish Environmental Protection Agency.

The Danish Environmental Protection Agency
Copenhagen, December 2013

Polycyclic Aromatic Hydrocarbons (PAH)

1 Introduction

Polycyclic aromatic hydrocarbons (PAH) contain three or more fused aromatic rings made up of carbon and hydrogen atoms. There are many natural and anthropogenic sources of PAH in the environment. PAH are formed in natural processes, such as carbonization and therefore are constituents in coal and crude oils. PAH are also formed and released during incomplete combustion or pyrolysis of organic matter during industrial processes and other human activities. PAH are emitted from a number of 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. Soils may be contaminated by PAH due to atmospheric fallout, urban runoff, deposition from sewage, and certain wastes, such as oil or gasoline spills.

PAH are chemically stable. In the presence of light, they are susceptible to oxidation and photo-degradation. 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.

Of the many PAH that can be formed, benzo[*a*]pyrene is the best studied and is often used as a marker for PAH.

For non-smokers, exposure to PAH occurs mainly by ingestion of food (>90 %) and by inhalation of air (< 10%). Other exposure routes are ingestion of contaminated dust and soil and dermal absorption from 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 areas varied between 0.1 and 1 ng/m^3; for urban areas levels were typically between 0.5 and 3 ng/m^3. Indoor sources to PAH exposure include tobacco smoke, cooking fumes, open fireplaces, and unvented heating sources. The additional benzo[*a*]pyrene exposure for a person smoking 20 cigarettes/day was estimated to be 210 ng per day (SCF 2002).

In food, PAH may be formed during processing and 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. Some marine organisms, such as mussels and lobsters are known to accumulate PAH from water, which may be contaminated, for example by oil spills. From surveys conducted in six EU countries, the SCF (2002) estimated the mean dietary intake of benzo[*a*]pyrene for an adult person in the range of 50 - 300 nanograms(ng)/day, corresponding to 1 – 5 ng/kg bw/day for a person weighing 60 kg.

The individual PAH included in the assessment by the SCF (2002) were the 33 compounds that were also included in the IPCS Environmental Health Criteria document on PAH (IPCS, 1998). They were selected due to availability of

information on their occurrence and toxic effects. Except for naphthalene that is not included in this evaluation, they are listed in Table 1.1.

Table 1.1 Polycyclic aromatic hydrocarbons included in the SCF assessment (SCF 2002).

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

2 Toxicological data

2.1 Toxicokinetics

2.1.1Absorption

Benzo(a)pyrene is rapidly absorbed after oral administration to rats. The extent of absorption of PAH from food is in the range of 20-50%. The absorption is influenced by the composition of the diet such that the bioavailability from food increases with increasing lipid content (SCF 2002; IPCS 1998).

2.1.2Distribution

In rodents administered PAH, detectable levels of PAH-derived material can be found in almost all organs. The organs rich in adipose tissue act as stores from which material is slowly released. Due to enterohepatic cycling, high levels can be found in the gastrointestinal tract irrespective of the route of administration (IPCS 1998).

Studies in pregnant mice and rats have shown that PAH cross the placenta being detectable in the fetuses. Benzo[*a*]pyrene, dibenz[*a,c*]anthracene and chrysene were reported to be present in human milk and umbilical cord blood at low levels (IPCS, 1998).

2.1.3Excretion

PAH metabolites are excreted in urine, bile and faeces. The bile is the major route of excretion accounting for 60% of an intraveneous dose of benzo[*a*]pyrene whilst the urinary excretion was 3%. The gastrointestinal microflora can hydrolyse glucuronic acid conjugates of PAH metabolites whereby the metabolites are released and can be re-absorbed, leading to enterohepatic cycling (IPCS, 1998).

2.1.4Metabolism

Metabolic pathways of PAH have been studied in whole animals and *in vitro* using hepatic homogenates, microsomes, cultured cells and explants from animals and humans. While the metabolites formed in many of the different *in vitro* systems are similar, the relative levels and rate of formation are specific for tissues, cells, species, and strain of animal (SCF 2002).

Metabolism and excretion has been studied in whole animals for anthracene, phenanthrene, pyrene, benz[*a*]anthracene, chrysene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and 3-methylcholanthrene (SCF 2002).

The general scheme of PAH metabolism involves oxidation to a range of primary (epoxides, phenols, dihydrodiols) and secondary (diol epoxides, triols, tetrahydrotetrols, phenol epoxides) phase 1 metabolites catalysed by several different cytochrome P450 (CYP) species and other enzymes, followed by conjugation to phase 2 metabolites with glutathione, glucuronic acid or sulphate (IPCS 1988, SCF 2002).

For benzo[*a*]pyrene, the initial stage of metabolism is the formation of several epoxides. These epoxides either spontaneously rearrange to phenols, are hydrolysed by epoxide hydrolase to dihydrodiols, or react with glutathione either chemically or catalysed by glutathione-S-transferases. Dihydrodiols can undergo further oxidative metabolism. For example, the 7,8-dihydrodiol forms the 7, 8-diol-9,10-epoxide. The diol epoxides and triols can be further metabolised to triol epoxides and pentols. Other enzymes such as prostaglandin H-synthase, myeloperoxidase, and lipoxygenases can also oxidatively metabolise the 7,8-diol to diol epoxides and tetraols. These enzymes may be of significance when levels of CYP are low such as in uninduced cells or in chronic irritation or inflammation (IPCS 1998, SCF 2002).

Many PAH 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. In rat liver microsomes the metabolically predominant isomer is (+)anti-benzo[*a*]pyrene-7, 8-diol-9,10-epoxide (BaPDE). This is the isomer that predominantly forms adducts by covalent binding to DNA following benzo[*a*]pyrene exposure in various mammalian cells and organs and also the isomer with the highest mutagenic and tumour inducing activity (IPCS 1998, SCF 2002).

The metabolism of other PAH is equally complex. Depending on the structure of the PAH metabolism can in many cases proceed via other routes than for benzo[*a*]pyrene. A number of diol-epoxides and other reactive metabolites of different PAH have been identified, including some presumed reactive metabolites,

which can form adducts with DNA and potentially induce mutations (IPCS 1998, SCF 2002).

Many of the enzymes involved in the metabolism of PAH have a polymorphic distribution, which mean that a genetic difference can be seen in at least 1% of the human population. Genetic polymorphism in the metabolising enzymes may change the ratio of activation/deactivation of a PAH (IARC, 1999). However, the exact influence of genetic polymorphism on PAH metabolism and toxicity is not known, because compensatory mechanisms or alternative enzymatic pathways may take over (SCF 2002).

2.2 DNA binding of PAH

The covalent binding of PAH to DNA *in vitro* and *in vivo* has been demonstrated in numerous studies (IPCS, 1998). The majority of PAH metabolites shown to react with nucleic acids are diol epoxides, often diol epoxides of the "bay region". Examples of other possibilities include activation of benzo[*j*]fluoranthene 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 (SCF 2002).

The available evidence suggests that the DNA adducts formed in humans are similar to those found in corresponding rodent tissues. The major stable adduct is formed on the N2 position of guanine. Diol epoxides are also thought to react with the N7 position of guanine, yielding labile adducts (SCF 2002).

The mechanism of mutagenicity of PAH has mainly been studied 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 vitro* and *in vivo* shows a prevalence of base-pair substitutions (G>T transversions). In addition, bulky DNA adducts of PAH 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. Studies in rats administered benzo[*a*]pyrene by the oral, dermal, or intratracheal routes, show that adduct formation occurs at both the site of contact and systemically (SCF 2002).

2.3 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 activation of the cellular arylhydrocarbon receptor (AhR) and the subsequent disturbance of cellular homeostasis. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most efficient ligand known for the AhR. The AhR is located in the cytoplasm and following binding of the ligand (e.g. a PAH) a series of biochemical events result in 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 1 and 2 enzymes, proteins involved in signal transduction, and growth factors (SCF 2002).

The AhR has been detected in most cells and tissues. 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.

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.

The AhR-inducing activity of many PAH has been demonstrated both *in vivo* and *in vitro*. The activity was found significantly lower for PAH than for TCDD (SCF 2002).

2.3.1AhR and carcinogenicity of PAH

Althoug the role of AhR is not entirely clear in PAH induced carcinogenesis it is thought to play an important role, especially in the promotional phase. Early animal studies using parenteral administration showed a correlation between inducibility of arylhydrocarbon hydroxylase (AHH) activity and PAH-induced lung carcinogenesis. Recent studies have demonstrated that benzo[*a*]pyrene induces tumours in wild type mice but not in the AhR null allele mouse, which lacks the receptor (SCF 2002).

2.3.2Other receptor mediated effects of PAH

The steric resemblance of PAH to steroid molecules has 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. In addition, an anti-androgenic effect of several PAH has been demonstrated *in vitro*. The significance of this is not known (SCF 2002).

2.4 Single dose toxicity

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 (IPCS, 1998).

2.5 Repeated dose toxicity

Because it is the genotoxic and carcinogenic potential of PAH that constitute the critical effects for hazard- and risk characterisations, the available database on other toxicological end-points from short- and long-term studies is limited.

The main tissues affected after short-term oral administration of PAH to experimental animals appear to be liver, bone marrow, and kidney.

Benzo[*a*]pyrene and other PAH have produced hepatic effect in rodents indicative of proliferative changes (preneoplastic hepatocytes, known as gamma-glytamyl transpeptidase foci, stimulation of hepatic regeneration, and induction of carboxylesterase and aldehyde dehydrogenase) following oral, intraperitoneal or subcutaneous administration (IPCS 1998).

Adverse haematological effects have also been observed in animals after exposure to benzo[*a*]pyrene. Aplastic anemia, pancytopenia, severe reduction in periphere blood leucocytes, and severe bone marrow depression with almost complete destruction of pluripotent haematopoietic stem cells has been seen in female DBA/2 mice after oral administration (125 mg/kg body weight/day) for 13 days. The targets for

benzo[*a*]pyrene toxicity are the proliferating haematopoietic cells of the bone marrow (SCF 2002).

The results of available oral short-term toxicity studies on PAH are summarised in table 2.5.1. (Adopted from SCF 2002)

Table 2.5.1 **Summary of NOAELs/LOAELs from short-term toxicity tests of a number of PAH following oral administration (gavage) (SCF 2002)**

Compound	Species	Duration	Critical effect	NOAEL
acenaphthene	mouse	90 days	liver toxicity	175 mg/kg bw/day
Anthracene	mouse	90 days	none	1000 mg/kg bw/day (highest dose)
Benzo[*a*]pyrene	rat	90 days	liver weight	3 mg/kg bw/day
Benzo[*a*]pyrene	rat	35 days	Immunotoxicity	3 mg/kg bw/day
Fluoranthene	mouse	13 weeks	liver/kidney toxicity	125 mg/kg bw/day
Fluorene	mouse	13 weeks	organ weight, haematology	125 mg/kg bw/day
Pyrene	mouse	13 weeks	kidney toxicity	75 mg/kg bw/day

2.5.1Effects on the immune system

The immunotoxicity of PAH is well-established from studies of benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene after subcutaneous or intraperitoneal injection. The effect most often reported following exposure to PAH is immunosuppression. Immunosuppression is associated with an increased susceptibility of the exposed animals to the development of cancers or infectious diseases (SCF 2002).

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. In any case, antigen and mitogen receptor signaling pathways are altered leading to proliferation and/or death (apoptosis) of immune cells (SCF 2002).

Treatment of female B6C3F1 mice with benzo[*a*]pyrene (50 or 100mg/kg bw per day for 5 days by intraperitoneal injection) induced a reduction in the thymic cellularity, alteration of thymocyte differentiation, and reduced cellularity of the bone marrow. Other studies in mice have demonstrated suppression of the antibody-forming cell response to sheep erythrocytes after PAH exposure. From studies on the immunosuppressive effect in female B6C3F1 mice of a mixture of 17 PAH, including PAH with 2, 3, or 4 or more rings it was concluded that the PAH with 4 rings or more were primarily responsible for the effect (SCF 2002).

Treatment of male Wistar rats by gavage for 35 days with 3, 10, 30, or 90 mg benzo[*a*]pyrene/kg bw/day (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, cited in SCF 2002).

Severe immunosuppressive effects have also been observed in the offspring of mice treated with benzo[a]pyrene (150 mg/kg bw) during the second trimester of the pregnancy. Immunodeficiency occurred early after birth and persisted for 18 months. After 12-18 months the progeny developed high incidences of hepatomas, lung adenomas and adenocarcinomas, reproductive tumours, and lymphoreticular tumours. When benzo[*a*]pyrene was administered postnatally (after 1 week) both immune suppression and tumour incidence were substantially lower (SCF 2002).

2.5.2Reproductive and developmental toxicity

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. Relative adduct levels were significantly higher in smokers than in non-smokers (SCF 2002).

Only benzo[*a*]pyrene has been studied in animals for reproductive toxicity. 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.

Oral and intraperitoneal administration of benzo[*a*]pyrene to mice have resultet in developmental toxicity in the form of embryonic and fetal death, reduced fetal weight and malformations. In mice, 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.

2.5.3Special 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).

However, 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 (SCF 2002).

2.6 Genotoxicity

Fifteen out of the 33 PAH considered by SCF (2002) show clear evidence of mutagenicity/genotoxicity in somatic cells *in vivo* (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, 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).

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 needed 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, three compounds (anthracene, benzo[*a*]fluorene, pyrene) gave totally or mainly negative results in a variety of short term tests (SCF 2002).

In general, within this group of PAH the evidence of genotoxicity shows considerable overlapping with carcinogenicity (see Table 2.7.1), in agreement with the mechanistic link between DNA adducts formation, mutations, and cancer outcome following PAH exposure (SCF 2002).

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.

2.7 Carcinogenicity

A number of PAH, as well as coal tars 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; ATSDR, 1994; IPCS, 1998; US EPA, 1984a and 1984b).

Most studies on single PAH have used dermal, subcutaneous, or intratracheal exposure. Only a limited number of studies have used oral administration.

2.7.1Carcinogenicity following oral administration

When administered by the oral route, benzo[*a*]pyrene most often has produced tumours of the gastrointestinal tract, liver, lungs and mammary glands of mice or 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, or fluorene (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 for 2 years. Equivalent doses are 0, 0.7, 3.6 or 14 mg benzo[*a*]pyrene/kg bw/day. 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 after 3.6 and 14 mg/kg bw/day. 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 control group (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 (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 (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 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 incidences of combined papilloma and carcinoma were 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 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, oral cavity, small intestine, and kidney (Kroese *et al.*, 2001).

2.7.2Carcinogenicity 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 some studies PAH were tested alone, but in most of the studies PAH were tested as initiators of skin cancer together with tumour promoters (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. 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 in newborn animals (ATSDR, 1994; IARC, 1973; IPCS, 1998).

The tumour development after PAH 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, and subcutaneous injection resulted in local sarcomas. However, tumour induction was not restricted to the sites of application (IPCS, 1998).

2.7.3Overall evaluation of the carcinogenicity of PAH
IPCS (1998) concluded that 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 should be considered carcinogenic. Benzo[*c*]phenanthrene and fluoroanthene were suspected of being carcinogenic.

The carcinogenicity of acenaphthene, acenaphthylene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*e*]pyrene, coronene, phenanthrene and pyrene was considered questionable.

Anthracene, benzo[*ghi*]fluoranthene, benzo[*ghi*]perylene, fluorene, 1-methylphenanthrene, perylene and triphenylene were considered to be not carcinogenic (Table 2.7.1).

Table 2.7.1 Evaluations of genotoxicity and carcinogenicity of selected polycyclic aromatic hydrocarbons

Common name (CAS no.)	**Genotoxicity (SCF, 2002)**	**Carcinogenicity (IPCS, 1998)***
Acenaphthene	inadequate data	questionable
Acenaphthylene	inadequate data	no studies
Anthanthrene	limited evidence	**positive**
Anthracene	**not genotoxic**	**negative**
Benz[*a*]anthracene	**genotoxic**	**positive**
Benzo[*b*]fluoranthene	**genotoxic**	**positive**
Benzo[*j*]fluoranthene	**genotoxic**	**positive**
Benzo[*k*]fluoranthene	**genotoxic**	**positive**
Benzo[*ghi*]fluoranthene	limited evidence	negative ?
Benzo[*a*]fluorene	probably not genotoxic	questionable
Benzo[*b*]fluorene	inadequate data	questionable
Benzo[*ghi*]perylene	**genotoxic**	negative ?
Benzo[*c*]phenanthrene	limited evidence	positive ?
Benzo[*a*]pyrene	**genotoxic**	**positive**
Benzo[*e*]pyrene	equivocal	questionable
Chrysene	**genotoxic**	**positive**
Coronene	inadequate data	questionable
Cyclopenta[*cd*]pyrene	**genotoxic**	**positive**
Dibenz[*ah*]anthracene	**genotoxic**	**positive**
Dibenzo[*a,e*]pyrene	**genotoxic**	**positive**
Dibenzo[*a,h*]pyrene	**genotoxic**	**positive**
Dibenzo[*a,i*]pyrene	**genotoxic**	**positive**
Dibenzo[*a,l*]pyrene	**genotoxic**	**positive**
Fluoranthene	equivocal	positive ?
Fluorene	inadequate data	**negative**
Indeno[1,2,3-*cd*]pyrene	**genotoxic**	**positive**
5-Methylchrysene	**genotoxic**	**positive**

Common name (CAS no.)	Genotoxicity (SCF, 2002)	Carcinogenicity (IPCS, 1998)*
1-Methylphenathrene	limited evidence	negative ?
Perylene	limited evidence	negative ?
Phenanthrene	equivocal	questionable
Pyrene	**not genotoxic**	questionable
Triphenylene	limited evidence	negative ?

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

2.7.4Carcinogenicity 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 alone (Culp *et al.*, 1998).

In a series of studies using skin painting, subcutaneous injection and intrapulmonary implantation of different fractions of condensates from car exhaust (gasoline, diesel), domestic coal stove emissions, and tobacco smoke it was shown that the 4-7 ring PAH fraction contained almost all their carcinogenic potential (SCF 2002).

2.8 Observations in humans

2.8.1Biomarkers of exposure to PAH

Several methods have been developed to assess internal exposure to PAH. In most studies, metabolites of PAH, such as 1-hydroxypyrene were measured in urine. Adducts of reactive metabolites (mainly diol epoxides) of benzo[*a*]pyrene and other PAH with DNA in peripheral lymphocytes, other tissues, and with proteins such as albumin have also been used.

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. However, in all populations studied, there is substantial interindividual variation in PAH-DNA adduct levels and 1-hydroxypyrene excretion in urine. This is probably due to differences in biotransformation, excretion, DNA adduct removal etc. (SCF 2002).

PAH-DNA adducts have been identified in peripheral white blood cells from humans exposed to tobacco smoke, living in polluted areas, or ingesting

charbroiled meat. In a prospective study, an increased lung cancer risk was obseerved among smoking individuals with a high level of aromatic DNA adducts in white blood cells (SCF 2002).

Exposure to PAH from food is a confounder when biomarkers are used in the evaluation of exposure to PAH by inhalation. There are indications that ambient air is a relatively unimportant source in comparison with dietary PAH and tobacco smoking. Thus, fire fighters working in the USA had higher levels of PAH-DNA adducts in blood cells than US army personnel fighting oil field fires in Kuwait, possibly because of a lower intake of charbroiled meat by the latter group (SCF 2002).

2.8.2Epidemiological and other studies

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. In addition, 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 (IPCS, 1998).

Mortality from lung cancer, especially among women, in a rural county, Xuan Wei in China, is five times the Chinese national average. The mortality rate was correlated with domestic use of 'smoky' coal as fuel for cooking and heating. Monitoring of air during cooking inside the homes showed that women were exposed to extremely high levels of PAH (IPCS, 1998).

Only one fully reported study and a published abstract on oral PAH exposure and health effects was identified by the SCF (2002). In the first, the exposure to wine stored in tar impregnated leather bottles was assessed by a self-administered questionnaire. Although an increased risk of gastric cancer was reported, the study population was too small to achieve statistically significant increases. In the abstract, an increased risk of colorectal adenomas was reported to be associated with dietary benzo[*a*]pyrene intake in a case-control study of 146 newly diagnosed cases and 226 controls (SCF 2002).

3 Hazard characterisation

3.1 Non-carcinogenic effects

Experimental studies on individual PAH have shown various non-carcinogenic effects, such as haematological effects, liver and kidney toxicity, reproductive and developmental toxicity, and immunotoxicity. NOAELs for individual PAH in subchronic studies are given in table 5.1. For benzo[*a*]pyrene a NOAEL at 3 mg/kg bw/day for effects on the liver was reported in a 90-day rat gavage study and for immunotoxicity in a 35 day rat gavage study. No NOAEL for reproductive and developmental toxicity has been identified for benzo[*a*]pyrene.

It would be possible to establish TDIs for the non-carcinogenic effects of some PAH. Thus, TDIs for chronic oral exposure to acenaphthene, anthracene, fluoranthene, fluorene, and pyrene have been established by the US EPA (IPCS 1998). However, because exposure to PAH in soil, air, or food is almost exclusively to a mixture of PAH, which include a number of genotoxic and carcinogenic PAH for which it is believed that no thresholds exist, TDIs of

individual components would not be relevant to the assessment of the risk of such mixtures.

3.2 Carcinogenic effects

Epidemiological data 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, no human data are available that can be used for the assessment of health effects of PAH exposure from ingestion. Therefore, the hazard characterisations for oral intake of benzo[*a*]pyrene alone or as a constituent of complex PAH mixtures, are based on results from carcinogenicity studies in experimental animals.

3.2.1Lifetime risk estimates for oral exposure to benzo[a]pyrene

The US EPA has established upper bound (95%) "slope" factor values for benzo[*a*]pyrene (human excess cancer risk from oral lifetime exposure to 1 mg benzo[*a*]pyrene/kg bw/day), ranging 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, 2004). The US EPA used data on the incidence of gastrointestinal tumours in mice (Neal and Rigdon, 1967) and rats (Brune *et al.*, 1981) exposed perorally to benzo[*a*]pyrene. 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 an oral "virtually safe dose" (dose associated with an excess cancer risk of zero to $1x10^{-6}$) of 0.14 ng benzo[*a*]pyrene/kg bw/day can be calculated via linear extrapolation (SCF 2002).

Kroese *et al.* (2001) used their recent chronic oral (gavage) rat study with benzo[*a*]pyrene (dosed 3, 10 and 30 mg/kg bw/day, 5 days/week during 2 years) 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. Applying a simple linear model, the authors calculated "virtually safe doses" that 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, 2004), i.e. it implies a lower risk at any particular dose level. This is because Kroese et al. (2001) not used a scaling factor to correct for differences between species (rat and man), and found it more appropriate to use a mean estimate instead of an upper bound 95% confidence limit.

When Kroese *et al.* (2001) applied the same extrapolation method on the results of Culp *et al.* (1998) a "virtually safe dose" of 5 ng benzo[*a*]pyrene/kg bw/day was also calculated based on forestomach tumours and number of tumour-bearing mice. 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) and a "virtually safe dose" of 3 ng benzo[*a*]pyrene/kg bw/day was calculated for mixture II (0.367% benzo[*a*]pyrene).

The Norwegian Food Control Authority has also used the study by Culp *et al.* (1998) to perform a hazard characterisation for benzo[*a*]pyrene in food. A simple linear extrapolation was used 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). When this simple 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, 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 correspond to a "virtually safe dose" of 0.57 ng benzo[*a*]pyrene/kg bw/day calculated for a risk level of $1x10^{-6}$ (SCF 2002).

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 potencies of the coal tar mixtures in mice were up to 5 times higher than what would be expected from their benzo[*a*]pyrene content.

Schneider *et al.* (2002) also 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 benchmark dose method to calculate the LED_{10} (0.052 - 0.08 mg benzo[*a*]pyrene /kg bw/day; the 95% lower confidence limit on the dose associated with 10% extra risk, adjusted for background), as a starting point for the extrapolation according to the US EPA revised cancer risk assessment guidelines (US EPA, 1999). Schneider *et al.* (2002) recommend that this slope factor be used for the risk assessment of PAH-contaminated soils. Using this oral "slope factor" of 11.5 per mg benzo[*a*]pyrene/kg bw/day as part of a PAH mixture 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 (SCF 2002).

It should be noted, that for substances that are both genotoxic and carcinogenic the EU Scientific Committee on Food (SCF) has never accepted the use of linear extrapolation from the observable range in animal experiments down to the much lower intake levels relevant for human exposures. Instead SCF has used 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 SCF 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 were considered to be without a threshold in their action on DNA.

3.2.2Hazard characterisation for other individual carcinogenic PAH

Relative potency factors, expressed as toxic equivalency factors (TEF) for individual PAH (relative to benzo[*a*]pyrene) have been established 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 (SCF 2002). 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.

The TEF approach relies on dose addition, in which case there is no interaction between the components in the mixture, and was initially developed to estimate the potential toxicity of mixtures of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), that all act via binding to the Ah-receptor (AhR). Use of the TEF approach, and thus dose addition, in risk assessment of chemical mixtures

is only scientifically justifiable when all the chemicals in the mixture act by the same mechanism and only differ in their potencies and should not be applied to mixtures of chemicals that act by a mechanism for which the additivity assumption is invalid.

Although a number of PAH have been shown to bind the AhR *in vitro*, there are no *in vivo* studies to clarify their potencies in this respect. In addition, AhR activation is not the only effect that determines the carcinogenic potency of a PAH. In contrat to PCDDs and PCDFs, DNA binding and induction of mutations are other significant effects in the carcinogenesis of PAH, and there is no indication that all carcinogenic PAH are activated via the same metabolic route, always 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 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.

The limitations in using the TEF approach for the assessment of PAH carcinogenicity following oral administration is illustrated when used on the carcinogenicity data in mice and the analytical data on the PAH composition in the coal tars tested 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 (SCF 2002).

Schneider *et al.* (2002) also examined the use of the TEF approach on 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 concluded that the benzo[*a*]pyrene TEFs did not adequately describe the potency of PAH mixtures and in most cases led to an underestimation of the carcinogenic potency.

Based on these considerations, the SCF (2002) did not find it appropriate to use the TEF approach for the risk assessment of PAH in food. Instead it was suggested to use benzo[*a*]pyrene as an indicator of occurrence and concentration in food of the higher-molecular mass PAH (from benzofluorenes upwards), i.e. the PAH with carcinogenic properties (see below).

3.2.3Use of benzo[a]pyrene as a marker of the carcinogenic PAH

The SCF (2002) examined the possibility of using benzo[*a*]pyrene, which almost always is included in any analysis of PAH occurrence, as a marker for PAH in foods. The patterns of PAH distributions (profiles) relative to benzo[*a*]pyrene in various foods, coke-oven fumes, and urban air were examined. The profiles were calculated as a set of ratios between the available concentrations of any individual PAH and benzo[*a*]pyrene ([PAH]/[BaP]). Profiles were also calculated for coal tars because new experimental studies on PAH carcinogenicity in mice after ingestion included a comparison of benzo[*a*]pyrene with coal tar administration in the diet (Culp *et al.*, 1998).

It was concluded, that the lower-molecular mass, i.e. 3- and 4-ring PAH (fluorene, anthracene, phenanthrene, fluoranthene and pyrene) showed markedly higher variability than higher-molecular mass PAH. Most notably, the profiles of the 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) were surprisingly similar in various foods, irrespective of the supposed origin of the PAH contamination, being it from contamination from air pollution or formation during production or cooking. Overall, these profiles seemed to vary within a factor of less than five. This suggested to the SCF that benzo[*a*]pyrene could be used as a marker for occurrence of the carcinogenic PAH in foods, but not for the lower-molecular mass PAH. Kazerouni *et al.* (2001) reached a similar conclusion in a validation study where 14 PAH were analysed in samples from each major food group.

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. 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 could be 10 times higher than expected on the basis of the benzo[*a*]pyrene content alone. This is also in line 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.

3.2.4Summary of the lifetime risk estimation for exposure to PAH

From the recent studies in mice and rats, 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.

A conservative assessment suggests that the carcinogenic potency of total PAH in an environmental mixture such as soil would be 10 times higher than expected on the basis of the benzo[*a*]pyrene content alone.

Applying this factor of 10, a "virtually safe dose" of benzo[*a*]pyrene as a marker of the mixture of carcinogenic PAH in soil would be in the range of 0.06 to 0.5 ng benzo[*a*]pyrene/kg bw/day. This is in line with Schneider *et al.* (2002) who calculated an oral "virtually safe dose" of 0.09 ng benzo[*a*]pyrene/kg bw/day in a PAH mixture for a risk level of $1x10^{-6}$ via linear extrapolation.

Overall, this would indicate an oral "virtually safe dose" of 0.1 ng benzo[*a*]pyrene/kg bw/day as a marker of the mixture of carcinogenic PAH in soil for a risk level of $1x10^{-6}$.

4 Quality criteria for PAH in soil using linear extrapolation

For PAH contamination of soils it is the potential oral intake of soil by small children (and the potential skin contact) that is regarded to be most critical. From the recent carcinogenicity studies in mice and rats, several authors have applied linear extrapolations to low dose human exposure indicating an overall oral "virtually safe dose" of 0.1 ng benzo[*a*]pyrene/kg bw/day in a PAH mixture for a lifetime risk level of $1x10^{-6}$.

4.1 Example 1 (old method)

It is assumed that a child weighing 10 kg ingests 0.2 g soil. Skin penetration is neglible due to the binding of PAH to soil particles.

A health based soil quality criteria of 0.005 mg benzo[*a*]pyrene/kg (covering all carcinogenic PAH) can be derived from the above mentioned lifetime risk estimate.

4.2 Example 2 (new method)

It is assumed that a child weighing 13 kg ingests 0.1 g soil. Skin penetration is neglible due to the binding of PAH to soil particles.

A health based soil quality criteria of 0.013 mg benzo[*a*]pyrene/kg (covering all carcinogenic PAH) can be derived from the above mentioned lifetime risk estimate.

5 Comments on the adequacy of using linear extrapolation to derive lifetime risk estimates for compounds that are both genotoxic and carcinogenic

While most toxicologists would agree that mathematical modelling of the dose response relationship in the observable range of toxicological studies is of value for the calculation of e.g. a benchmark dose (see below), most would have severe reservations about using a linear extrapolation from high-dose animal tumour data in order to estimate risks to humans at much lower exposures to substances that are both genotoxic and carcinogenic, such as PAH.

Our current understanding is that genotoxicity is the most plausible mechanism involved in the initiation of PAH carcinogenicity, and because DNA binding of PAH and other genotoxic compounds show linearity at very low doses, it is commonly believed that there would also be no threshold for the carcinogenic effect of a compound when it is also genotoxic. However, the correlation between DNA adducts, mutagenesis and carcinogenesis of PAH is not straightforward. Comparable levels of DNA adducts and gene mutations 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.* (1998) also found that cytotoxicity and cell proliferation, rather than DNA binding, were the critical factors in the tumour induction in mice. This indicates 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.

Therefore, in addition to its effect on the initiation stage, the carcinogenicity of PAH observed at high 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 immunosuppression 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 assumption that there is a theoretical possibility of an effect following exposure to a single molecule of a compound has recently been questioned. This is based on current knowledge of repair mechanisms and other homeostatic processes (EC, 2000), and the application of biologically based threshold models for risk assessment of both genotoxic and non-genotoxic and carcinogenic compounds (Butterworth and Bogdanffy, 1999) have been proposed. However, due to the lack of the detailed information necessary for the construction of biologically based models, cancer risk assessment still requires assumptions and default approaches.

In order to avoid the use of quantitative risk estimates that cannot be scientifically supported, but nevertheless may be taken very litteraly by risk managers and laypeople, approaches applying safety faxtors or uncertainty factors have been proposed also for compounds that have genotoxic and carcinogenic properties.

For Australia and New Zealand the National Health and Medical Research Council (NHMRC) has prepared a technical guidance document on "Toxicity Assessment for Carcinogenic Soil Contaminants". The outcome of the risk assessment for a particular chemical is the derivation of a Guideline Dose (GD) (NHMRC, 1999).

The proposed point of departure for the assessment is a modified benchmark dose. The concept of the benchmark dose was originally put forward by Crump (1984) as an alternative to the NOAEL and LOAEL for non-cancer health effects because it provides a more quantitative alternative to the first step in the dose-response assessment. The BMD approach is based on a mathematical model being fitted to the experimental data within the observable range and estimates the dose that causes a low but measurable effect (e.g. a 5% or 10% increase in tumours compared to background). In most cases the BMDL (benchmark dose lower confidence limit) is used instead of the BMD. The BMDL is a calculated point on the experimental dose-response curve that corresponds to a statistical lower 95% confidence limit of the BMD (US EPA, 1995).

NHMRC suggests to use a modified-BMD corresponding to the 5 per cent extra risk in the animal bioassay or human study as the starting point for deriving the Guideline Dose. This contrasts with the traditional BMD, which is associated with the 95 per cent statistical limit associated with the chosen response rate (BMDL). Guideline Doses are then obtained by applying safety factors (SF) to the modified-BMD. Derivation of the factors involves four primary decision points:

- Assessment and evaluation of the data relating to differences between experimental animals and humans. A SF of < 1 – 10 is proposed.
- Assessment and evaluation of the data relating to individual variability within the human population. A SF of 1 – 10 is proposed.
- Assessment and evaluation of the quality of the data available. A SF of 1 – 10 is proposed.
- Assessment and evaluation of the seriousness of the cancer response. An overall SF of 1 – 50 is proposed. This SF is composed of a factor of 1 – 5 for potential genotoxicity and of 1 - 10 depending on the degree of malignancy and the appearance of the tumour in a critical organ.

The value of the overall factor may thus range up to 50 000 (NHMRC, 1999).

This method was used for the assessment of benzo(a)pyrene in soil. A composite safety factor of 4500 was applied on a modified-BMD of 0.362 mg benzo(a)pyrene /kg bw/day derived from the studies in mice by Culp *et al.* (1998). The safety factor consisted of a factor of 10 for differences between experimental animals and humans, a factor of 10 for individual variability within the human population, a factor of 1 for the quality of the data, a factor of 5 for the genotoxicity of benzo(a)pyrene, and a factor of 9 for the seriousness of the cancer response. Consequently, a guideline dose of 80 nanogram benzo(a)pyrene/kg bw/day was proposed (Fitzgerald *et al.*, 2004).

Using benzo[*a*]pyrene as a marker for the occurrence and effect of the carcinogenic PAH in environmental mixtures and food this guideline dose can be converted into a guideline dose of 8 ng benzo[*a*]pyrene/kg bw/day from all sources, including soil.

A recent (2002) dietary study from the UK have shown an average intake of about 4 ng benzo[*a*]pyrene/kg bw/day for children aged 1.5 – 2.5 years. The 97.5 percentile was about 6 ng benzo[*a*]pyrene/kg bw/day (SCF 2002). Assuming that Danish children have a similar dietary intake of PAH, an additional intake of 4 ng benzo[*a*]pyrene/kg bw/day from soil might be tolerable, based on the overall

guideline dose of 8 ng benzo[*a*]pyrene/kg bw/day from all sources. This would result in a health based soil quality criteria of 0.20 mg benzo[*a*]pyrene/kg (covering all carcinogenic PAH) when the old method is used and of of 0.28 mg benzo[*a*]pyrene/kg (covering all carcinogenic PAH) when the new method is used.

6 Quality criterion in soil

A health based quality criterion based on a lifetime risk estimate will for compounds such as benzo[a]pyrene and other PAHs that act not only as initiatiors of the carcinogenic process but also as tumor promotors overestimate the cancer risk as it involves extrapolation of the tumour risk observed as a result of high dose (promotional) administration to low dose (initiating and non-promotional).The result is a very conservative soil quality criteria. An alternative use of a threshold approach would result in a quality criterion that is at least one-two order of magnitudes greater. This illustrates the difficulty of finding a scientifically sound numerical value as the basis for a health based quality criterion in soil.

As a health based quality criterion based on a lifetime risk estimate would be below the background concentration in Danish soils an *administrative* based soil quality criterion is proposed, based on the approach suggested in section 5. With an adminsitrative soil quality criterion of 0.3 mg BaP/kg the daily BaP exposure from ingestion of soil (0.1 g/d) would be equal to the average daily BaP ingested from food for a young child. However, this exposure from soil is only estimated to occur for a certain period of time during young childhood (1 to 3 years old children). Thus an administrative based soil quality criterion of 0.3 mg benzo[a]pyrene/kg may still be considered as a preventive level although it theoreticcally represents an increased risk level compared to the traditional lifetime risk level of 10^{-6} when the conservative non-threshold risk assessment approach is used.

6.1 Administrative based quality criterion in soil

Quality criterion (administrative): 0.3 mg benzo[*a*]pyrene/kg soil (covering all carcinogenic PAH).

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Polyaromatic Hydrocarbons (PAH)

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to poly aromatic hydrocarbons (PAH) and an estimation of a quality criterion in soil. This resulted in 2004 in the present report which includes an administrative based quality criterion in soil.

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