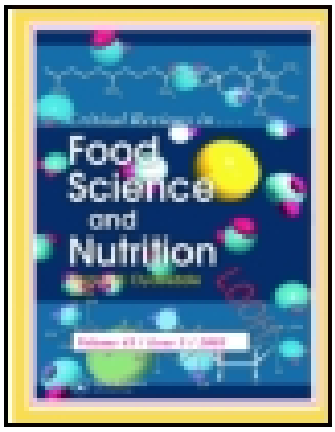

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A Critical Review about the Health Risk Assessment of PAHs and their Metabolites in Foods

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are a family of toxicants that are ubiquitous in the environment. These contaminants generate considerable interest, because some of them are highly carcinogenic in laboratory animals and have been implicated in breast, lung, and colon cancers in humans. Dietary intake of PAHs constitutes a major source of exposure in humans. Factors affecting the accumulation of PAHs in the diet, their absorption following ingestion, and strategies to assess risk from exposure to these hydrocarbons following ingestion have received very little attention. This review, therefore, focuses on concentrations of PAHs in widely consumed dietary ingredients along with gastrointestinal absorption rates in humans. Metabolism and bioavailability of PAHs in animal models and the processes, which influence the disposition of these chemicals, are discussed. Finally, based on intake, disposition, and tumorigenesis data, the exposure risk to PAHs from diet is presented. This information is expected to provide a framework for refinements in risk assessment of PAHs.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); PAHs metabolites; food; bioaccumulation; biotransformation; exposure and risk; biomarkers; chemopreventive compounds.

Contents

1. General description

1.1. Physical and chemical properties

The term polycyclic aromatic hydrocarbons (PAHs) refers to the compounds made up of carbon and hydrogen atoms grouped into rings containing five or six carbon atoms. Their physical and chemical properties are governed by the size (number of carbon atoms) and shape (ring linkage pattern) of the individual molecule. In this way, the lower molecular weight PAHs (<3 rings), are generally not carcinogenic and are found in the gas phase. The larger molecular weight PAHs (≥ 4 rings), are most stable and toxic and are mainly associated with airborne particles. PAHs are soluble in lipid (fat), and are essentially insoluble in aqueous systems and chemically inert (Ishizaki et al., 2010). The aqueous solubility decreases with increasing molecular size. Vapour pressure for PAHs are low and decrease with increasing molecular size. A summary of some relevant physical-chemical properties for a number of selected PAHs is provided in **Table 1**. Their octanol-water partition coefficients (K_{ow}) are relatively high, indicating a relatively high potential for adsorption to suspended particulates in the air and in water, and for bioconcentration in organisms (NRCC 1983; Slooff et al., 1989). Transport and partitioning of PAHs in the environment are determined to a large extent by physicochemical properties such as water solubility, vapor pressure, Henry's law constant, K_{ow}, and organic carbon partition coefficient (K_{oc}).

1.2. Sources and environment fate

The widespread occurrence of PAHs is largely due to their formation and release in all processes of incomplete combustion of organic materials. They originate from two main sources: these are natural (biogenic and geochemical) and anthropogenic (Bamforth and Singleton 2005).

PAHs naturally occur in fossil fuels, but are also formed during the incomplete combustion of organic materials such as coal, diesel, wood and vegetation. This results in airborne PAH contamination, which is the main route for PAH transport over long distances. More minor sources of PAHs include tobacco smoke and burnt food. Natural processes can also provide a source of PAHs, such as volcanic eruptions and forest fires. PAHs can also have a geochemical origin as they are formed during pyrolysis, which involves the exposure of sediments to high temperatures during sediment diagenesis (Bamforth and Singleton 2005).

However, the most part of PAHs released into the environment are via anthropogenic processes like burning of coal, wood, municipal refuse, expulsion of fumes from manufacturing industries such as coke, aluminum, petroleum, domestic activities such as smoking, cooking (El-Shahawia et al., 2010).

Their hydrophobic properties, allow adsorption onto atmospheric (Bodzek et al., 1993; Subramanyam et al., 1994; Van Jaarsveld et al., 1997; Wild and Jones 1995) particles and direct deposition in sediments, soils and plants. The phase distribution of any PAH depends on the vapor pressure of the PAHs, the atmospheric temperature, the PAH concentration, K_{oc}, and the nature and concentrations of the particles (Baek et al. 1991). In general, PAHs having two to three rings are present in air predominantly in the vapor phase, those that have four rings exist both in the vapor and particulate phase, and PAHs having five or more rings are found

predominantly in the particle phase (Subramanyam et al., 1994; Van Jaarsveld et al., 1997; Wild and Jones 1995; Wania and Mackay 1996). Atmospheric residence time and transport distance depend on the size of the particles to which PAHs are adsorbed and on climatic conditions.

Consequently, environmental PAHs can be introduced into the food chain mainly by both plants and animals. Several studies confirmed that diet is the major source of human contamination to PAHs (Falcó et al., 2003; Ibánez et al., 2005; Stolyhwo and Sikorski 2005; Fontcuberta et al., 2006; Lee and Shim 2007; Varlet et al., 2007; Martí-Cid et al., 2008; Martorell et al., 2010).

It should be taken into account chemical reactions of PAHs in environment. The most important are reactions in the air between PAHs adsorbed on the particle surfaces and oxidant gases like NO_2, O_3, and SO_3 that do not appear to be influenced by exposure to UV irradiation and photooxidation of PAHs irradiated either under solar radiation or simulated sunlight which produces a variety of oxidized derivatives such as quinones, ketones, or acids (Vu Duc and Huynh 1991). Kamens et al. (1986) estimate that, even in highly polluted air, photolysis is the most important factor in the decay of particle-sorbed PAHs in the atmosphere, followed by reaction with NO_2, N_2O_5, and HNO_3. In water PAHs can be volatized, photolyzed, biodegraded and bind to suspended particles or sediments, or accumulated in aquatic organisms.

The most important processes contributing to the degradation of PAHs in water are photooxidation, chemical oxidation, and biodegradation by aquatic microorganisms (Neff 1979). Hydrolysis is not considered to be an important degradation process for PAHs (Radding et al., 1976). The contribution of the individual processes, to the overall fate of a PAH will depend largely on the temperature, depth, pollution status, flow rate, and oxygen content of the water.

In soil, PAHs can be volatized or undergo biotic or abiotic degradation, mainly photolysis and oxidation. Moreover, these compounds can also enter groundwater and be transported within an aquifer. The rate and extent of biodegradation of PAHs in soil are affected by environmental factors such as the organic content, structure and particle size of the soil, characteristics of the microbial population, the presence of contaminants such as metals and cyanides that are toxic to microorganisms, and the physical and chemical properties of the PAHs (Wilson and Jones 1993). Others environmental factors that may influence the rate of PAH degradation in soil include temperature, pH, oxygen concentration, PAH concentrations and contamination history of soil, soil type, moisture, nutrients, and other substances that may act as substrate co-metabolites (Sims and Overcash 1983). The size and composition of microbial populations in turn can be affected by these factors. Moreover, the rate of biodegradation may be altered by the degree of contamination. At hazardous waste sites, half-lives may be longer since other contaminants at the site may be toxic to degrading microorganisms. Bossert and Bartha (1986) reported reduced biodegradation of PAHs in soil containing a chemical toxic to microorganisms. Sorption of PAHs to organic matter and soil particulates also influences bioavailability, and hence, biotransformation potential. Sorption of PAHs by soil organic matter may limit biodegradation of compounds that would otherwise rapidly undergo metabolism (Manilal and Alexander 1991; Weissenfels et al., 1992).

Although there are differences in the biodegradation half-life values estimated by different investigators (Park et al., 1990; Wild and Jones 1993) their results suggest that the biodegradation half-lives of PAHs with more than three rings will be considerably longer (>20 days to hundreds of days) than the PAHs with three or fewer rings.

1.3. Sampling and Analytical Methods

The sampling, sample preparation and analytical detection procedures are very important for determination of PAHs in foodstuffs. The general criteria were established in the European Commission Directive 2005/10/EC that establishing the sampling methods and the methods of analysis for the official control of the levels of benzo[*a*]pyrene in foodstuffs. The present provisions are set in European Commission Regulation (EC) No. 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3- monochloropropane-1,2-diol (3-MCPD) and benzo[*a*]pyrene in foodstuffs.

1.3.1. Sampling

The requirements for sampling methods are different depending on type and weight of lot. Several precautions should be taken in order to avoid changes in the composition of the sample and therefore, the analyst should to avoid the samples do not become contaminated during preparation. In order to avoid the contamination, the material shall be rinsed with high purity acetone or hexane before use.

Moreover the equipment and the material that coming into contact with the sample shall be made of inert material such as aluminum, glass or stainless steel. Plastics shall be avoided because the PAHs can be adsorbed onto these materials (EFSA 2008).

During sample collection and storage the samples not be exposed to tobacco smoke, light and high temperatures (leading to volatilization and/or chemical conversion) (EFSA 2008). Exposure to tobacco smoke may increase the PAH levels in the sample (FAO/WHO 2006). These compounds are light sensitive and they can decompose by photoirradiation and oxidation therefore the light exposure has been carefully controlled (Mottier et al., 2000; Diletti et al., 2005).

The extended storage of the samples before analysis may cause the reaction of some PAHs with components of the food matrix.

Many methods have been developed to isolate, separate and quantitate PAHs in foods. Following homogenization of the foodstuff, PAHs are extracted using different techniques prior to clean up and purification.

1.3.2. Analytical methods

The extraction method used depends on the nature of the food matrix. Conventional techniques such as saponification followed by liquid-liquid extraction (LLE) (Moret and Conte 2000; Mottier et al., 2000; Simon et al., 2008) and extraction with organic solvent are the most often used methods for solid and liquid fat food samples (e.g. meat, fish and their products, as well as vegetable oils).

For solid food matrices automated extraction techniques, such as pressurized liquid extraction (PLE) (Janska et al., 2004; Martínez et al., 2004; Veyrand, et al., 2007; Jira 2004; Djinovic et al., 2008a; Houessou et al., 2007) and supercritical fluid extraction (SFE) (Ali et al., 2002; Veyrand et al., 2007), are also applied, but less frequently. In the same way, for liquid

food matrices the application of less-solvent-consuming techniques, such as matrix-solid phase dispersion (MSPD) (Bogusz et al., 2004; Pensado et al., 2005), ultrasound-assisted solvent extraction (UASE) (Rey-Salgueiro et al., 2008 and 2009) and solid-phase microextraction (SPME) techniques such as headspace (HS) (Arrebola et al., 2006), HS-SPME (Vichi et al., 2005; Viñas et al., 2007; Aguinaga et al., 2008a;) and SPME, has been reported (Purcaro et al., 2007a; Purcaro et al., 2007b; Wang et al., 2009; Ishizaki et al., 2010; Ishizaki et al., 2011). Recently the ultrasound-assisted emulsification-microextraction (USAEME) has been also applied for determining PAHs in tap water samples (Cheng et al., 2011).

The performance of clean-up stages is also requested for most of applications, but the utilization of techniques such as SPME, USEAME or HS-SPME has permitted the reduction of the pre-treatment stage. The clean-up stage is still a time-consuming step, especially in fatty matrices. Column chromatography, SPE and gel permeation chromatography (GPC) are the main sample purification techniques used for isolating PAHs from interfering matrix substances. Recently especially the automatic GPC technique has been applied for cleaning up PAH sample extracts (Fontcuberta et al., 2006; Llobet et al., 2006; Navarro et al., 2006; Ballesteros et al., 2006; Fromberg et al., 2007; Reinik et al., 2007;; Djinovic et al., 2008a; Djinovic et al., 2008b; Suchanová et al., 2008).

In relation to analytical detection techniques nowadays there are two main techniques for identify and quantify the PAHs. These are the liquid chromatography (LC) coupled to a fluorescence detector (FLD) and gas chromatography (GC) coupled to mass spectrometry (MS). Both methods are sensitive enough for determining PAH concentrations usually found in foods. In the past, LC with an ultraviolet (UV) (Chen et al., 1996; Chiu et al., 1997; Lin and Zhu, 2004;

Bishnoi et al., 2005; Danyi et al., 2009) or a photo-diode array (PDA) detector GC with a flame ionization detector (FID) were also used but today they are not the most appropriate methods due to their poorer selectivity and sensitivity.

LC-FLD has been extensively applied for determination of PAHs in very different matrices due to their selectivity and sensitivity. Also it is a cheap a simple method in comparison to other detection methods. For this reason this technique has been largely used for determination of the EPA priority list of PAHs (Moret and Conte 2002; Barranco et al., 2003; Barranco, et al., 2004; Janska et al., 2004; Gomes-Zuin et al., 2005; Galinaro et al., 2007; Luo et al., 2007; Tfouni et al., 2007; Windal et al., 2008; Djinovic et al., 2008b; ; Ramalhosa et al., 2009: Rey-Salgueiro et al., 2008a, 2008b, 2009a, 2009b; Ramalhosa et al., 2012) and has been the basis of different official methods for the analysis of PAHs in food (Wenzl et al., 2006; EPA 2010). Nevertheless, some authors describe certain selectivity problems due the presence of alkylated PAHs (Simko 2002), which are considered the main impurities of PAHs fractions. Another disadvantage of this technique is the impossibility of using isotopically labeled compounds because of FLD cannot distinguish these ones from native PAHs.

As the LC-FLD technique can still show a lack of selectivity, the GC-MS could be applied in order confirm the positive results (Mottier et al., 2000; Rojo Camargo and Toledo 2003; Houessou et al., 2006; Viñas et al., 2007; Kumari et al., 2012). The GC-MS methods have become popular methods for analyzing PAHs in foods and they are the main alternative to LC-FLD. Although GC-MS is lower sensitive than LC-FLD, the use of the GC-MS shows some advantages in comparison to LC-FLD. This is due to the selectivity of the MS-detector, the use of mass spectrum data for reliable confirmation of PAHs, and the possibility to use isotope

labeled PAHs as internal standards. Besides GC-MS permits the determination of non fluorescence PAHs (Cai et al., 2009) and the identification can be carried out in a single step while with using LC-FLD the re-injection of samples by GC-MS for confirmation is often reported. As the case of LC-FLD, there are official methods for the analysis of PAHs by GC-MS (EPA 2010; Poster et al., 2006). GC-MS-based methods are more frequently found in the more recent bibliography (Veyrand et al., 2007; Purcaro et al., 2007a; Purcaro et al., 2007b; Rodil et al., 2007; Martin and Ruiz 2007; Aguinaga et al., 2008a; Aguinaga et al., 2008b; Djinovic, et al., 2008a; Nacher-Mestre et al., 2009; Gómez-Ruiz and Wenzl 2009; Duedahl-Olesen et al., 2010; Wang and Guo, 2010; Forsberg et al., 2011; Wu and Yu, 2012). Most of the studies use single quadrupole MS but the use of tandem mass spectrometry is increasing because it gives more specific mass fragments (daughter ions) and hence improves specificity and sensitivity of the MS methods (; Al-Omair and Helaleh 2004; Diletti et al., 2005; Anyakora et al., 2005; Ballesteros et al., 2006; Varlet et al., 2007; Veyrand et al., 2007; Jie and Kai-Xiong 2007; Jung et al., 2011; Hollosi and Wenzl, 2011).

1.4. Legislation

In view of disparities caused by different maximum levels (ML) for PAHs in food in several Member States, the Commission set harmonized ML for benzo[a]pyrene for the first time in 2005 by European Commission Regulation (EC) No 208/2005 amending European Commission Regulation (EC) No 466/2001 as regards PAHs. Benzo[a]pyrene was chosen because the Scientific Committee on Food (SCF) concluded in its opinion of 4 December 2002

that this compound can be used as a marker for the occurrence and effects of carcinogenic PAHs in food. All the same, SCF suggested that this evaluation should be accompanied by additional analysis of other PAHs in order to establish a PAH contamination profile in food commodities (SCF 2002). Later, in 2007, the European Food Safety Authority (EFSA) pointed out that the supposition that benzo[*a*]pyrene was a good indicator of any PAH contamination was uncertain (EFSA 2007). Moreover, the monitoring of other PAHs has been strongly recommended by the UE (European Commission Recommendation No 2005/108/EC).

Until recently the determination of benzo[*a*]pyrene has been widely used as a marker for the carcinogenic PAHs in food and to evaluate the risk assessment of the carcinogenicity of PAHs in food on the basis of level of benzo[*a*]pyrene. However, in 2008 the EFSA established that benzo[*a*]pyrene is not a suitable indicator for the occurrence of PAHs in food. As a result, a system of two specific substances called PAH2 (benzo[*a*]pyrene + chrysene), of four specific substances called PAH4 (PAH2 plus benzo[*a*]anthracene benzo[*b*]fluoranthene) or eight specific substances called PAH8 (PAH4 plus benzo[*k*]fluoranthene, benzo[*ghi*]perylene, dibenzo[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene) were considered as the most appropriate indicators (EFSA 2008).

Formerly, the maximum levels (ML) for benzo[*a*]pyrene was laid down in the Commission Regulation (EC) No 1881/2006 . In 2011, new PAHs maximum levels were established for the content of PAHs in foods. These new maximum levels are lain down in the Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs.

These maximum levels (MLs) for benzo[*a*]pyrene and PAH4 are show in the **Table 2**.

The lowest MLs are set for food for infants and young children and dietary foods for special medical purposes. The need for reliable data about the concentration of PAHs in food is increasing in order to establish new maximum permitted levels, specially of 15 mutagenic/genotoxic PAHs such as PAH8 plus benzo[*j*]fluoranthene, cyclopenta[*cd*]pyrene, dibenzo[*ae*]pyrene, dibenzo[*ah*]pyrene, dibenzo[*ai*]pyrene, dibenzo[*al*]pyrene and 5-methylchrysene. Legal regulation is limited, partly because of the difficulty of defining safe levels of these complex mixtures.

2.- Toxicity

It is long been known that PAHs can have serious deleterious effects to human health (Bamforth and Singleton 2005). In 1761, John Hill recognized the link between the snuff and nasal cancer (Cerniglia 1984). Following Hill's findings, research into PAH toxicity continued, resulting in the identification of their carcinogenic, mutagenic and teratogenic properties (Fisher 1999).

2.1. Toxicokinetics

2.1.1. Absorption

There are three main routes of absorption in humans, lung and respiratory tract following

inhalation of aerosols or particulates containing PAHs, dermal following skin contact and gastrointestinal tract following ingestion in water or food (SCF 2002).

The absorption rate of PAHs from the diet is determined by the size, lipophilicity and aqueous solubility of the molecule, the presence of bile in the digestive tract, the dose ingested and the lipid content of the diet. Rahman et al. (1986) showed the presence of bile increased the intestinal absorption of PAHs in rats. Kawamura et al. (1988) demonstrated that the composition of the diet influenced the PAHs absorption in rats. They suggest that the bioavailability of PAHs from food will be in the range of 20-50% and that it increases with increasing content of lipophilic components in the food.

The absorption of PAHs from the gastro-intestinal tract appears to vary per animal species in the case of experimental animals this absorption especially benzo[*a*]pyrene is well documented.

Studies in rats showed that first a direct absorption occurs 1-2 hours after feeding. After 3-4 hours a second increase in serum concentration occurs due to entero-hepatic circulation (van Schooten et al., 1997). In contrast, a study from Grova et al. (2002) showed that activity from radio-labeled benzo[*a*]pyrene was not traced in blood and milk from orally exposed lactating goats. Hoogenboom (2005) concluded from this study that the heavier PAHs are apparently not absorbed from the gastro-intestinal tract (and transferred to milk). Since the rat seems more relevant as a model for human uptake, it is considered that in humans PAHs may be readily absorbed from the gastro-intestinal tract.

2.1.2. Distribution

The distribution of PAHs and their levels in tissues are influenced by several factors such as the route and the vehicle of administration, time of tissue sampling after treatment and presence or absence of inducers or inhibitors of hydrocarbon metabolism. However three common traits are observed; there are detectable levels of PAHs (probably more accurately PAH-derived material) in almost all organs, those organs rich in adipose tissue act as depots from which material is slowly released and high levels are found in the gastrointestinal tract irrespective of the route of administration (SCF 2002). They tend to be stored mostly in the kidneys, liver, and fat. Smaller amounts are stored in the spleen, adrenal glands, and ovaries (ATSDR 1995).

Saunders et al. (2002) demonstrated that significant amounts of benzo[*a*]pyrene and metabolites (benzo[*a*]pyrene-*trans*-4,5-dihydrodiol (±), benzo[*a*]pyrene-*trans*-7,8-dihydrodiol (±), benzo[*a*]pyrene-*trans*-9,10-dihydrodiol (±), benzo[*a*]pyrene-3,6-dione, 3-hydroxybenzo[*a*]pyrene, and 9-hydroxybenzo[*a*]pyrene) were found in brain of rats administered by gavage a single dose of benzo[*a*]pyrene (12.5, 25, 50 and 100 mg/kg b.w.). The diol metabolites (4,5; 7,8; 9,10-diols) were predominant in cerebellum, and cortex relative to plasma across various time points. From the standpoint of time-course distribution of various metabolites, the diol metabolites dominated in the earlier time points (up to 12 h post exposure) and the hydroxyl metabolites in the later (from 24 to 96 h post exposure) time points. These results are in accordance with previous data (Lipniak and Brandys 1993; Modica et al., 2006) published on other PAHs (benzo[*a*]anthracene, chrysene, fluoranthene, pyrene, and benz[*a*]anthracene) and confirms the ability of these compounds or their metabolites to cross the

blood-brain barrier (EFSA 2008).

Studies in pregnant mice and rats have shown that PAHs (benzo[*a*]pyrene, 7,12-dimethylbenzo[*a*]anthracene and 3-methylcholanthrene) were widely distributed in maternal tissues and was detected in fetuses, showing that they crossed the placenta (EC 2002).

In a small study in humans, samples of milk, placenta, maternal and umbilical cord blood were taken from 24 women and analyzed for selected PAHs. The highest levels of benzo[*a*]pyrene, dibenzo[*a,c*]anthracene and chrysene were observed in milk and umbilical cord blood but levels were only above the detection limit in half of the samples. Nevertheless the authors concluded that both fetuses and infants were exposed to PAHs, which were presumed to be from the maternal diet (Madhavan and Naidu 2006).

Limited data are available describing measurement of tissue levels of PAHs in autopsy or biopsy samples from humans. Gräf and Schrmair (1975) reported average benzo[*a*]pyrene levels of 0.32 µg/100 g dry tissue in liver, spleen, kidney, heart and skeletal muscle and 0.20 µg/100 g dry tissue in lung based on autopsy samples from normal humans with a wide age range (Gräf and Schrmair 1975; IPCS 1998).

2.1.3. Metabolism

The aim of the metabolization of PAHs is to increase their polarity to obtain hydrophilic substances in order expedite their excretion. PAHs are readily metabolized by resulting in a detoxification or activation (Bauer et al., 1995; Pelkonen and Nebert 1982; Shou et al., 1994).

The metabolism of PAHs has been studied in a number of human cells and tissues including bronchus, colon, mammary cell aggregates, keratinocytes, monocytes, lymphocytes

and bronchial macrophages (SCF 2002).

Metabolism of xenobiotics is divided into two phases; phase I generally involves alteration of the structure of the compound to increase polarity and the formation of a functional group that can undergo further conjugation or in some cases react with macromolecules such as DNA, and phase II addition of polar groups. PAHs are metabolized to a complex mixture of quinines, phenols, dihydrols, triols and tetrols in the biological system. Pyrene and benzo[*a*]pyrene are two of the best characterized PAHs (European Commission 2002). The general scheme of PAH metabolism involves oxidation to a range of primary (epoxides, phenols, dihydrodiols) and secondary (diol epoxides, tetrahydrotetrols, phenol epoxides) phase I metabolites followed by conjugation to phase II metabolites with glutathione, glucuronide or sulphate (SCF 2002). In invertebrates, PAH biotransformation has so far been considered species specific. Phase II biotransformation products identified in many invertebrate species include pyrene-1-sulfate, pyrene-1-glucuronide, and pyrene-1- glucoside (Beach et al., 2009; Giessing and Lund 2002; Jørgensen et al., 2008). Recently, pyrene-1-glucuronide, pyrene-1-sulfate, pyrenediol disulfate, and pyrenediol glucuronide sulfate have been reported as metabolites of hydroxylated pyrene in the whelk Neptunea lyrata (Beach et al., 2010). This previous exposure showed that, at lower exposure levels, biotransformation to pyrenediol glucuronide sulfate was the predominant fate of hydroxylated pyrene. At higher body burdens, accumulation of unmetabolized hydroxylated pyrene became a more important part of the fate. The bioaccumulation and biotransformation of PAH has yet to be reported for the common northern whelk, *Buccinum undatum* (Beach et al., 2010).

The most prominent biomarker of exposure to PAHs in humans is the level of 1-

hydroxypirene, hydroxynaphtalenes and hydroxyphenanthrenes because they are excreted in urine at substantial extent (EFSA 2008).

Though this mechanism produces a desintoxication, some PAHs are metabolized to active mutagen or carcinogen substances, which are capable of attacking cellular DNA. Moreover, some assays with animals have shown that some PAH metabolites are suspected to be endocrine disruptors acting like hormones.

2.1.4. Excretion

Excretion is the final phase of elimination of PAHs and their metabolites of the body. The majority of PAHs and their metabolites are excreted in urine, bile and faeces. A small amount is eliminated through sweat, saliva, tears or milk (Grova et al., 2002; Lapole et al., 2007; Grova et al., 2006). Urinary excretion has been studied more extensively than faecal excretion, although research on faecal metabolism has increased following recognition of the importance of enterohepatic cycling of PAH metabolites (SCF 2002).

2.2. Toxicological database for PAHs

The formation of DNA-adducts by electrophilic metabolites is generally regarded as one of the earliest steps in PAH carcinogenesis, but determination of DNA-adducts in whole tissues provides only a rough indication of cancer risk (Luch and Glatt 2004). Some authors (Goldstein et al., 1998; Kroese et al., 2001) concluded that other factors additional to DNA-adduct formation apparently are critical to tumor development by benzo[*a*]pyrene and they postulated

that local cell proliferation might be this critical factor. Adduct formation follows the prior conversion of lipophilic parent PAH to nucleophilic reactive metabolites by xenobiotic metabolizing enzymes. Genetic differences between humans with regard to enzymatic formation as well as enzymatic degradation of these active metabolites have been shown to correlate with adduct formation (Benford et al., 2010a).

2.2.1. Margin-of-exposure (MOE) approach

EPA and others have been developing a new approach to cancer risk characterization. The new approach is based on benchmark doses (BMD's) and margin-of-exposure (MOE) characterizations. The BMD 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 response. The MOE is defined as the ratio of the no-observed-adverse-effect level (NOAEL) or benchmark dose lower confidence limit (BMDL) for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration (WHO 2009).

The MOE approach can be applied both to individual substances and exposure sources and to chemical classes and aggregate exposures. It is considered to be the most scientifically credible and practical approach because it takes into account both the dietary exposure and the available data on the dose-response relationship, without extrapolation beyond the observed dose-range or generation of uncertain risk estimates (Barlow et al., 2006). The magnitude of the MOE gives an indicator of the level of concern, but is not a precise quantification of risk: the larger the MOE, the smaller the potential risk posed by exposure to the selected compound. In this way it a substance with an MOE of 1000 cannot be assumed to represent 10 times the cancer

risk of a different carcinogen with an MOE of 10,000. Therefore, further discussions in order to promote understanding of the utility and relevance of the MOE approach are recommended (Benford et al., 2010b).

Benford et al. (2010b) selected twelve genotoxic and carcinogenic chemicals, such as PAHs, that could be present in food. They try to assess the applicability of the MOE approach to genotoxic carcinogens in food and to evaluate whether it is possible to develop a practical banding approach by which it would be possible to categorize these substances according to their MOEs in high, medium and low classes concern to human health. Nevertheless, they concluded that the MOE calculation is dependent on the quality of the estimated dietary exposure data as well as the carcinogenicity data used to derive a BMDL. Different exposure scenarios can result in MOEs ranging over several orders of magnitude. If MOEs for different substances are to be compared, the dietary exposure assessment needs to be made as comparable as feasible.

Benford et al. (2010a) modeled the number of tumor-bearing mice resulted in a $BMDL_{10}$ of 0.122 mg benzo[*a*]pyrene /Kg-bw/day, which was lower than that for any of the individual tumors and was considered to be most appropriate since the different PAH may have different mechanisms of carcinogenicity. Average dietary exposure estimates of 0.0080 µg benzo[*a*]pyrene/Kg-bw/day was identified from the range of national estimates. The calculated MOE was 15,000.

EFSA has proposed also the use of BMD methodology (EFSA 2005). In this way, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) used a MOE approach based on dietary exposure for average and high level consumers to benzo[*a*]pyrene, PAH2, PAH4 and PAH8, respectively and their corresponding $BMDL_{10}$ values from the studies of Culp et al.

(1998). The resulting MOES were 17,900 for benzo[*a*]pyrene, 15,900 for PAH2, 17,500 for PAH4 and 17,000 for PAH8 and therefore, PAH2, PAH4 and PAH8 could be used as alternatives as markers to benzo[*a*]pyrene. The values obtained indicate a low concern for human health at the average estimated dietary exposure but when high level consumers are considering, MOES close to 10,000 are obtained and a high concern for human health is reached.

2.2.2. Relative Potency Factors (RPFs)

EPA's current approach to assessing cancer risk for PAHs uses the relative potency factor (RPF), which estimates the cancer risk of individual PAHs relative to benzo[*a*]pyrene. USEPA (1993) indicated that the data for PAHs did not meet the criteria for the development of toxicity equivalency factors (TEFs).

There is a large PAH database on carcinogenicity in animal bioassays, genotoxicity in various test systems, and bioactivation to tumorigenic and/or genotoxic metabolic intermediates. The External Review draft of EPA (EPA 2010) collected studies of rodent carcinogenicity bioassays (all routes) in which one or more PAH was tested at the same time as benzo[*a*]pyrene. In addition, in vivo and in vitro data for cancer-related endpoints in which one or more PAH and benzo[*a*]pyrene was tested simultaneously were obtained, including studies on the formation of DNA adducts, mutagenicity, chromosomal aberrations, sister chromatid exchange frequency, aneuploidy, DNA damage/repair/recombination, unscheduled DNA synthesis, and cell transformation. Studies in which benzo[*a*]pyrene was not tested simultaneously with another PAH were not considered in the RPF calculations.

In this way, EPA's Office of Research and Development (ORD) has developed a database

of primary literature relevant to the RPF approach for PAHs by performing a comprehensive review of the scientific literature dating from the 1950s through 2009 on the carcinogenicity and genotoxicity of PAHs (EPA 2010). They evaluated 74 PAHs for the RPF analysis and established final RPFs based on tumor bioassay data for 27 PAHs.

3. Contamination of food with PAHs

3.1. Sources of food contamination

In general, data about contaminants in food are gathered to obtain information on background concentrations in view of intake and associated risk assessments. In addition, food ingestion is the major route of exposure compared to inhalation for a large section of general population exposed to PAHs (Butler et al., 1993; Van Rooij et al., 1994).

In vivo studies suggest a transfer in intestinal epithelium by diffusion, which appears extensively governed by the physicochemical properties of PAHs, particularly lipophilicity. However, other mechanisms, such as metabolism, are considered to intervene (Srogi 2007). Food-animal transfer pathways of PAHs are so far poorly known due to the absence of investigations involving tracers (Srogi 2007). Laurent et al. (2002) reported a study of portal absorption of PAHs using two ^{14}C-tagged compounds: ^{14}Cphenanthrene and ^{14}C-benzo[*a*]pyrene in the growing pig. The obtained results indicate that the two studied molecules have a quite different behavior during digestion and absorption. Phenanthrene was greatly absorbed and its absorption occurs via the blood system, whereas benzo[*a*]pyrene was partly and weakly absorbed

respectively. However, these two molecules are mainly absorbed via the portal vein.

PAH contamination of food arises from two sources, environment and food-processing technique.

In regard to environmental sources, food could be contaminated by deposition (air), by transfer (soil) or by deposition and transfer (water). Raw foods should usually not contain high levels of PAHs. In remote areas from urban or industrial activities, the levels of PAHs found in unprocessed foods reflect the background contamination, which originates from long distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires (SFC 2002). Unprocessed food consists mainly of vegetables, fruits, grains, seafoods… For plants (leafy vegetables and tubers), uptake through atmosphere and soil are prime sources of contamination. The level of contamination is governed by where the vegetables are grown. Vegetables are considerably contaminated if they are grown in soils close proximity to highways or industrial areas (Zhong and Wang 2002; Tao et al., 2004). Samsøe-Petersen et al. (2002) have experimentally demonstrated the uptake of PAHs by fruit and vegetables grown in contaminated soils. Air pollution by dust and particle containing large quantities of PAHs may contaminate plants via atmospheric fallout during the growing period and this superficial contamination can be transferred to the final product (Lee et al., 1981; Bories 1988; Dennis et al., 1991; Simoneit 2002). In this way, Rey-Salgueiro et al. (2008a) quantified impacts of regional fires on PAHs deposition in vegetation of the Spanish town of Caldas de Reis. The results revealed that the company fire did not constitute a health risk, due to the limited exposure of the plants to smoke through the smooth winds blowing in the direction of the intensive horticultural area. Surface area appeared to be an important factor controlling PAH levels in various exposed

tissues of plants. Similar results were found by Costopoulou et al. (2010). They investigated dioxin, furan, PCB and PAH contamination of olive oil produced in fire-affected areas and found normal levels in oils producing areas after the fire and 1 year later. This factor is more significant in industrial areas and close to highways than in rural areas, where contamination of vegetables could be 10 times higher (Derache 1990; Grova et al., 2002). Broad-leaved vegetables such as lettuce can have particularly high levels of PAHs have due to their larger surface area that is ideal for deposition of airborne particles containing PAHs (Wickstrom et al., 1986). In cereals and beans, PAHs levels are low and the contamination of these food products is due to aerial deposition (Jones et al., 1989). The residue levels of PAHs in nuts, roots, and tubers are low (Dennis et al., 1991).

For animal origin foods, especially livestock, the accumulation of PAHs is due to consumption of contaminated pastures and vegetation (Crepineau et al., 2003). Grova et al. (2002) assessed milk PAH contamination sources of milk samples collected from the tank milk at farms located near potential contaminating emission sources. For all potential contaminating sources, PAHs were detected with similar profiles and at low concentrations except for fluorene and naphthalene. These results motivated later analysis to verify the metabolization PAHs and their migration to milk (Grova et al., 2006). They reported the impact of chronic exposure to PAHs on milk contamination, evaluating oral administration of a mixture of fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*k*]fluorene, benzo[*a*]pyrene and benzo[*g,h,i*]perylene at 0.020 mg/kg to lactating goats for 28 days. The results evidenced three main conclusions: (1) benzo[*k*]fluorene, benzo[*a*]pyrene and benzo[*ghi*]perylene were not detected in the milk; (2) unexpectedly, the concentration of fluorene, phenanthrene, anthracene,

fluoranthene, pyrene and chrysene did not change with time; (3) monohydroxylated PAH metabolites, namely 2- hydroxy -fluorene, 3- hydroxy -phenanthrene and 1- hydroxy -pyrene were detected shortly after administration. The concentrations of 2- hydroxy -fluorene and 3-hydroxy-phenanthrene reached, respectively, maxima of 0.41 and 0.22 ng/mL during the first exposure week, whereas the concentration of 1- hydroxy pyrene increased to reach a maximum of 0.97 ng/mL on day 14, then slightly decreased during the last two exposure weeks (Srogi 2007).

PAHs were also determined in baby formula milks and in this way, the food standards agency (FSA) determined PAHs in 97 samples of infant formulae milk obtained from across the UK (FSA, 2006). B[a]P was detected in 39 samples in concentrations levels lower than 1.0 µg/Kg. Kishikawaa et al. (2003) analyzed three infant formulae samples, finding total levels of PAHs about 2.0 ± 0.30 µg/kg, ranging B[a]P concentration between 0.27 and 0.39 µg/kg . Rey-Salgueiro et al. (2009) analyzed PAHs and two hydroxylated PAHs metabolites (1-OH-pyrene and 3-OH-benzo[*a*]pyrene) and their conjugates in commercial milk formulae and infant cereals but no samples exceeded the limit for benzo[*a*]pyrene and also no hydroxy PAH metabolites were detected.

Zanieri et al. (2007) determined PAHs in breast milk samples from 32 smoking and no-smoking mothers. The results obtained have shown the impact of tobacco in milk pollution. PAH levels of the 11 breast milk samples from smoking mothers were about 11 µg/Kg for naphthalene and acenaphthene, 0.10 µg/Kg for anthracene and benzo[*k*]fluoranthene. About 0.70 µg/Kg benzo[*a*]pyrene was detected in six milk samples. In no-smoking mothers no quantifiable benzo[*a*]pyrene levels were detected. Kishikawaa et al. (2003) determined also human milk and

total PAH levels ranging from 0.19 and 2.2 μg/kg.

PAHs in the atmosphere can be deposited in the aquatic environment. Once there they are readily taken up by aquatic organisms and so enter the food chain (Meador et al., 1995). Sources of contamination could include oil spills and run off from land of industrial effluent, the use of creosote-treated wood to support the cultivation of mussels…PAHs become concentrated in marine sediments, especially in coastal waters, where bottom-feeding fish and filter-feeding invertebrates are particularly prone to exposure and accumulation of the compounds (Phillips 1999). So PAHs in fish and shellfish are a result of contamination of fresh and coastal waters. The residue levels of PAHs in aquatic organisms depend on contamination of their habitat and ability of these organisms to metabolize the contaminants. In general, fish have a greater ability to metabolize PAHs than do mollusks, so the compounds tend to persist more in the latter (Meador et al., 1995).

Besides the food contamination by environmental sources, PAHs may be formed directly in food as result of various processing and heating processes. Processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting, frying) are major sources of PAH contamination (Guillén and Sopelana 1997; Phillips 1999). The type of cooking, cooking temperature, time, amounts of fat and oil influence the formation of PAHs (Vainiotalo and Matveinen 1993; Perez et al., 2002). The preservation of food by curing it with wood smoke is a process that has been used since antiquity. Smoke curing of meat and fish is now largely a highly industrialised process involving modern controlled kilns; nevertheless, traditional smokehouses are still used fairly widely. Since the generation of wood smoke is an example of incomplete combustion, PAHs are generated. In smoked foods the data reported in the literature

are highly variable. These variations can be due to the different procedures used to evaluate the presence of PAHs or the difference in procedures used for smoking. The variables that affect the process are: the type and composition of wood, type of generator, oxygen accessibility, temperature of smoke generation and smoking time (García-Falcón et al., 2005a).

In a detailed analysis of smoked food, Duedahl-Olesen et al. (2010) carried out the determination of PAHs in 180 industrially smoked fish products. It was shown that PAHs increased to a certain level with increasing smoking time and combustion temperatures. A discussion of the use of wood material for smoke formation indicated that mixtures of wood might be one of the critical parameters. Penetration of PAHs into the fish muscle of the smoked product illustrated that the skin acted as a barrier to smoke particles and the PAH compounds were mainly detected in the outer layers. In order to diminish the contamination of smoked fish by PAHs, the recommendation is clearly to use indirectly smoking or preferably cold smoking, stick to traditional woods such as beech for smoke formation, and to smoke whole fish. García-Falcón et al. (2005b) examined the dependence on the nature of the wood used for smoking on the formation of eight PAHs (selected as markers of PAHs) and transfer of these PAHs into traditional Spanish smoked chorizo sausages with collagen and tripe casings. The results showed that the kind of combustion performed on different materials seriously affected the PAH levels in the smoke generated, as well as ignition and firing of the material with a flame compared with heating. Santos et al. (2011) collected the contamination levels in traditional dry fermented sausages manufactured in Alentejo (South of Portugal), 66 samples were collected from regional producers. PAH8 represented less than 0.50 % of the total contamination profile, with benzo[*a*]anthracene and chrysene being the most concentrated compounds, irrespective of the

product type analyzed. Blood sausages were potentially more risky, since total PAH8 contents were generally higher and because its presence in inner parts were significantly superior than that found in casings, comparatively to meat counterparts, which expressed superior benzo[*a*]pyrene toxic equivalents.

Roasting and drying of coffee beans and tealeaves increase the PAH content (Stall et al., 1988; Houessou et al., 2006). However, the transfer of PAHs from coffee powder to coffee brew during coffee making has been reported to be relatively low (Hischenhuber and Stijve 1987; Kruijf 1987; García-Falcón et al., 2005b). The treating of wooden barrels for aging of alcoholic beverages may also lead to contamination of these beverages. However, the migration of PAHs from wood to wine is very low (García-Falcón et al., 2005c; Chatonnet and Scobessa 2007). Drying techniques used for cereal preservation such as combustion gas heating increase the PAH concentrations (Tateno et al., 1990).

Vegetable oils and fats are a significant source of PAHs 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). Contamination of cereal and vegetable oils with PAHs is mostly related to the drying processes of the seeds where combustion gases may come into contact with the seeds (Speer et al., 1990). The levels of PAHs in crude edible oils vary widely. So the variations in refining processes contribute to the differences in PAH concentrations in oils of plant origin (Guillén and Sopelana 2003). PAHs migration from pickle mussels to the vegetable oil in pickle sauce could also occur. In this way Rey-Salgueiro et al. (2009) confirmed PAHs in vegetable oil after a 3-months migration assay. In fact, the migrated benzo[*a*]pyrene % in oil increased when

the time to sell-by date decreased.

PAHs are also formed as a result of certain food preparation such as grilling and roasting. High PAH concentrations have been reported in charcoal grilled/barbecued foods. When food, particularly meat, is cooked over an open flame, PAHs are formed. If the meat is in direct contact with the flame, pyrolysis of the fats in the meat generates PAHs that can become deposited on the meat. Even if not in direct contact, fat dripping on to the flame or hot coals generates the compounds which are then carried back on to the meat (Phillips 1999). Although the exact mechanism of formation of PAHs in grilled/smoked foods is not precisely known, it is generally considered that at least three possible mechanisms exist (Alomirah et al., 2011). The pyrolysis of organic matter such as fat, protein and carbohydrates at temperatures above 200 ºC (PAH formation is favored at a temperature range of 500-900 ºC). The second mechanism is the yield of direct contact of lipids dripping at intense heat directly over the flame. The third mechanism is the incomplete combustion of charcoal, which can generate PAHs that are brought onto the surface of the food.

Differences in the PAH levels could be found in grilled products depending on the heat source as well as the type and geometry of the grill. The strong influence of the cooking method and the type of heat source on the PAH levels in the processed food was also demonstrated by Larsson et al. (1983). The geometry of the barbecue on the PAHs formation was investigated by Saint-Auberrt et al. (1992). The amount produced increases with increased fat content, longer exposure of the food to the flames and closeness to the heat source. The influence of the fat content of the meat upon the amount of PAHs formed during charcoal grilling was demonstrated by Mottier et al. (2000). In order to check PAHs generated from toasting in sandwich bread,

several treatment conditions were evaluated: direct toasting or indirect toasting by Rey-Salgueiro et al. (2008a). The used toasted technique would strongly affect in PAH levels in the final product. Differences between different ways of toasting could be ascribed to deposition of PAHs from smoke.

Charred food of almost any composition will contain PAHs; however, normal roasting or frying food does not produce copious quantities of PAHs (Howard and Fazio 1980). In contrast, the absence of PAHs from meat broiled in electric or gas broilers or cooked above charcoal in a vent pan, which catches the melted fat and prevents contact with the flames, shows that deposition of PAHs on the meat can be prevented by sequestering the meat surface from the flames (EFSA 2008). Moreover, with respect to home cooking practices, in general there is little evidence of PAH formation during the grilling, frying, roasting and toasting experiments.

3.2. Indicators of PAH sources

Usually, in environmental and food samples, the molecular patterns of PAHs are like fingerprints, what make possible to hypothesize about which processes generate them by studying their distribution in samples (Orecchio and Papuzza, 2009; Mansuy-Huault et al., 2009). The ratios commonly used in the literature are phenanthrene/anthracene, anthracene/anthracene+phenanthrene, fluoranthene/pyrene, fluoranthene/fluoranthene+pyrene, benzo[*a*]anthracene/chrysene and benzo[*a*]anthracene/benzo[*a*]anthracene+chrysene (Budzinski et al., 1997; Tam et al., 2001; Yunker et al., 2002; Orecchio and Papuzza2009). Generally, phenanthrene/anthracene ratios higher than 10 could show petrogenic inputs and

phenanthrene/anthracene < 10 for the dominance of pyrolytic sources. For the other hand, higher fluoranthene/fluoranthene+pyrene ratios than 0.40 and higher benzo[*a*]anthracene/benzo[*a*]anthracene+chrysene ratios than 0.35 belong to pyrolytic sources. The same consideration could be applied to the fluoranthene/pyrene and benzo[*a*]anthracene/chrysene ratios. Values greater than 1.0 and 0.40, respectively, are classically related to pyrolytic origins, namely to coal combustion (Sicre et al., 1987). Nevertheless, sometimes it is difficult the use of fluoranthene/fluoranthene+pyrene and benzo[*a*]anthracene/benzo[*a*]anthracene+chrysene ratios, because the PAH composition can differ between diesel fuels or between the vapor and particulate phases for diesel and crude-oil (Westerholm et al., 2001) but the higher mass PAHs usually are minor contributors to refined petroleum products. Orecchio and Papuzza (2009) examined the fingerprint PAHs in bread samples using wood as fuel for baking to confirm that all the PAHs identified in the bread samples were from combustion processes.

Other authors use PAH profiles ranging from 2 to 7 rings, i.e. low molecular weight PAHs (LMW, containing 2-3 aromatic rings) and high molecular weight PAHs (HMW, containing more than 3 aromatic rings). They used them a reliable tool for discriminating the petrogenic/pyrolytic origin of PAHs (Orecchio and Papuzza 2009). The lower the LMW/HMW ratio, the higher the prevalence of pyrolytic on petrogenesis origin of PAHs is. In this way, Kobayashi et al. (2008) carried out a pilot study of agricultural crops. Since wheat grain is one of the major food crops consumed internationally by many including infants and children, it could be useful to study a human exposure pathway for environmental contaminants using wheat as a model crop. They found that further research on the fate of more volatile PAHs present in grain

during storage, processing and cooking is needed to examine retention of these compounds in the final food products. Alomirah et al. (2011) suggested that LMW PAHs arise from smoke generated during meat grilling, as these PAHs are more volatile than HMW PAHs. Roseiro et al. (2011) also used these profiles for determining the occurrence of selected PAHs in traditional dry/fermented sausage along distinct stages of processing under two different technological procedures (traditional and modified processes). They proved that both, raw materials and final products, showed PAH profiles with light compounds representing about 99 % of the total PAHs, mostly accounted by those having two rings (28 % naphthalene) or three rings (17 % acenaphtene; 27 % fluorene; 20 % phenanthrene and 3.9 % anthracene). Guillén et al. (2011) determined the PAH contamination degree of a traditionally smoked cheese. They established that HMW PAH are very scarce in contrast with LMW. They also used certain ratios between phenanthrene/anthracene and naphthalene/acenaphthylene to provide information on the PAH contamination source. They found differences depending on the position of the cheeses in the smokehouse; those placed in the path followed by the smoke being were more contaminated.

The Scientific Committee on Food (SCF) calculated two correlation coefficient matrices using, firstly the eight PAHs found in the coal tar mixtures (eight PAHs considering in PAH8) and combinations of these PAHs in the studied samples, and then for the 15 priority PAHs. They founded that the PAH4 and PAH8 combinations reached a sufficient accuracy for contaminated samples and therefore, PAH4 and PAH8 could be also suitable indicators for occurrence of total PAHs (EFSA 2008).

3.3. Measures to reduce PAH contamination of food

In respect to cooking techniques the level of PAHs formed during cooking of foods depends of the conditions used. There are simple practices that results in reduce of the contamination by PAHs (Lijinsky, 1991; Knize et al., 1999). These practices include the use of lean meats and fish, the use of less fat for grilling, avoid the food contact with flames for barbecuing and in general cooking at lower temperature for a longer time (García-Falcón and Simal-Gándara 2005a; Rey-Salgueiro et al., 2008a). It is not suitable that the fat of the foods dripping onto the flame for avoid so the formation of smoke that could achieve the foods. The use of medium to low heat, and to place food farthest from the heat source can significantly reduce the formation of PAHs.

In smoked products, in the past two decades the traditional smoking process is being replaced by the use of liquid smoke flavorings. This is due to smoke flavorings are produced from smoke that is subjected to fractionation and purification processes and consequently its PAH content is lower. So, the risks to health are minor compared to the traditional smoking (EFSA2008).

For the vegetables oils contaminated as result drying processes, the PAH levels could be reduced by treatment of these oils with activated charcoal (Larsson et al., 1987). Moreover, oil-refining processes (based on the deodorization step) reduce also the concentration of a number of the lower molecular weight compounds. However for the higher molecular weight PAHs this effect not was observed. This refining method has been reported (Dennis et al., 1991).

In the case of fruit and vegetables, contaminated as a result of deposition of certain environmental PAHs, concentrations are usually higher on plant surface (peel, outer leaves) than

in internal tissue. Consequently, a careful washing may remove, on average, up to 50% of the total PAHs (SFC 2002).

4. Dietary exposure assessment to PAHs

Dietary intake of PAHs constitutes a major source of human exposure (Xia et al., 2010) because recent epidemiological studies have revealed that dietary exposure to PAHs (Brody et al., 2007; Lee and Shim, 2007) is associated with an increased risk of some human cancers (**Figure 1**). In recent years, environmental PAH concentrations have increased in many industrialized and developing countries, leading to high levels of PAHs in foodstuffs. However, reports concerning dietary exposure of PAHs are quite limited. Recently the Scientific Committee on Food (SCF) has assessed the health risk to consumers associated with exposure to PAHs in foodstuffs (EFSA 2008). The background for this risk assessment was that the European Commission levels for PAHs in food at Community level. The report of the SCF shows that presently there are no reliable data for the content of PAHs in several products and those intensive studies showed that animal feed/feed ingredients are one of the major sources of contamination. They used 11 food categories corresponding also with food consumption (**Table 3**) and calculated four groups of PAHs to study the exposure in foodstuffs: benzo[*a*]pyrene, PAH2, PAH4 and PAH8. The contribution of each category to the total exposure was calculated from the mean consumption of consumers, defined as subjects consuming the food category under consideration at least once during the survey duration. The highest contributors to the dietary exposure were cereals and cereal products; vegetables, nuts and pulses; meat and meat

products; seafood and seafood products as well as fish and fishery products (**Table 3**).

They also estimated the average dietary exposure to PAHs in the EU countries. They concluded that the overall average dietary exposure for which data are available assuming a body weight of 60 kg was 235 ng/day for benzo[*a*]pyrene, 641 ng/day for PAH2, 1168 ng/day for PAH4 and 1729 ng/day for PAH8. They pointed out the importance of high consumption of barbecued foods, which lead to a high exposure to PAHs.

In Arabian countries, dietary exposure to PAHs from grilled and smoked foods was also studied (Alomirah et al., 2011). They concluded that meat tikka, whole grilled chicken, meat burger and grilled vegetables were the major contributors to the daily intake of benzo[*a*]pyrene, PAH8 and ∑PAHs for children/adolescent and adult population. They founded that total mean dietary intakes for children/adolescents and adults for benzo[*a*]pyrene (8.1 ng/day, 9.2 ng/day), PAH8 (84 ng/day, 96 ng/day) and ∑PAHs (974 ng/day, 1100 ng/day) were comparable. The same authors reported also results in olive oil (extra virgin olive oil, virgin olive oil, olive oil, pomace olive oil and blended olive oil), cooking oil (corn oil, sunflower oil, sesame oil, palm olein oil, soya oil, canola oil, mustard oil, peanut oil and mixed vegetable oil) and fat (butter and table margarine) collected from retail stores in Kuwait (Alomirah et al., 2010). Approximately 20% of the samples within the olive oil and cooking oil sub-categories exceeded the EU maximum tolerable limit for benzo[*a*]pyrene, with the highest level of 6.8 and 118 µg/Kg, respectively. The Kuwaiti general population's dietary exposure to the genotoxic PAH8 was estimated to be 196 ng/day (assuming an average adult body weight of 60 kg).

Dietary exposure of PAHs in high lung cancer incidence areas was developed by Cai et al. (2012) in China. The daily exposure doses of benzo[a]pyrene and total PAHs were 458 ng/d

and 14 532 ng/d, respectively. High vegetarian food uptake (rice and potato) resulted in low dietary PAH exposure. In this case, PAHs with 2–4 rings occupied a significant percentage of total PAHs in food samples. Nevertheless, they concluded that dietary exposure was not the main exposure route of PAHs and should not account for the abnormal high lung cancer incidence in these areas. Also Shen et al. (2008) studied the incidence of lung cancer to ingestion of food, which are exposed to carcinogenic coal emissions. They observed that an increased intake of rice, green vegetables, mushrooms and fresh meat was associated with an increased risk of lung cancer and therefore, dietary intake of PAH-contaminated foods may be an important route of exposure.

5. Bioaccumulation

The need to understand chemical movement within food webs of persistent organic pollutants (POPs) has been of great interest in the last 50 years. In this way, various food web models have been developed and applied to supplement our understanding of field and laboratory studies of the behavior and fate of POPs within organisms and through the food web (Thomann et al., 1992; Gobas, 1993; Campfens and Mackay 1997; Arnot and Gobas, 2003, 2004; Gobas and Wilcockson 2003; Webster and Ellis 2012). The bioaccumulation of chemicals in biota and the potential for biomagnification through the food chain represent essential elements for a proper hazard and risk assessment of chemical substances (Alonso et al., 2008).

Bioaccumulation can be defined as the process by which chemicals are absorbed and removed from the water directly by organisms or through the ingestion of food (Froehner et al., 2011). Usually, bioaccumulation is assessed by analyzing the compound characteristics, such as K_{ow}, bioconcentration factor from water (BCF), bioaccumulation factor (BAF), the biota-sediment accumulation factor (BSAF) and biomagnification factor (BMF) (Gobas and Morrison 2000; Arnot and Gobas 2004).

5.1. Biotransformation

Biotransformation, also referred to as metabolism, is widely recognized as the most important and most uncertain determinant of bioaccumulation. In the past in the absence of measured biotransformation rates it was standard practice to assume an infinite biotransformation half-life, i.e., no biotransformation (Webster and Ellis 2012).

Biotransformation refers to the entire modification of chemical molecules occurring in the organisms. PAH and metabolites that bioaccumulate in species low on the food chain can then be taken up by higher trophic levels through dietary exposure [Palmqvist et al., 2006]. The tendency for such trophic transfer depends on numerous factors, including the chemical properties of the contaminant, the food source, and also the food source's residence time within and the pH of the gut of the organism [Granberg and Forbes 2006]. These factors all affect the bioavailability of a contaminant from a food source to the consumer.

Parent PAHs are preferentially accumulated in the lipid-rich part of the organism, whereas metabolites accumulated in bile and urine before excretion [Beach et al., 2010]. Because

of the ease of analysis of metabolites in these biofluids, this approach has been adopted as a biomarker of exposure and effects [Johnson-Restrepo et al., 2008]. In smaller species of invertebrates, the absence of an easily accessible biofluid makes tissue analysis necessary to detect metabolites. This, as well as concerns associated with occupational exposure to PAHs, has meant that PAH biotransformation has been more carefully studied in vertebrate species, especially humans and finfish, than in invertebrates [Beach et al., 2010].

Biotransformation could also be estimated *in vitro*. A significant improvement came with the development of the use of quantitative structure-activity relationship (QSAR) models to estimate biotransformation, such as applications in fish (Arnot et al., 2008a, 2008b and 2009). The resulting models intended to provide predictions of biotransformation. Nevertheless, estimations models do not account for the variability between species.

5.2. Bioremediation

It can be described as “the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state”. The aim of this technique is to remove pollutants from natural environment and/or convert the pollutants to a less harmful product, such as carbon dioxide and water, using the indigenous microbiological community of the contaminated environment (Bamforth and Singleton 2005).

Bioremediation technique based on the optimization of biodegradation has been developed as an agricultural soil clean-up technique. It has advantages over thermal and some physico-chemical techniques in terms of cost and because the soil as a living system suitable for

plant growth is not destroyed. The degradation process may be enhanced by changing chemical or physical conditions in the soil, such as soil pH, moisture, and aeration, and also by nutrient addition. Bioremediation of PAH-contaminated soils, sediments and water can be accomplished in a variety of ways: *in situ* or *ex-situ* treatments.

In situ treatments

The contaminated agricultural soil is treated in place and essentially remains undisturbed during in-situ treatment (Wilson and Jones, 1993). The most common form of in-situ treatment is the biodegradation of contaminants within the saturated zone of the soil. This involves the addition of nutrients, an oxygen source (usually hydrogen peroxide), and sometimes specifically adapted micro-organisms to enhance degradation and may be achieved by drilling a series of wells throughout the contaminated area and directly injecting the appropriate solutions. The success of in-situ treatment is highly dependent on the soil permeability owing to the necessity for oxygen transfer. In situ remediation includes techniques such as bioventing, biosparging, bioslurping and phytoremediation along with physical, chemical, and thermal processes.

***Ex-situ* treatments**

Ex situ remediation techniques involve removing the soil from the subsurface to treat it. Ex situ remediation includes techniques such as bio-piling and composting methods.

Biopiling is an in situ process that is also known as the heap technique. The first step in the biopiling process is to perform laboratory tests that will determine the biological degradation capabilities of the soil sample. The next step involves the mechanical separation of the soil, which will homogenize the sample and remove any disruptive material such as plastics, metals, and stones. The stones will then be crushed into smaller pieces and then depending on the degree

of contamination will either be added to a pile or sent out for reuse. The soil is then homogenized, meaning that the pollution concentration is averaged out across the entire soil sample. Homogenization allows for biopiling being more effective (Schulz-Berendt 2000).

Composting is a form of prepared-bed type of treatment that has been used to treat highly contaminated material. This is a specific process involving a succession of mesophilic and thermophilic microorganisms and consists in piling the soil and mixing with an organic bulking agent, such as straw or wood chips. The pile is aerated by either forced aeration or pile turning, and the moisture content, pH, and nutrient content, etc., are controlled. Composting has not been widely applied for treatment of hydrocarbon contaminated soil (Wilson and Jones, 1993).

6. The challenges of scientific research in PAHs

Advances should be made in understanding the clinical toxicology of PAHs. These include the development of transfer factors from feed to food, knowledge on formation of PAHs and their metabolites, validation of biomarkers of exposure, and search on chemopreventive agents stimulating detoxification pathways.

6.1. Transfer of PAHs from feed to animal products

Animal feed materials may contain elevated concentrations of PAHs due to

environmental contamination or through direct drying processes. Although very little data are available on carry-over rates, it is generally assumed that PAHs are not transferred from animal feed to animal products such as milk (Bulder et al., 2008). Nevertheless, some authors Kan et al. (2003) indicated that the low molecular PAHs are transfer to milk (and other edible tissues) in contrast to high molecular PAHs. Other studies by Grova et al. (2002) and Lutz et al. (2006) included the metabolites in their analysis. The present authors developed a study about feeds and their corresponding footprints of residual PAHs and polychlorinated biphenyls based on their constituents (Yebra-Pimentel et al., 2012; Fernández-González et al., 2012). We found that PAH pollution in feed and ingredients is produced not only due to their fiber content but also due to the other ingredients such as minerals and additives. Contamination of these products (cereals, fiber products, minerals and additives) by PAHs could occur at source (e.g. by atmospheric deposition on crops), but mainly during intense thermal processing (drying and toasting). It was also observed that the concentration of low-molecular PAHs was higher than the high-molecular PAHs, as the other authors have been reported (Bulder et al., 2006).

The intake of PAHs by animals is higher than by humans, in cows is 65 to 1000 times. Cancer of the gastro-intestinal tract is seen in cows, which could be an indication for carcinogenic potential of compounds present in animal feed (Bulder et al., 2006).

Bulder et al. (2006) took a transfer rate for the calculated human intake from the study of Grova et al. (2002), which showed a transfer of 0.20 % benzo[*a*]pyrene related activity from radiolabeled PAHs from feed to milk in goat. Since benzo[*a*]pyrene was not detected in cow's milk in other studies, it could be possible that the transfer in cows is different than in goats. They concluded that detailed analysis on occurrence and effects of metabolites of benzo[*a*]pyrene (and

other PAHs) would be recommended for facilitating more accurate risk assessment. Therefore, more information on the transfer of PAHs from feed to animal products is needed.

6.2. Formation and carry-over of PAHs and their metabolites

As it was previously commented, in 2008 the EFSA established that benzo[a]pyrene is not a suitable indicator for the occurrence of PAHs in food and a system of two specific substances called PAH2 (benzo[a]pyrene + chrysene), of four specific substances called PAH4 (PAH2 plus benz[a]anthracene benzo[b]fluoranthene) or eight specific substances called PAH8 (PAH4 plus benzo[k]fluoranthene, benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) were considered as the most appropriate indicators (EFSA 2008). These considerations are based on the total diet. However focusing on animal products (edible tissues and milk) only the profile of PAHs might be different due to the differential transfer of the individual PAHs (Bulder et al., 2006).

In addition the PAHs are extensively metabolized in vivo (Cavret et al., 2004), there is evidence that the metabolites of the high molecular PAHs are transferred at a higher rate to milk than the original compounds (Lutz et al., 2005) and also, that these metabolites are important because the carcinogenic potency of the high molecular PAHs can be attributed (at least partly) to these metabolites (ATSDR 1995). Further research on the formation and carry-over of metabolites is clearly needed. In this way Shailaja et al. (2006) investigated how NO_2^- affected the biological impact of PAH-containing industrial effluents in fish. They concluded that the presence of NO_2^- in the environment could influence the overall carcinogenicity of PAH-

containing effluents, because NO_2^- upregulates induction of cytochrome P450 activity by PAH and leaded to enhancement of metabolism of benzo[*a*]pyrene.

New challenges on the formation of PAHs are also important to study their impact on food. On January 2012 an international team discovered a novel route to form PAHs in ultra-cold regions of interstellar space (Science daily 2012). These findings have crucial implications not only to reduce the emission of PAHs as toxic byproducts from internal combustion engines, but also rationalize the synthetic routes to a key class of organic molecules in the interstellar medium associated with the origins of life.

6.3. Biomarkers of exposure to PAHs

One of the most pressing problems for PAH toxicity assessment is the determination of exposure. Because most species can affectively metabolize PAHs, determining if individuals have been exposed is often very difficult. In mostly invertebrates that do not extensively metabolize PAHs, a tissue concentration-response relationship could be established. Future work linking biological responses to biomarkers of exposure or specific metabolites found in bile may be valuable in defining dose-response relationships for vertebrates exposed to PAHs. For example, the determination of fluorescent aromatic compounds (FACs) in bile as a biomarker for PAH exposure may be a useful way to link exposure and response metrics. Other biomarkers of exposure include DNA adducts in various tissues and activation of certain enzymes, such as cytochrome P450-1A. At this point, there are no generalized correlations between the actual dose and biomarkers of exposure. For most of these biomarkers, such correlations have rarely been

explored.

Some properties make mosses suitable as monitors for air deposition of organic micropollutants. In this case, the capacity to absorb large organic molecules is the key parameter. The first applications of mosses as monitors of the deposition of organic micropollutants were made in the early 1980s (Thomas and Herrmann 1980). Most of the work concentrated on the deposition of organochlorine compounds and also in some studies a few PAHs were studied as well (Wegener et al., 1992).

6.4. Chemopreventive agents for PAHs exposure effects

There are a large number of compounds that can prevent the occurrence of cancer (**Table 4**). The chemical diversity of the inhibitors indicates that inhibition of carcinogenesis is not a highly selective phenomenon and that multiple strategies exist for bringing about this desired effect. It also makes it probable that additional compounds with chemopreventive properties will be identified in the future, again adding to the choices that will be available. Chemopreventive agents may inhibit adduct formation or remove adducted nucleotides and damaged cells through several pathways: 1) metabolic inactivation of chemical carcinogens; 2) enhancement of DNA repair; and 3) induction of cellular apoptosis (Zhou et al., 2011).

Recently, natural chemopreventive agents have received great attention for cancer prevention, such as ascorbic acid, α-tocopherol and β-carotene. The hope has been that these compounds could be administered with very little risk to human subjects and that they would produce a significant preventive effect. Ascorbic acid is effective in preventing the formation of

nitroso carcinogens from precursor compounds but in most instances is not effective in inhibiting carcinogenesis resulting from administration of preformed carcinogens (Mirvish, 1975). There is only a small amount of published evidence showing inhibition of carcinogenesis by α-tocopherol and by β-carotene (Wattenberg and Loub 1978; Mathews-Roth 1982). Thus, while the risk of the use of these compounds would appear to be minimal, it is quite possible that their administration to humans will not show positive results. If this proves to be the case, recognition should be made of the considerations involved in their early choice for studies in the human. These negative results should not discourage further endeavors to explore the use of more effective chemopreventive compounds.

Zhou et al. (2011) tested *in vivo* the hypothesis that levels of carcinogenic PAH-DNA adducts can be diminished by dietary fish oil. They concluded that fish oil has a potential to be developed as a cancer chemopreventive agent, as it was observed by other authors (Hong et al., 2005; Fan et al., 2011).

7. Conclusions

Polycyclic Aromatic Hydrocarbons (PAHs) may end up in edible products and as such impose a possible risk for consumers. A carry-over study on Polycyclic Aromatic Hydrocarbons (PAHs) from feed to milk showed the availability and health-related effects of PAHs originating from animal feed for the consumer. Certain PAHs are transferred as native compounds to milk, in

particular those PAHs with less than five rings. In addition, it appears that metabolites of some PAHs are transferred to milk. The carry-over of metabolites and its toxicological relevance are generally underestimated in risk assessments. The carcinogenic properties of PAHs can be contributed to the PAH metabolites, however a complete toxicological profile of the metabolites is not available. The metabolites of PAH have not been considered in monitoring programs yet, thus only limited data are available, which is severely hampering the risk assessment. In this respect, it is of paramount importance to promote studies with the aim of obtaining:

1. Data on non-carcinogenic effects of Polycyclic Aromatic Hydrocarbons (PAHs), which are very limited. Similar applies to the oral carcinogenic potential of PAHs other than Benzo(a)Pyrene (BaP).

2. Data on transfer rates of PAHs to cow's milk and other animal products, which are also very limited. Metabolized PAHs are generally not considered in the transfer of PAHs from feed to food. It is likely that PAHs with more than 5 rings are transferred as metabolites.

3. Data of PAHs in food should not be expressed solely in BaP equivalents. Analysis of PAHs in animal products should also include metabolites of PAHs.

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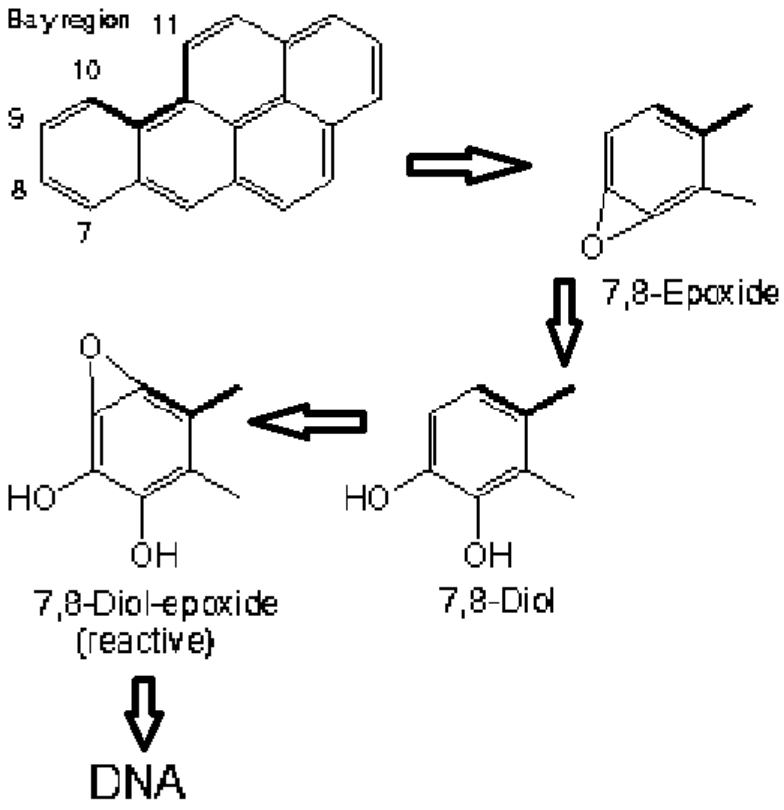
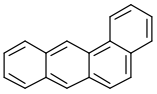
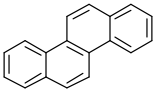
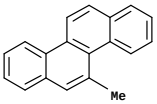
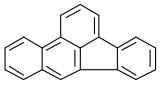
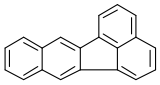
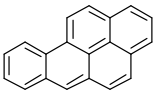
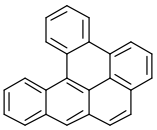
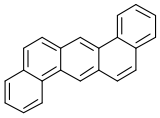
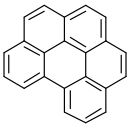
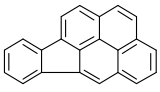
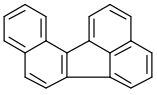
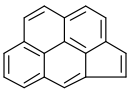
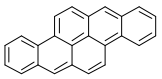


Figure 1. PAH carcinogenic activation.

Table 1. The 15 Polycyclic aromatic hydrocarbons mutagenic/genotoxic PAHs and also two of their main hydroxy metabolites.

COMPOUND	MW	CAS	STRUCTURE
Benzo[a]anthracene	228	56-55-3	
Chrysene	228	218-01-9	
5-MethylChrysene	242	3697-24-3	
Benzo[b]fluoranthene	252	205-99-2	
Benzo[k]fluoranthene	252	207-08-9	
Benzo[a]pyrene	252	50-32-8	
Dibenzo[al]pyrene	302	191-30-0	
Dibenzo[a,h]anthracene	278	53-70-3	
Benzo[ghi]perylene	276	191-24-2	
Indeno[1,2,3-cd]pyrene	276	193-39-5	
Benzo[j]fluoranthene	252	205-82-3	

Cyclopenta[cd]pyrene	226	27208-37-3	
Dibenzo[a,h]pyrene	303.3	189-64-0	

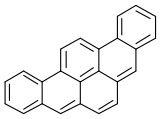
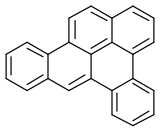
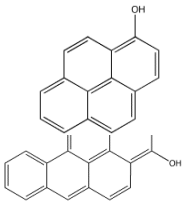
COMPOUND	MW	CAS	STRUCTURE
Dibenzo[a,i]pyrene	302.3	189-55-9	
Dibenzo[a,e]pyrene	302.3	192-65-4	
1-OH-Pyrene	218	5315-79-1	
3-OH-Benzo[a]pyrene	268	13345-21-6	

Table 2. Maximum levels (MLs) for benzo[a]pyrene and PAH4.

	Foodstuff	Maximum levels (µg/kg)	
		Benzo(a)pyrene	**PAH4** (1)
1	Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	2.0	10
2	Cocoa beans and derived products	5.0 fat as from 1/4/2013	35 fat as from 1/4/2013 until 31/3/2015 30 fat as from 1/4/2015
3	Coconut oil intended for direct human consumption or use as an ingredient in food	2.0	20
4	Smoked meat and smoked meat products	5.0 until 31/8/2014 2.0 as from 1/9/2014	30 as from 1/9/2012 until 31/8/2014 12 as from 1/9/2014
5	Muscle meat of smoked fish and smoked fishery products, excluding fishery products listed in points 6 and 7. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen. In case of smoked crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages.	5.0 until 31/8/2014 2.0 as from 1/9/2014	30 as from 1/9/2012 until 31/8/2014 12 as from 1/9/2014
6	Smoked sprats and canned smoked sprats (*sprattus sprattus*); bivalve molluscs (fresh, chilled or frozen) ; heat treated meat and heat treated meat products sold to the final	5.0	30

	consumer		
7	Bivalve molluscs (smoked)	6.0	35
8	Processed cereal-based foods and baby foods for infants and young children	1.0	1.0
9	Infant formulae and follow-on formulae, including infant milk and follow-on milk	1.0	1.0
10	Dietary foods for special medical purposes intended specifically for infants	1.0	1.0

(1) Lower bound concentrations are calculated on the assumption that all the values of the four substances below the limit of quantification are zero.

Table 3. Consumer exposure to benzo[*a*]pyrene (B[*a*]P), PAH2, PAH4 and PAH8.

Category food consumption	Category occurrence	Consumption Median g/day	Exposure % B[a]P ng/day	% PAH2 ng/day	% PAH4 ng/day	% PAH8 ng/day
Cereals and cereal products	Cereals	257	2.61E-08	5.02E-08	**1.00E-07**	**1.53E-07**
Sugar and sugar products including chocolate	Chocolate	43	1.16E-08	3.02E-08	5.81E-08	9.07E-08
Fats (vegetable and animal)	Fats and oil*	38	6.84E-08	3.39E-08	2.15E-08	1.59E-08
Vegetables, nuts, pulses including carrots, tomato	Vegetables and nuts	194	2.58E-08	6.39E-08	1.14E-07	1.95E-07
Fruits (dried fruit 4%, other fruit 96%)	Dried fruit	153	3.27E-09	2.61E-08	5.24E-08	5.69E-08
Coffee, tea, cocoa (expressed as liquid)	Coffee powder	601	3.49E-09	9.15E-09	1.76E-08	2.60E-08
Alcoholic beverages	Alcoholic beverages	413	9.69E-10	2.91E-09	6.05E-09	1.79E-08
Meat and meat products and substitutes	Meat	132	3.18E-08	8.11E-08	**1.48E-07**	**2.11E-07**
Seafood and their products	All mollusc	27	**1.33E-07**	**5.19E-07**	**5.19E-07**	**1.56E-06**
Fish and their products (processed fish 30%, fresh and frozen fish 70%)	All processed fish	41	5.12E-08	**2.05E-07**	**4.15E-07**	**5.12E-07**
Cheese	Smoked cheese	42	1.43E-08	2.86E-08	4.67E-08	7.14E-08

*excluding cocoa butter and pomace oil

Table 4. Inhibitors of carcinogen-induced neoplasia.

Category of inhibitor	Chemical class	Examples of inhibitory compounds
Compounds preventing formation of carcinogen from precursor compounds	Reductive acids	Ascorbic acid
	Tocopherols	α-Tocopherol, γ-tocopherol
	Phenols	Caffeic acid, ferulic acid, gallic acid, propyl gallate
Blocking agents	Phenols	2(3)-tert-Butylhydroxyanisole, butylated hydroxytoluene, hydroxyanisole, ellagic acid, caffeic acid, ferulic acid, p-hydroxycinnamic acid
	Indoles	Indole-3-acetonitrile, indole-3-carbinol, 3,3'-diindolymethane
	Aromatic isothiocyanates	Benzyl isothiocyanate, phenethyl isothiocyanate, phenyl isothiocyanate
	Coumarins	Coumarin, limettin
	Flavones	β-Naphthoflavone, α-naphthoflavone, quercetin pentamethyl ether
	Dithiothiones	5-(2-Pyrazinyl)-4-methyl-1,2-dithiol-3-thione, 3-(p-methoxyphenyl)-1,2-dithiol-3-thione
	Diterpenes	Kahweol palmitate
	Dithiocarbamates	Tetraethylthiuram disulfide (disulfiram), sodium diethyldithiocarbamate, bis(ethylxanthogen)
	Phenothiazines	Phenothiazine
	Barbiturates	Phenobarbital
	Trimethylquinolines	6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin)
Suppresing agents	Retinoids and carotenoids	Retinyl palmitate, retinyl acetate, 13-cis-retinoic acid, ethyl retinamide, 2-hydroxyethylretinamide, retinyl methyl ether, N-(4-hydroxyphenyl)retinamide, β-carotene
	Selenium salts	Sodium selenite, selenium dioxide, selenious acid, sodium selenide

	Protease inhibitors	Leupeptin, antipain, soybean protease inhibitors
	Inhibitors of arachidonic acid metabolism	Indomethacin, aspirin
	Cyanates and isothiocyanates	Sodium cyanate, tert-butyl isocyanate, benzyl isothiocyanate
	Phenols	2(3)-tert-Butylhydroxyanisole
	Plants sterols	B-Sitosterol
	Methylated xanthenes	Caffeine
	Others	Dehydroepiandrosterone, fumaric acid