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Benzo(b)fluoranthene (205-99-2)
Benzo(k)fluoranthene 207-08-9

Mammal

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Polycyclic aromatic hydrocarbons (polynuclears) in foods

By

MEI-TEIN LO* and EMIL SANDI*

Contents

I.	Introduction	35
II.	Formation and general properties	36
III.	Origin in foods	42
	a) Natural occurrence	42
	b) Polluted environments	42
	c) Food additives and food packagings	42
	d) Curing smokes and other pyrolysis products	43
IV.	Analysis	44
	a) Isolation and separation	44
	b) Identification and determination	45
V.	Occurrence and concentration in foods	45
	a) Vegetable oils, fats, and shortenings	45
	b) Fish and other marine foods	47
	c) Meat and meat products	48
	d) Fruit and other plant products	52
	e) Other data	54
VI.	Metabolism	55
	a) Absorption, distribution, and excretion	55
	b) Degradation	55
	c) Reactivity of intermediate metabolites	63
VII.	Carcinogenicity	64
	a) *In vitro* bioassay	65
	b) Whole animal studies	66
Conclusions		66
References		76

I. Introduction

Ever since benzo(a)pyrene (BaP)[1] was recognized as a carcinogen, at the beginning of this century, the presence of it and of other poly-

* Toxicological Evaluation Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada, K1A 0L2.

[1] All abbreviations of chemicals used in text are listed in Table XXV.

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9233

06/05/2001 07:43 5054243202 JILL PODOLSKY, EHA PAGE 01

06/05/2001 07:43 5054243202 JILL PODOLSKY, EHA PAGE 02

cyclic aromatic hydrocarbons (PAH) in the environment has received continuous attention. As a result numerous reviews have been published, the most recent ones being the following: ANDELMAN and SUESS (1970), *U.S. NATIONAL ACADEMY OF SCIENCES* (*US NAS* 1972), *U.S. ENVIRONMENTAL PROTECTION AGENCY* (*US EPA* 1975), and *INTERNATIONAL AGENCY FOR RESEARCH ON CANCER* (*IARC* 1973). A symposium on PAH has also been held recently (FREUDENTHAL and JONES 1976).

However, these reviews deal chiefly with the environmental aspects of PAH and only marginally with the area of foods. Reviews on analysis, occurrence and residues of PAH in food are available from before 1969: GUNTHER and BUZZETTI (1965), GUNTHER *et al.* (1967), HAENNI (1968), HOWARD and FAZIO (1969), and TILGNER and DAUN (1969).

The objectives of the present review are (1) to bring together for ready reference the information available today (end of 1976) on the occurrence of PAH in foods, and (2) to list and evaluate the toxicological and carcinogenic studies performed in this field.

II. Formation and general properties

PAH, also known as polynuclear aromatic hydrocarbons, can be defined as organic compounds containing two or more benzenic ring structures which may or may not have substituted groups attached to one or more rings. To date about 100 PAH have been identified in the environment and in foods (TILGNER and DAUN 1969, ANDELMAN and SUESS 1970, GUNTHER *et al.* 1967, *U.S. NAS* 1972, *U.S. EPA* 1975). These are listed, along with their occurrence and carcinogenic potential in Table I.

Table I. *PAH found in the environment and in foods and their carinogenic activity.*[a]

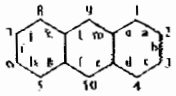
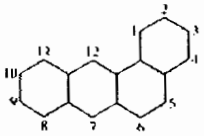
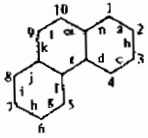
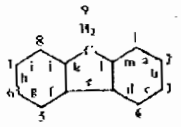
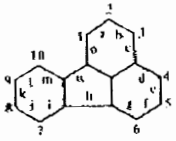
Group	Compound[b]	Carcinogenic activity[c]	Occurrence[d]
Anthracene	Anthracene (A)	—	E, F, S
	2-Methylanthracene	?	E
	10,11-Dihydro-9-H-benzo(a) cyclopent(i)anthracene	?	E
	2,3-Dihydro-1-H-benzo(a) cyclopent(h)anthracene	?	E
Benz(a)anthracene	Benz(a)anthracene (1,2-benzanthracene)(BaA)(BA)	+	E, F, S
	7,12-Dimethylbenz(a)anthracene (9,10-dimethyl-1,2-benzanthracene) (DMBaA) (DMBA)	+++	E, S
	Methylbenz(a)anthracenes (MBaA) (MBA)	?	E, F, S

Table I. (*continued*)

Group	Compound[b]	Carcinogenic activity[c]	Occurrence[d]
	Dibenz(a,j)anthracene (1,2-7,8-dibenzanthracene) (DBajA)	+	E, F, S
	Dibenz(ah)anthracene (1,2-5,6-dibenzanthracene) (DBahA) (DBA)	+++	E, F
	Dibenz(a,c)anthracene (1,2-3,4-dibenzanthracene) (DBacA)	+	F
	Dibenz(a,i)anthracene (DBaiA)	?	F, S
	7H-Benz(d,e)anthracen-7-one	?	E
Phenanthrene	Phenanthrene (PA)	—	E, F, S
	Benzo(c)phenanthrene (3,4-benzphenanthrene) (BcPA)	+++	E
	Dibenzo(b,h)phenathrene	?	E
	3-Methyl phenanthrene(3-MPA)	?	F
	2-Methyl phenanthrene	?	F
	9-Methyl phenanthrene	?	F
	3,6-Dimethyl phenanthrene	?	F
	13H-Indeno(1,2,e) phenanthrene	?	E
Fluorene	Fluorene (F)	—	E, F, S
	Benzo(a)fluorene (1,2-benzfluorene)(BaF)	—	E, F, S
	Benzo(b)fluorene (2,3-benzfluorene)	—	E, F
	Benzo(c)fluorene (3,4-benzfluorene)	—	E, F
	Dibenzo(a,c)fluorene (1,2-3,4-dibenzfluorene)	±	E
	Dibenzo(a,g)fluorene (1,2-5,6-dibenzfluorene) (DBagF)	+	E
	Dibenzo(a,h)fluorene (1,2-6,7-dibenzfluorene)	±	E
	Dibenzo(a,i)fluorene	?	E
Fluoranthene	Fluoranthene (FL)	—	E, S
	Benzo(b)fluoranthene (2,3-benzofluoranthene) (BbFL)	++	E, F

06/05/2001 07:43 5054243202 JILL PODOLSKY, FHA PAGE 03

Table I. (*continued*)

Group	Compound[b]	Carcinogenic activity[c]	Occurrence[d]
	Benzo(j)fluoranthene (7,8-benzofluoranthene) (BjFL)	++	E, F
	Benzo(k)fluoranthene (8,9-benzofluoranthene) (BkFL)	—	E, F
	3,4-Benzofluoranthene (3,4-BFL)	++	E
	10,11-Benzofluoranthene (10,11-BFL)	++	E
	11,12-Benzofluoranthene (11,12 BFL)	—	E
	Benzo(ghi)fluoranthene	—	E, F
	Dibenzo(e,l)fluoranthene	?	E
	Alkyl fluoranthene	?	E
	8-Methylfluoranthene	?	E
	2-Methylfluoranthene (2-MFL)	+	E
Aceanthrylene	Aceanthrylene (AC)	—	E
	Benz(e)aceanthrylene	?	E
	Benz(j)aceanthrylene (cholanthrene) (BjAC)	++	E, S
	3-Methylcholanthrene (MCA)	+++	E
	20-Methylcholanthrene	—	S
	Dibenz(aj)aceanthrylene	?	E
Naphthacene	Naphthacene (Benz(b)anthracene)	—	E
	Naphtho(2,1,8-qra)naphthacene	—	E
	Dibenzo(a,l)naphthacene	?	E
	Dibenzo(a,j)naphthacene	?	E
	Dibenzo(a,c)naphthacene	?	E
	Benzo(a)naphthacene	?	E
Pyrene	Pyrene (P)	—	E, F, S
	1-Methylpyrene	?	E
	4-Methyl pyrene (4-MP)	?	E, F, S
	2,7-Dimethyl pyrene	?	E
	Indeno(1,2,3-cd)pyrene (o-phenylenepyrene) (IP)	+	E, F
	Alkylpyrene	?	E

Table I. (*continued*)

Group	Compound[b]	Carcinogenic activity[c]	Occurrence[d]
Benzo(a)pyrene	Benzo(a)pyrene (1,2-benzpyrene) (3,4-benzpyrene[e]) (BaP)	+++	E, F, S
	Benzo(e)pyrene (4,5-benzopyrene) (1,2-benzopyrene[e]) (BeP)	+	E, F, S
	Methylbenzo(a)pyrene (MBaP)	?	E, S
	Hydroxybenzo(a)pyrene	?	E
	Dibenzo(a,i)pyrene (2,3-6,7-dibenzpyrne) Benzo(rst)pentaphene) (DBaiP)	+++	E, S
	Dibenzo(a,l)pyrene (2,3-4,5-dibenzpyrene) (Dibenzo(b,def)chrysene) (DBalP)	±	E, S
	Dibenzo(a,e)pyrene (1,2,4,5-dibenzopyrene) (DBaeP)	?	E, S
	Dibenzo(a,h)pyrene (1,2-6,7-dibenzpyrene) (Dibenzo(b,def)chrysene) (DBahP)	+++	E, F, S
	Dibenzo(cd,jk)pyrene (anthanthrene) (dibenzo(def,mno)chrysene (AT)	—	E, F, S
	Dibenzo(cd,jk)pyrene-6,12-dione	?	E
	Dibenzo(e,l)pyrene (DBelP)	+	E
	Tribenzo(a,e,i)pyrene (dibenzo(b,rst)pentaphene (TBP)	+	E
Chrysene	Chrysene (1,2-benzophenanthrene) (CH)	+	E, F, S
	Alkyl chrysene	?	E
	Naphtha(1,2,3,4 def)chrysene	?	E
	Benzo(b)chrysene (BbCH)	?	F
	Benzo(g)chrysene	?	E
	Methylchrysene	?	S
Perylene	Perylene (PR)	—	E, F, S
	Benzo(ghi)perylene (BghiPR)	—	E, F, S
	Dibenzo(b,pqr)perylene	?	E
	1,12-Benzperylene (BPR)	—	E

06/05/2001 07:43 5054243202 JILL PODOLSKY, CHA PAGE 04

Table I. (*continued*)

Group	Compound[b]	Carcinogenic activity[c]	Occurrence[d]
Other	Acenaphthene (ACP)	?	E, F
	Acenaphthalene (ACT)	?	F
	Acenaphthylene (AN)	?	E, F
	Alkylnaphthalenes	?	E
	Anthanthrene	—	E
	Benzo(e)acephenanthrylene	?	E
	Benzo(k)acephenanthrylene	?	E
	Benzo(a)pyrnequinone	?	E
	Coronene (CR)	—	E
	Dibenzofuran	?	E
	Dibenzothiophene	?	E
	Diphenylenoxide	?	F
	1-Methylnaphthalene	?	F
	2-Methylnaphthalene	?	E, F
	3-Methylnaphthalene	?	S
	Naphthalene (NA)	?	E, F
	Naphtho(2,1,8,7-klmn)xanthene	?	E
	Phenalen-1-one	?	E
	Triphenylene (TP)	—	S
	Xanthene-9-one	?	E

[a] Sources: GUNTHER *et al.* (1969), TILGNER and DAUN (1969), ADELMAN and SUESS (1970), *U.S. NAS* (1972), and *U.S. EPA* (1975).
[b] Commonly used other names or abbreviations are in parentheses.
[c] +++ or ++ = strongly carcinogenic, + = carcinogenic, ± = uncertain or weakly carcinogenic, — = not carcinogenic, and ? = unknown.
[d] E = water, air, tobacco smoke, and gasoline or diesel exhaust; F = foods; and S = curing smoke.
[e] Old Richter nomenclature.

The PAH found in foods, apart from minute amounts of geochemical or biosynthetic origin, are formed during the pyrolysis of organic matter (Fig. 1.) (BADGER 1962). During this series of reactions free radicals of one, two, or more carbon atoms combine rapidly and form PAH molecules.

Table II lists some of the chemical properties of various carcinogenic PAH, reviewed by *IARC* (1973). These data show that the solubility of PAH in water is extremely low, whereas most are very soluble in organic solvents. Their solubility in water can be increased considerably in the presence of other organic compounds. This, and other characteristics

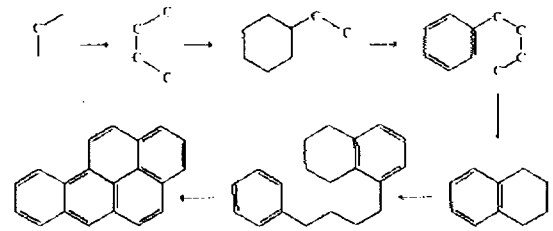


Figure 1. Mechanism of benzo(a)pyrene formation (BADGER 1962).

such as sorption on surfaces and micelle formation by surface-active agents, are probably the relevant phenomena in relation to the appearance of PAH in environmental waters and in foods (ANDELMAN and SUESS 1970). Other properties including absorption spectra, volatility, stability, and chemical reactivity of these compounds are also available in the literature (*IARC* 1973).

Table II. *Properties of some PAH* (IARC 1973).[a]

Compound	M.W.	bp (°C)	mp (°C)	Solubility: Water (mg/L)	Solubility: Organic solvents
BaA	228	450	160	0.011	s(most o.s.)
BbFL	252	—	168	—	—
BjFL	252	195	165	—	—
BaP	252	310	176	0.012	s(bz,to,xyl); S(al,me)
BeP	252	492	178	0.075	—
CH	228	448	255	—	S(al,to,bz,xyl)
DBahA	278	—	266	0.0005	s(most o.s.); S(al,eth)
TBP	352	—	320	—	—
DBaeP	302	—	241	—	s(most o.s.)
DBahP	302	—	308	—	—
DBaiP	302	—	162	—	S(g.a., bz)
DBalP	302	—	162	—	—
IP	276	—	162	—	—

[a] Abbreviations:
s = soluble
S = slightly soluble
al = ethyl alcohol
to = toluene
bz = benzene
xyl = xylene
me = methanol
eth = ether
g.a. = glacial acetic acid
o.s. = organic solvents

06/05/2001 07:43 5054242292 JILL PODOLSKY, PHA PAGE 05

III. Origin in foods

a) Natural occurrence

In 1947 PAH were found to be present in marine and other sediments (KERN 1947). Later, several were found in concentrations ranging from 100 to 1,000 ppb in forest soils far from human habitation, and also in river sediments (BINET and MALLET 1963, MALLET 1966). Some 12 PAH were identified in rural soils in the eastern U.S., the concentration of BaP being 40 to 1,200 ppb (BLUMER 1961). A recent study suggests that such PAH are formed mainly in forest and prairie fires and deposited in surface sediments (BLUMER and YOUNGBLOOD 1975).

Another natural source of PAH is the endogenous formation by plants or other organisms. Evidence of this was provided by the appearance of BaP at a level of ten to 20 ppb dried material in hydroponically grown wheat and rye seedlings (GRAF 1965, MALLER and HEROSS 1962). Also, bacteria can synthetize BaP (KNORR and SCHENK 1968), and the fresh water algae *Chlorella vulgaris* was reported to produce several types of PAH (BORNEFF *et al.* 1968).

b) Polluted environments

Man-made emission of PAH, measured as BaP, injected into the atmosphere in the U.S. alone, was estimated to be about 1,320 tons/year, of which the main contributors are heat and power generation 500 tons, refuse burning 600 tons, coke production 200 tons and motor vehicles 20 tons (*U.S. NAS* 1972). The deposition of such vast quantities is bound to contribute to the PAH content of foods. Various studies show that this is indeed the case. About ten % of BaP found in lettuce, kale, spinach, leeks, and tomatoes can be removed by cold water washing, an indication that it was originally deposited externally (GRIMMER 1966). Plants with the smallest surface area exposed to the atmosphere, such as tomatoes, contained the least amount of BaP. In culture studies DORR (1965) found that BaP in the soil can be absorbed by barley roots and translocated to the shoots.

Oysters collected from moderately polluted waters (petroleum oil contamination) contained two to six ppb BaP (CAHNMANN and KURATSUNE 1957). Clams in petroleum-contaminated areas may contain BaP and benzo(ghi)perylene (BghiPR) in concentrations of up to 16 and 25 ppb, compared to 1.5 and 4.5 ppb in those obtained from "unpolluted" areas (GUERRERO *et al.* 1976).

c) Food additives and food packaging

Of more than 200 refined petroleum wax samples used to treat packaging materials ("dairy wax") analyzed, only six contained PAH: two at $\geqslant 0.01$ ppm and four at $\geqslant 0.1$ ppm levels, but none of the PAH identified were established carcinogens (SHUBIK *et al.* 1962). Another set of

data on seven commercial wax samples showed 13 to 240 ppb PAH, mainly benzo(e)pyrene (BeP), pyrene (P), fluoranthrene (FL), and chrysene (CH) (HOWARD and HAENNI 1963).

Hexane, a common solvent used to extract edible vegetable oils, has also been identified as a possible source of PAH contamination. Anthracene (A), benzanthracene (BaA), FL, BghiPR, and P were reported in hexane at levels ranging from 0.4 to 280 ppb, and one sample contained BaP and BaA in amounts of 21 and 280 ppb, respectively (LIJINSKY and RAHA 1961). The same study revealed also the presence of BeP, BghiPR, and P in other solvents, including toluene, isooctane, and benzene.

In a survey of hexane samples collected in 11 plants in the U.S. no carcinogenic PAH were found in any of the 15 samples examined, but up to 35 ppb were found of P, FL, A, and phenanthrene (PA), as well as substituted PA. These data are shown in Table III (HOWARD *et al.* 1968 a).

Table III. *PAH found in hexane solvents used in the extraction of vegetable oils* (HOWARD *et al.* 1968 a).

	Parts per billion*				
Solvent	P	FL	A	PA	Substituted PA
Pure hexane	0.8 (1/5)	0.8 (1/5)	8.1 (2/5)	4.6–35.0 (3/5)	5.3 (2/5)
Pure Skelly B	— (0/1)	— (0/1)	— (0/1)	— (0/1)	— (0/1)
Recycled hexane	0.7–2.6 (2/6)	1.5–2.3 (2/6)	2.4 (1/6)	6.0–14.4 (2/6)	0.6 (1/6)
Recycled Skelly B	— (0/3)	— (0/3)	— (0/3)	— (0/3)	— (0/3)

* Data in parentheses are no. of samples with PAH over no. of samples tested.

A recent survey performed by PANALAKS (1976 b) revealed that food-grade carbon blacks may contain CH, BaA, benzo(k)fluoranthrene (Bk-FL), 3-methylcholanthrene (MCA), and BeP up to 100 ppb.

d) Curing smokes and other pyrolysis products

The PAH of this origin are probably the most important ones because they usually represent the bulk of PAH found in foods. TILGNER and DAUN (1969) in their review identified 24 PAH in this category (Table I). In "liquid smoke" P, FL, BghiPR, CH, and BaA were found by LIJINSKY and SHUBIK (1964 and 1965 a). A, PA, P, FL, triphenylene (TP), and methylbenzo(a)pyrene (MBaP) were identified by WHITE *et al.* (1971); their respective levels were 9, 35, 4, 3, 7 and 2 ppb.

The PAH content of smoke condensates, smoked hams and sausages, and of "liquid smokes" was extensively studied by TOTH and BLAAS (1972 a, b, and c), by TOTH (1969 and 1971), and by FILIPOVIC and TOTH (1971). BaP, P, A, PA, FL, CH, BaA, 3,4-benzofluoranthene (3-4-BFL), perylene (PR), BeP, 11,12-benzofluoranthene (11,12-BFL), and

06/05/2001 07:43 5054243202 JILL PODOLSKY, FHA PAGE 06

1,12-benzoperylene (1,12-BPR) were found in the condensates and products. Washing the smokes by bubbling in cold water and subsequent filtration through glass wool, the quantities of PAH in the treated meat products could be reduced by 30 to 90%.

Besides smoking, the heat treatment of foods during cooking plays also an important part in contributing PAH to the finished product. Carbohydrates and fats seem to be the most important sources of PAH, the quantities produced depending on the temperature and mode of cooking. Starch heated at 370 to 390°C in the absence of air yields 0.7 ppb BaP in its distillation condensate; at 650°C the yield is 17 ppb (DAVIES and WILMSHURST 1960). In a similar study with carbohydrates, amino acids, and fatty acids, MASUDA *et al.* (1967) found no PAH at 300°C, but at 500 to 700°C 19 PAH, including BaP, were detected. By cooking meats under relatively severe conditions the BaP concentration can reach 50 ppb in steaks, and other PAH are formed as well; the authors suggested that their origin is the pyrolysis of fats (LIJINSKY and ROSS 1967).

IV. Analysis

The determination of PAH in foods is very cumbersome and time-consuming. For this reason most early investigators limited their studies to the detection and determination of BaP; only more recently were other PAH also investigated. The entire analytical procedure can generally be divided into two distinct steps: (a) isolation and separation and (b) identification and determination.

a) Isolation and separation

Organic solvents are normally used to extract PAH from food or other products. Further separation is achieved by elution, with solvents of different polarity, from chromatographic columns (HAENNI 1968, TILGNER and DAUN 1969, SOOS 1972). In the case of "liquid smokes" partition between different solvents is the more often used method. This consists of isooctane extraction, successive washes with alkali solution, water, phosphoric acid, and dimethylsulfoxide, followed by column or thin-layer chromatographic separation (WHITE *et al.* 1971). This technique has also been used for the isolation of PAH from commercial solvents employed in the food industry in the production of edible vegetable oils (HOWARD *et al.* 1966 c and 1968 a).

The procedure is more complicated in the case of foods of high fat content, such as smoked meats. To eliminate the interference of the fats an initial extraction is commonly carried out with acetone in a Soxhlet extractor. After evaporation of the solvent the extract is treated with alcoholic potassium hydroxide and the unsaponifiable fraction is dissolved in cyclohexane. This is followed by chromatographic separation with cyclohexane-benzene as eluting solvent (BAILEY and DUNCAL 1958).

Similar methods also were developed by HAENNI *et al.* (1962 a and b), HOWARD and HAENNI (1963), and HOWARD *et al.* (1965). Further improvement made it possible to isolate also BaA, BeP, BghiPR, P, and 4-methylpyrene (4-MP) from smoked foods (HOWARD *et al.* 1966 a). The refined method consists of extraction with ethanol, saponification with potassium hydroxide, further extraction with isooctane, and partition on paper or thin-layer chromatography. A comprehensive multidetection procedure including BaA, BaP, dibenz(a,h)anthracene (DBahA), BghiPR, and a method for BaP in "total diet" composite samples was also developed (HOWARD *et al.* 1968 b).

A more recent method involving liquid-liquid partition systems based on methanol-water-cyclohexane and dimethylformamide-water-cyclohexane systems has the capacity to extract and separate 19 different PAH from high protein foods, vegetable oils, and fats (GRIMMER and BOHNKE 1975).

Several methods are available for the separation of PAH from marine foods. Methanolic extraction followed by methanol-cyclohexane or methanol-benzene-hexane partition was found to be suitable for oysters and barnacles (CAHNMANN and KURATSUNE 1957, ZECHMEISTER and KOE 1952). Aqueous caustic digestion followed by ether-hexane extraction seems also to be effective for the separation of the hydrocarbons from clams (WARNER 1974).

The separation of individual PAH from the hydrocarbons extracted from foods is generally achieved by chromatographic methods, as summarized in Table IV.

b) Identification and determination

Identifications of PAH are mainly based on direct comparison of physical and physico-chemical properties of isolated compounds with those of standard samples. Techniques and instruments frequently employed are listed in Table IV. Ultraviolet absorption spectra can be used for qualitative identification and quantitative determination; fluorescence spectra are mainly used for qualitative confirmation of data arrived at by other methods (TILGNER and DAUN 1969). Recently methods based on gas-chromatography/mass spectrometry and on high-pressure liquid chromatography (HPLC) have also been proposed, but have not been extensively used yet in the area of foods (GRIMMER and BOHNKE 1975, WARNER 1974, GUERRERO *et al.* 1976).

V. Occurrence and concentration in foods

a) Vegetable oils, fats, and shortenings

The review of HOWARD and FAZIO (1969) lists eight PAH (P, BaP, BeP, PA, FL, BaA, CH, and PR) occurring in edible refined oils as determined during 1962–65 by European workers. In the U.S. some soybean,

Table IV. *Techniques for separation, identification, and determination of PAH in foods.*

Chromatography	Elution	Identification[a]	Determination[a]	Compound	Reference
Column (Silica gel)	CH_2Cl/ether	GC/MS		F, PA, MPA	Warner (1974)
Column (Silica gel; Al_2O_3 and Sephadex LH)	Cyclohexane; isopropanol	UV (lamp)	GLC (flame ionization) UV (baseline tech.)	19 PAH[d]; 14 PAH[e]	Grimmer & Böhnke (1975), Grimmer & Hildebrandt (1972)
Column (Florisil), thin-layer (cellulose acetate)	Benzene; DMFA[b]/isooctane-ethanol-toluene-water	UV (spectrum)	UV (baseline tech. and SF[c])	A, PA, P, TP, MBP	Howard *et al.* (1966 c), Howard *et al.* (1968 b), White *et al.* (1971)
Paper (Whatman #1) and thin-layer (cellulose acetate)	Isooctane/DMFA[b]; ethanol-toluene-water	UV (spectrum)	UV (baseline tech. and SF[c])	BaP, BaA, DBahA, BghiPR	Howard *et al.* (1966 a)

[a] GC/MS = gas chromatography/mass spectrometry, GLC = gas-liquid chromatography, and UV = ultraviolet.
[b] DMFA = *N,N*-dimethylformamide.
[c] SF = spectrophotofluormetry.
[d] 19 PAH = PA, A, P, FL, CH, BaA, BaP, BeP, BkFL, PR, AT, BghiPR, DBahA, CR, F, BbFL, BjFL, DBajA, and IP.
[e] 14 PAH = the first 14 PAH listed in the above footnote.

cottonseed, corn, olive, and peanut oils, but not safflower oil, were reported to contain 0.4 to 1.6 ppb of BaP and BeP, and 0.5 to 3.3 ppb BaA, BghiPR, FL, and P (Howard *et al.* 1966 c). Oils being used in deep-frying of foods were analyzed by Lijinsky and Ross (1967) and by Malanoski *et al.* 1968): these contained 0.9 to 1.9 ppb of BaP and six to 12 ppb of FL and P. The relatively high levels of PAH found indicate that the heating such oils were exposed to might have led to a slight increase of PAH concentrations, mainly of the noncarcinogenic types.

In a total diet study Howard *et al.* (1968 b) found only trace amounts, less than 0.5 ppb, of BaP, BkFL, benzo(b)fluoranthene (BbFL), BeP, BghiPR, BaA, and PA in the composite sample containing the fats, oils, and shortenings.

b) Fish and other marine foods

Fish from unpolluted waters usually do not contain detectable amounts of PAH, but smoked or cooked fish contain varying levels. The presence of PAH in smoked fish has been recognized for over two decades: most of the relevant studies were conducted outside North America (Howard and Fazio 1969). In smoked fish BaP was found at levels of two to 50 ppb, the difference was probably due to various parameters such as type of smoke generator, temperature of combustion, and degree of smoking (Draudt 1963). In a later study of Thorsteinsson (1969) on commercially smoked fish from Iceland, the BaP was found to be only at a level up to one ppb in the edible portion (see Table V).

Table V. *PAH in commercially-smoked fish from Iceland* (Thorsteinsson 1969).

Fish	No. of analyses	Parts per billion[a]								
		P	ACT	A	BeP	BaP	FL	BaA	F	PA
Eel	2	6	t	4	t	1	4	—	9	37
Lumpfish	2	1	t	t	t	0	2	t	5	10
Trout	3	5	4	26	t	t	12	—	67	52

[a] In edible portion; t = trace.

In North America Lijinsky and Shubik (1965 b) found the following amounts, respectively, in smoked salmon and haddock: BaA zero and 0.2 ppb, BaP 1.0 and 0.3 ppb, BeP zero and zero ppb, BghiPR 1.5 and 0.2 ppb, fluorene (F) 6.0 and 1.1 ppb, and P 3.0 and 0.6 ppb.

In an FDA-sponsored study PAH levels in unsmoked and smoked fish were compared (Table VI) (Howard *et al.* 1966 a and b). As a consequence of this, a further study was initiated by FDA-USDA in which 60 different foods, including nine smoked fish samples, were analyzed. The results showed (Table VII) that BaP is present at levels up to seven ppb but only trace amounts of F and P were found (Malanoski *et al.* 1968).

Four different types of smoked fish products were investigated in

05/05/2001 07:43 5054242202 JILL PODOLSKY, PHA PAGE 08

Table VI. *PAH found in smoked and nonsmoked fish* (HOWARD *et al.* 1966 a and b).

Fish	Parts per billion					
	BaA	BaP	BeP	BghiPR	F	P
Smoked						
Herring	—	—	—	—	3.0	2.2
Herring (dried)	1.7	1.0	1.2	1.0	1.8	1.8
Salmon	0.5	—	0.4	—	3.2	2.0
Sturgeon	—	0.8	—	—	2.4	4.4
White	—	—	—	—	4.6	4.0
Nonsmoked						
Haddock	—	—	—	—	1.6	0.8
Herring (salted)	—	—	—	—	0.8	1.0
Salmon (canned)	—	—	—	—	1.8	1.4

Table VII. *PAH found in smoked fish* (MALANOSKI *et al.* 1968).

Fish	Parts per billion[a]							
	BaA	BaP	BeP	BghiPR	F	P	CH	PR
Chubs	ND	1.3 (1/3)	ND	ND	ND	ND	ND	ND
Salmon	ND	— (0/1)	ND	ND	ND	ND	ND	ND
Whitefish	—	6.6 (2/4)	—	2.4	—	0.5	—	0.4
Kippered Cod	—	4.5 (1/1)	—	2.2	—	0.6	1.4	0.7

[a] Data given in parentheses are no. of samples with BaP over no. of samples examined; ND = not determined.

Japan by MASUDA *et al.* (1966 a) and MASUDA and KURATSUNE (1971). Several PAH were found in all samples, sometimes in extremely high levels (Table VIII).

In addition to fish, various other marine organisms were investigated and found to contain PAH. For example, barnacles were noted to contain A, PA, BaP, CH, FL, and coronene (CR) (KOE and ZECHMEISTER 1952, ZECHMEISTER and KOE 1952). Oysters collected from moderately contaminated areas were shown to contain BghiPR one to five ppb, BaP two to six ppb, BaA ten ppb, BkFL eight to 14 ppb, BeP 20 ppb, CH 20 to 40 ppb, P 100 to 160 ppb, and FL 600 to 1,000 ppb (CAHNMANN and KURATSUNE 1957).

c) Meat and meat products

Whereas raw meat does not normally contain PAH, smoked or cooked meat may contain varying amounts. Steaks prepared "well done" on a "standard" charcoal broiler contained 15 different PAH, and so did steaks and ribs barbecued in a restaurant (LIJINSKY and SHUBIK 1964 and 1965 b). The quantitative data from this study are summarized in Table IX. The same authors could not detect more than occasional trace amounts of PAH in untreated bacon.

Table VIII. *PAH in smoked and/or broiled Japanese fish*[a] (MASUDA *et al.* 1966 a, MASUDA and KURATSUNE 1971).

Compound	Parts per billion						
				Japanese horse mackerel[d]			
				Electric broiler		Gas broiler	
	Katsuo-bushi[b]	Saba-bushi[c]	Urume-bushi[c]	Smoke	Scorch	Smoke	Scorch
NA	–	—	—	—	—	167	—
AN	77– 392	160	45	—	—	22	—
F	205– 210	130	122	2.6	—	8.2	3.2
A	138– 435	115	23	1.9	0.2	2.3	2.0
PA	584–1,650	610	206	9.0	1.0	11.0	8.0
P	318–1,020	170	70	3.6	0.2	4.0	7.0
FL	320– 896	200	71	5.2	0.2	3.6	7.0
BaF	40– 90	21	5	1.0	—	0.6	1.7
BaA	55– 59	34	6	1.2	—	0.6	2.9
CH	42– 173	36	10	4.3	—	0.4	2.1
PR	1– 6	1	t[e]	t[e]	—	t[e]	0.2
BaP	9– 37	7	2	0.2	—	0.3	0.9
BeP	7– 29	7	3	0.5	—	0.2	1.2
BbFL	12– 37	7	4	0.2	—	0.1	1.2
BjFL	5– 23	5	2	0.5	—	t[e]	0.5
BkFL	3– 12	2	1	0.2	—	—	0.2
BghiPR	2– 10	2	1	0.2	—	0.3	2.2
CR	–	—	—	—	—	t[e]	t[e]

[a] Fresh Horse mackerel contained no PAH.
[b] Made from *Katsuwonus vagans* (*Euthynnus pelamis*) = bonito.
[c] Made from *Scomber japonicus* or *Saronnia melanosticta*.
[d] *Trachurus japonicus*.
[e] t = trace.

Table IX. *PAH found in charcoal broiled steaks and barbecued ribs* (LIJINSKY and SHUBIK 1964 and 1965 b).

Compound	Parts per billion		
	Charcoal broiled steaks		Barbecued ribs
	Lab. prep.	Commercial	Commercial
AT	2	0.6	1.1
A	4.5	—	7.1
BaA	4.5	1.4	3.6
Alkyl-BaA	2.4	—	—
BbCH	0.5	—	—
BghiPR	4.5	6.7	4.7
BaP	8	5.8	10.5
BeP	6	5.5	7.5
CH	1.4	0.6	2.2
CR	2.3	3.2	4.2
DBahA	0.2	—	—
FL	20	43	49
PA	11	21	58
P	18	0.9	1.5
PR	2	35	42

06/05/2001 07:43 5054243292 JILL PODOLSKY, FDA PAGE 09

Table X. *PAH concentration in laboratory cooked meats* (LIJINSKY and ROSS 1967).

Food	Parts per billion								
	BaA	BaP	BeP	BghiPR	F	P	BaF	CH	CR
Charcoal broiled									
Hamburger, fat,[b] hot[a]	2.7	2.6	—	14.9	13.3	7.7	—	1.7	1.0
Hamburger, fat,[b] cool[a]	—	—	—	—	6.4	3.9	—	t[c]	—
Hamburger, lean,[b] hot[a]	—	—	—	0.9	0.3	1.6	—	—	—
Hamburger, lean,[b] cool[a]	—	—	—	—	1.3	0.6	—	0.3	—
Hamburger, no-drip pan	—	—	—	t[c]	0.2	0.1	—	t[c]	—
Hamburger, (frozen), hot[a]	4.5	11.2	4.6	10.7	4.9	7.0	—	3.2	3.0
Pork Chop, hot[a]	8.2	7.9	5.9	1.3	22.5	24.2	—	6.3	—
Chicken, hot[a]	3.2	3.7	3.4	0.8	1.1	0.9	—	2.2	—
Sirloin steak, hot[a]	10.3	11.1	6.4	24.3	12.6	9.0	4.3	9.3	4.0
T-bone steak, hot[a]	31.0	50.4	17.6	12.4	19.8	19.1	5.7	25.4	8.0
Flame broiled									
T-bone steak, hot[a]	3.9	4.4	5.7	6.2	19.0	20.0	—	2.0	9.0

[a] Cool = 25 cm from heat source, hot = seven cm from heat source.
[b] Fat = 21% fat, lean = seven % fat.
[c] t = trace.

Further investigations have shown that the presence of PAH in broiled meats and especially the presence of the carcinogenic PAH is directly proportional to the temperature of the treatment. Broiling at high temperature (seven cm from heat source) led to the production of BaA, BaP, or BeP, indicating that most probably the pyrolysis of fats is the origin of these PAH (Table X) (LIJINSKY and ROSS 1967).

Other than pyrolysis of fats, incomplete combustion of the charcoal can also contribute to the PAH content of the meat. When T-bone steak was charcoal-broiled, its PAH content increased two- to ten-fold compared to a similar product which was flame broiled (see Table X). THORSTEINSSON and THORDARSON (1968) reported that when sheep and seabirds were singed with coal both BeP and BaP were found in them in concentrations well above detection limits, but if propane was used, neither of them was detected.

European samples of smoked sausages and smoked mutton were reported to contain 0.3 to 50 ppb BaP (HOWARD and FAZIO 1969), but in North America, except for smoked ham, most smoked meats contained much less carcinogenic PAH (HOWARD *et al.* 1966 a and b) (Table XI).

Table XI. *PAH in some smoked and nonsmoked foods* (HOWARD *et al.* 1966 a and b).

Foods	Parts per billion					
	BaA	BaP	BeP	BghiPR	F	P
Smoked						
Beef (chipped)	0.4	—	—	—	0.6	0.5
Cheese (Gouda)	—	—	—	—	2.8	2.6
Ham[a]	2.8	3.2	1.2	1.4	14.0	11.2
Pork (roll)	—	—	—	—	3.1	2.5
Frankfurters	—	—	—	—	6.4	3.8
Nonsmoked						
Cheese (Cheddar)	—	—	—	—	0.8	0.7

[a] Additional PAH determined: methylchrysene (0.5) and 4-MP (1.9).

In a follow-up examination performed by FDA-USDA on 38 smoked meat products, seven were found to contain up to 3.5 ppb BaP; some samples also contained other PAH (Table XII; MALANOSKI *et al.* 1968).

Table XII. *PAH in some smoked meats* (MALANOSKI *et al.* 1968).

Meat	Parts per billion						
	BaA	BaP	BeP	BghiPR	F	P	CH
Ham	1.3–9.6	0.7	—	—	0.6–2.9	0.2–0.9	0.5–2.6
Barbecued beef	13.2	3.5	1.7	4.3	2.0	3.2	9.6
Hot sausage[a]	0.5	0.4	—	—	—	1.5	1.0

[a] Additional PAH determined: 4-MP, 2.0 ppb.

In Iceland, home and commercially smoked meats were compared by THORSTEINSSON (1969). While very little BaP was detected in smoked sausage and mutton, considerable amounts of BaP and other PAH were found in home-smoked mutton and lamb, either with or without being covered with cellophane or cotton fabric (Table XIII). About 60 to 75% of the BaP was found to be in the superficial layer of the meat. This low penetration has also been noted by RHEE and BRATZLER (1970), who observed that in smoked bologna sausage the BaP is located within 1.5 mm from the surface. Cellulose casing is more effective as a barrier to the passage of PAH than animal casing (SIMON *et al.* 1969).

06/05/2001 07:43 5054243202 JILL PODOLSKY, FHA PAGE 10

Table XIII. *PAH in commercially and home smoked foods* (THORSTEINSSON 1969).

Food	Parts per billion[a]								
	P	ACT	A	BeP	BaP	FL	BaA	F	PA
Commercially smoked									
Mutton	8	5	13	5	t[c]	18	2	117	104
Mutton sausages	2	t[c]	2	t[c]	t[c]	6	0.5	10	17
Home smoked									
Close to stove without cover	402	253	388	27	107	303	115	2,024	873
Close to stove with cover[b]	34	27	41	1	3	35	3	215	80
Distance from stove without cover	68	14	70	2	21	47	5	280	88
Home-smoked lamb	127	29	162	4	23	158	115	811	443

[a] In edible portion.
[b] Cotton fabric or cellophane.
[c] t = trace.

Ten different brands of bacon and ham were examined in Germany by TOTH (1971). Seven of them contained BaP at levels ranging from 0.2 to 1.6 ppb. The other three heavily smoked products contained 4.0 to 14 ppb and, in addition, in these three samples many other PAH were also present. In a further study Yugoslav sausages were analyzed by FILIPOVIC and TOTH (1971) both with their casings and with their casings removed. Data from these studies, and additional data from TOTH and BLASS (1972 a) are summarized in Table XIV.

FRETHEIN (1976) analyzed several Norwegian smoked meat products: these contained lower PAH levels as a result of the lighter smoking methods of this country (Table XV).

d) Fruit and other plant products

Various European workers have reported the presence of PAH in fruits and related products, as summarized in Table XVI (*IARC* 1973). No similar study was reported from North America.

KURATSUNE and HUEPER (1958 and 1960) published PAH data on roasted coffee and coffee soot (Table XVII). Carcinogenic PAH, particularly BaP and BeP, may vary from zero to 0.3 ppb in roasted coffee.

In Japan MASUDA *et al.* (1966 a and b) analyzed whiskey, roasted barley, shoju (soy sauce), and caramel. Only whiskey and roasted barley contained some PAH, at very low levels.

SOOS (1974) determined a number of carcinogenic PAH in Hungarian grain, collected in unpolluted and industrially polluted areas. Wheat, barley, and rye samples were analyzed and P, FL, BaA, CH, BaP, BeP, DBahA, and BPR were found in the samples, in low concentrations. The

Table XIV. *PAH content of smoked meat products from Germany and Yugoslavia* (FILIPOVIC and TOTH 1971, TOTH and BLASS 1972 a).[a]

Compound	Sausage				Salami	Mortadella	Ham			Bacon
	Cold smoked		Hot smoked							
	With casing	Casing removed	With casing	Casing removed	Hot smoked, casing removed	Hot smoked, casing removed	Light smoked ham	Heavily smoked ham	Heavily smoked bacon
A	25.4	2.4	10.6	5.6	0.7	2.8	2–3	12–57	20
P	34.2	5.8	21.8	13.0	5.2	15.0	2–11	35–161	27
FL	40.0	7.2	35.2	13.0	5.6	22.0	4–14	48–156	35
CH	9.2	2.4	4.4	2.8	1.2	3.4	0–3	12–66	Q
BaA	9.0	0.8	6.4	2.2	0.6	2.8	0.5–3.0	6–33	29
3,4-BP	2.9	0.5	0.7	1.1	0.8	0.7	3–4	3.8–55	3.6
3,4-BFL	3.5	0.7	1.3	1.1	Q	1.8	0–3	6–33	3.6
PR	0.5	Q	0.1	Q	3.2	0.1	0–0.9	0.7–13	0.9
1,2-BP	Q	Q	4.4	0.9	0.2	1.8	0–7	4–26	Q
1,12-BPR	Q	Q	0.3	0.1	Q	0.4	0–1.4	2.5–25	3.0
11,12-BFL	Q	Q	Q	Q	Q	Q	Q	Q	Q
MP	Q	Q	Q	Q	Q	Q	Q	Q	Q
MFL	Q	Q	Q	Q	Q	Q	0–15	Q	Q
PA	188	Q	Q	Q	Q	Q	Q	Q	Q

[a] Q = qualitatively detected; values in ppb.

06/05/2001 07:43 5054242202 JILL PODOLSKY, PHA PAGE 11

Table XV. *Maximum amounts of PAH in smoked sausage produced in Norway* (FRETHEIM 1976).

Compound	Parts per billion	
	Fermented and dried	Cooked bologna
BcPA	0.25–2.15	0.15–0.70
BaA	0.05–0.30	0.04–0.55
CH and TP	0.07–1.95	0.15–1.20
BkFL and DMBaA	0.05–1.35	0.05–2.15
BaP	0.04–0.15	0.04–0.08
PR	0.04–0.15	0.04–0.07
MCA	0.04–0.15	0.04–0.04
DBahA	0.07–0.60	0.04–1.10
Picene	0.20–1.70	0.05–6.75
BghiPR	0.25–0.50	0.04–0.20

Table XVI. *PAH in fruit and plant products* (IARC 1973).

Food	Parts per billion					
	BaA	BbFL	BaP	BeP	CH	DBahA
Spinach	16	—	7.4	6.9	28	0.3
Salad	4.6–15.4	—	2.8–5.3	3.7–14.7	5.7–26.5	0.6–1.0
Kale	43–230	—	12.6–48.1	1.1–67.2	58–395	0.1–2.6
Soybean	—	—	3.1	4.3	—	—
Apples	—	—	0.1–0.5	—	—	—
Tomatoes	0.3	—	0.2	0.2	0.5	0.04
Other fruits	—	—	2–8	—	—	—
Yeast (dried)	2.5–93	—	1.8–40.4	3.1–55	42–203	0.9
Tea	—	—	3.9–21.3	—	—	—
Whisky[a]	0.04–0.08	0.05	0.04	0.03	0.04–0.06	—
Prunes (dried)	—	—	0.2–1.5	—	—	—
Coffee (malt or heavily roasted)	43[b]	1.2–3.0	15	20.5	—	—
Cereals	0.4–6.8	—	0.2–4.1	0.3–4.9	0.8–14	0.1–0.6
Tea extracts	—	—	—	1.9–22	4.6–6	—
Roast peanuts	0.9	—	—	0.4	0.01–0.7	—

[a] One out of 15 samples.
[b] Up to this value.

data showed that industrial pollution is the probable source of these PAH.

e) Other data

Recently some data are available for various foods collected from the Canadian market (Tables XVIII and XIX, PANALAKS 1976 a and b). With the exception of smoked marine foods and charcoal-broiled meat products, which exceeded 20 ppb BaA, BaP, or BeP, the other samples were very low in BaP or other carcinogenic PAH.

Table XVII. *PAH in coffee and coffee soots* (KURATSUNE and HUEPER 1958 and 1960).

Compound	Parts per billion		
	Coffee soots	Roasted coffee	
		Moderate dark	Darkest
PA	130–300	–	–
P	260–720	2–8	0–17
FL	340–1000	1–7	0–15
CH	530–670	0	0–0.5
BaA	16–150	0	0–2
PR	280–660	0	0–0.8
BeP	190–370	0	0–3
BaP	200–400	0	0–4
BkFL	70–140	0	0–0.8
BghiPR	100–140	0	0–4

VI. Metabolism

a) Absorption, distribution, and excretion

The absorption, distribution, and excretion of PAH in experimental animals have been studied extensively (*U.S. NAS* 1972). However, in most of the studies the test compounds were administered by parenteral routes (i.v., i.m., or i.p. injection) and the results may not have much relevance in the evaluation of orally ingested PAH.

The only available recent information in this field seems to be that of DANIEL *et al.* (1967): radioactive DBahA, MCA, 7,12-dimethylbenz(a) anthracene (DMBaA), and dibenz(a,c)anthracene (DBacA) were given orally to rats. Most of the radioactivity was rapidly absorbed and excreted through biliary excretion in the feces and in urine. Some of the absorbed material was retained in the adrenals, the ovaries, and in the body fat and could be detected after eight days. None was detected in brain lipids.

b) Degradation

Since the discovery of quinones and of 4-hydroxybenzanthracene (4-OH-BA) as metabolites, respectively, of BaP and BaA (BERENBLUM and SCHOENTAL 1943 a and b, BERENBLUM *et al.* 1943) many studies of PAH metabolism have been made in intact animals, in tissue cultures, or in *in vitro* systems.

HEIDELBERGER *et al.* (1953) identified three quinones in the liver of mice treated with DBahA. These were 1,2,5,6-dibenz-9,10-anthraquinone (DB-9,10-AQ), 1,2,5,6-dibenz-3,4-anthraquinone (DB-3,4-AQ), and dihydro-1,2,5,6-dibenz-9,10-anthraquinone (diOH-DB-9,10-AQ). HARPER (1959 b) administered BaA to rabbits and detected 2-hydroxybenzanthracene (2-OH-BA) and 4-OH-BA excreted with the feces. BOYLAND and

Table XVIII. *PAH found in smoked and charcoal broiled food products* (PANALAKS 1976 a).

Foods	No.[a] analyzed	Parts per billion										
		BbFL	BaA	BeP	BaP	DBahP	IP	DBajA	CR	BghiPR	DMBA	PR
Smoked												
Bologna	2/5	—	—	5.0	2.0	0.5	—	—	—	—	—	—
Frankfurters	2/4	—	1.5	—	2.0	—	—	—	2.0	—	—	—
Salami	1/3	—	1.5	—	2.0	—	—	—	2.0	—	—	—
Pepperoni	1/2	—	0.2	2.0	—	—	1.0	—	—	—	—	—
Various sausages	1/15	—	0.5	2.5	1.0	8.0	1.0	—	—	—	—	—
Ham	3/3	—	0.2	0.2	2.0	1.0	0.5	—	—	—	—	—
Wesphalian ham	1/1	—	—	5.0	2.0	—	—	—	—	—	—	—
Bacon	4/5	—	0.5	—	0.5	1.0	—	0.6	4.0	—	—	—
Smoked beef	2/3	—	8.0	—	—	—	0.5	—	—	—	—	—
Smoked pork	2/2	—	—	—	0.3–0.2	—	—	—	—	—	—	—
Smoked herrings	7/7	—	20.0	2.0	15.0	—	0.2–9.0	—	8.0	—	—	—
Various smoked fish	5/7	—	1.0	1.5	0.5	5.0	—	—	10.0	—	—	—
Canned smoked fish	1/6	—	—	1.0	—	—	—	—	—	—	—	1.0
Gouda cheese	1/1	—	—	—	0.5	—	—	—	—	—	—	—
Canned (smoked) oysters	5/5	25.0	30.0	16.0	2.0	—	5.0	1.0	—	4.0	38.0	20.0
Canned (nonsmoked) oysters	2/2	—	30.0–15.0	1.0	—	—	2.0	—	—	—	25.0	—
Charcoal-broiled												
Porterhouse steak	1/1	—	1.0	4.0	3.0	—	—	—	—	—	—	2.0
Barbecued chicken	1/1	—	—	2.0	—	—	10.0	8.0	—	15.0	2.0	2.0
Hamburger	1/1	30.0	50.0	20.0	20.0	—	—	—	—	—	10.0	—
Frankfurter	1/1	2.0	2.0	5.0	5.0	—	—	—	—	—	—	—
Chinese sausages	1/1	30.0	40.0	—	25.0	—	—	—	—	—	—	20.0

[a] No. of samples with PAH over no. of samples tested.

Table XIX. *PAH found in "liquid smokes," fish oils, and coconut oil* (PANALAKS 1976 b).

Foods	Parts per billion[a]								
	BaA	BaP	BeP	BbFL	CH	DBajA	MCA	IP	BghiPR
Liquid smokes	—	10(1/8)	10(1/8)	10(2/8)	8(1/8)	—	—	—	—
Hydrogenated flat fish oil	—	—	8	5	—	4	—	—	—
Hydrogenated anchovy oil	—	—	8	—	—	—	—	—	—
Cod liver oil	100	—	50	80	80	—	—	—	—
Cod liver oil (high test)	80	—	20	—	—	—	10	20	20
Halibut liver oil	40	20	25	20	—	—	5	20	20
Coconut oil	5–80(3/3)	8–10(3/3)	8–20(2/3)	60(1/3)	—	—	5	5–8	20

[a] Data in parentheses are no. of samples with PAH over no. of samples tested.

06/05/2001 07:43 5054243202 JILL PODOLSKY, FHA PAGE 12

06/05/2001 07:43 5054243202 JILL PODOLSKY, FHA PAGE 13

SIMS (1964 b) also injected BaA to rats, rabbits, and mice. No 2-OH-BA was detected, but one conjugated derivative [*N*-acetyl-*S*-(5,6-dihydro-6-hydroxy-5-benzanthracenyl)-4-cysteine] plus four phenols and four dihydrodiols, all as sulfuric and glucuronic acid conjugates, were found in the urine, bile, and feces of the treated animals. The eight metabolites found were:

5,6-dihydro-5,6-dihydroxybenzanthracene (BA-5,6-diol)
8,9-dihydro-8,9-dihydroxybenzanthracene (BA-8,9-diol)
10,11-dihydro-10,11-dihydroxybenzanthracene (BA-10,11-diol)
3,4-dihydro-3,4-dihydroxybenzanthracene (BA-3,4-diol)
3-hydroxybenzanthracene (3-OH-BA)
4-hydroxybenzanthracene (4-OH-BA)
8-hydroxybenzanthracene (8-OH-BA)
9-hydroxybenzanthracene (9-OH-BA)

FALK *et al.* (1962) injected radioactive BaP into rats and then collected bile, from a biliary fistula, for 48 hr. Within six hr 70 to 80% of the radioactivity was excreted and most of the metabolic products fell into two main categories: the primary oxidation products 3-hydroxybenzo(a)pyrene (3-OH-BP) and 6-hydroxybenzo(a)pyrene (6-OH-BP), and the secondary oxidation products 1,6-dihydro-1,6-dihydroxybenzo(a)pyrene (BP-1,6-diol), and 3,6-dihydro-3,6-dihydroxybenzo(a)pyrene (BP-3,6-diol). All these compounds were found to be conjugated with sulfuric or glucuronic acids.

The metabolism of noncarcinogenic PAH was also studied by some authors. Dihydrodiols, phenols, and quinones have been found to be the most common metabolites of PA, P, CH, and A in animal studies, as summarized in Table XX. (BERENBLUM and SCHOENTAL 1949, BOYLAND

Table XX. *PAH metabolites found in rat and mouse studies.*[a]

PAH	Metabolites		
	Group	*In vitro*	*In vivo*
A	Dihydrodiols	1,2-diH-1,2-diOH-A	1,2-diH-1,2-diOH-A
		—	9,10-diH-9,10-diOH-A
	Phenols	—	9,10-diOH-A
		—	2,9,10-triOH-A
	Quinones	—	A-9,10-dione
PA	Dihydrodiols	1,2-diH-1,2-diOH-PA[b]	1,2-diH-1,2-diOH-PA
		3,4-diH-3,4-diOH-PA	3,4-diH-3,4-diOH-PA
		9,10-diH-9,10-diOH-PA[b]	9,10-diH-3,4-diOH-PA
	Phenols	1-OH-PA	1-OH-PA
		2-OH-PA	2-OH-PA
		3-OH-PA	3-OH-PA
		4-OH-PA	4-OH-PA
BaA	Dihydrodiols	5,6-diH-5,6-diOH-BA[b,c]	5,6-diH-5,6-diOH-BA
		7,8-diH-7,8-diOH-BA[b]	3,4-diH-3,4-diOH-BA
		8,9-diH-8,9-diOH-BA	8,9-diH-8,9-diOH-BA
			10,11-diH-10,11-diOH-BA

Table XX. (*continued*)

PAH	Metabolites		
	Group	*In vitro*	*In vivo*
	Phenols	2-OH-BA	—
		3-OH-BA	3-OH-BA
		4-OH-BA	4-OH-BA
		—	8-OH-BA
		—	9-OH-BA
	Quinones	BA-7,12-dione	BA-7,12-dione
P	Dihydrodiols	4,5-diH-4,5-diOH-P[c]	4,5-diH-4,5-diOH-P
		1,6-diH-1,8-diOH-P[b]	—
	Phenols	1-OH-P	1-OH-P
		—	1,6-diOH-P
		—	1,8-diOH-P
	Quinones	—	P-1,6-dione
DBahA	Dihydrodiols	1,2-diH-1,2-diOH-DBA	—
		3,4-diH-3,4-diOH-DBA[c]	—
		5,6-diH-5,6-diOH-DBA[b,c]	—
	Phenols	3-OH-DBA	—
		4-OH-DBA	—
	Quinones	—	DBA-9,10-dione
		—	DBA-3,4-dione
		—	4,8-diOH-DBA-9,10-dione
DBacA	Phenols	p.m.	—
MBaA	Dihydrodiols	5,6-diH-5,6-diOH-MBA[c]	—
		8,9-diH-8,9-diOH-MBA[b]	—
	Phenols	p.m.	—
	Derivatives	7-HM[b]	
DMBaA	Dihydrodiols	8,9-diH-8,9-diOH-DMBA[b]	—
		5,6-diH-5,6-diOH-DMBA[c]	—
	Phenols	p.m.	—
	Derivatives	7-HM[b]	—
		12-HM[b]	—
		7,12-diHM	—
		other HM	—
MCA	Dihydrodiols	11,12-diH-11,12-diOH-MCA[b]	—
	Phenols	1-OH-MCA[b]	—
		2-OH-MCA[b]	—
		1,2-diOH-MCA	—
	Derivatives	3-HM	
BaP	Dihydrodiols	1,2-diH-1,2-diOH-BP	—
		9,10-diH-9,10-diOH-BP	—
		4,5-diH-4,5-diOH-BP[d]	—
		7,8-diH-7,8-diOH-BP[d]	—
		9,10-diH-9,10-diOH-BP[d]	—
		—	1,6-diH-1,6-diOH-BP
		—	3,6-diH-3,6-diOH-BP
	Phenols	1-OH-BP[d]	1-OH-BP
		3-OH-BP	3-OH-BP
		7-OH-BP[d]	—
		9-OH-BP	—
		—	6-OH-BP
		—	1,6-diOH-BP
		—	3,6-diOH-BP

06/05/2001 07:43 5054243292 JILL PODOLSKY, FDA PAGE 14

Table XX. (*continued*)

PAH	Group	Metabolites *In vitro*	Metabolites *In vivo*
	Quinones	BP-1,6-dione	—
		BP-3,6-dione	BP-3,6-dione
		BP-6,12-dione[a]	—
BeP	Dihydrodiols	4,5-diH-4,5-diOH-BeP	—
	Phenols	p.m.	—
CH	Dihydrodiols	1,2-diH-1,2-diOH-CH	—
		3,4-diH-3,4-diOH-CH	—
	Phenols	p.m.	3-OH-CH
NA	Dihydrodiols	1,2-diH-1,2-diOH-NA	1,2-diH-1,2-diOH-NA
	Phenols	1-OH-NA	1-OH-NA
		2-OH-NA	2-OH-NA
			1,2-diOH-NA

[a] Sources: BERENBLUM and SCHOENTAL 1949; BOYLAND and WOLF 1950; BOYLAND and SIMS 1962 a and b and 1964 a; HARPER 1959 a; SIMS 1962; BOYLAND and SIMS 1965 a and b and 1967 a; BOYLAND *et al.* 1964; CONNEY 1957; SIMS 1966, 1967 b and c, and 1970 b; KINOSHITA *et al.* 1973; SELKIRK *et al.* 1971; KEYSELL *et al.* 1970; FREUDENTHAL *et al.* 1975; SELKIRK *et al.* 1974 a and b and 1976 a. Abbreviations used to describe metabolites: diH = dihydro, diOH = dihydroxy, triOH = trihydroxy, p.m. = phenolic metabolites (not specified), HM = hydroxymethyl derivatives and diHM = dihydroxymethyl derivatives.

[b] Major metabolites.

[c] K-region dihydrodiols.

[d] Detected by HPLC.

and WOLF 1950, BOYLAND and SIMS 1962 a and b and 1964 a, HARPER 1959 a, SIMS 1962).

The *in vitro* metabolism of a number of PAH was also investigated, in the presence of rat liver homogenates and rat liver microsomes. The data from these studies are presented alongside other ones (*in vivo* and *in vitro* data) in Table XX (BOYLAND and SIMS 1965 a and b and 1967 a, BOYLAND *et al.* 1964, CONNEY 1957, SIMS 1966, 1967 b and c, and 1970 b, KINOSHITA *et al.* 1973, SELKIRK *et al.* 1971, KEYSELL *et al.* 1973).

Except for 7-methylbenz(a)anthracene (MBaA), DMBaA, and MCA, which are convertible into hydroxymethyl derivatives in liver preparations, the rest of the findings indicated that most PAH are similarly metabolized both in intact animals and in liver preparations, giving rise to phenols, quinones, and dihydrodiols. Some of the studies referred to above indicated that the rate of metabolism of the PAH can be increased considerably if the animals are pretreated with various PAH.

Although no studies on whole animals were done with MBaA, DMBaA, and MCA, these compounds have been examined along with BaP, BeP, and DBA in mouse prostate cell cultures (HUBERMAN *et al.* 1971 a), and in mouse embryo cultures (SIMS 1970 a). Results of these studies generally indicated that the metabolic patterns of PAH in cell cultures are

comparable to those in liver preparations, with phenols and dihydrodiols plus hydroxymethyl derivatives as major metabolites; however, in cell cultures most PAH tested also yielded unidentified water-soluble metabolites; this phenomenon had been described by DIAMOND *et al.* (1968) and by DUNCAN *et al.* (1969).

High-pressure liquid chromatographic (HPLC) techniques were recently used for the separation and identification of BaP metabolites formed by rat liver microsomes. New metabolites were identified with this method by FREUDENTHAL *et al.* (1975), SELKIRK *et al.* (1974 a and b and 1976 a), which, together with metabolites previously known, are listed in Table XX. These data suggest that at least 14 different compounds can be formed during metabolism of BaP in the rat.

HPLC techniques were also used by LEBER *et al.* (1976) to analyze the metabolites of BaP formed at incubation with squirrel and rhesus monkey liver microsome preparations. The chromatographic profiles obtained revealed the presence of 9,10-dihydro-9,10-dihydroxybenzo(a)-pyrene (BP-9,10-diol), 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene (BP-4,5-diol), 7-8-dihydro-7,8-dihydroxybenzo(a)pyrene (BP-7,8-diol), benzo-(a)pyrene-1,6-dione (BP-1,6-quinone), benzo(a)pyrene-3,6-dione (BP-3,6-quinone), 9,hydroxybenzo(a)pyrene (9-OH-BP), and 3-OH-BP, in addition to a number of unidentified peaks that appeared in the dihydrodiol region of the preparations. When BaP was incubated with rhesus monkey lung microsome preparations the overall pattern of the HPLC profiles was different from what was seen in the liver preparations and only BP-9,10-diol, BP-4,5-diol, 9-OH-BP, and 3-OH-BP could be identified.

The metabolite pattern of BaP breakdown in human beings was also studied recently. Liver microsome preparations were obtained from car accident victims and from individuals killed by sterile brain damage. SELKIRK *et al.* (1976 b) found in these preparations the same metabolites as LEBER *et al.* (1976) found in experiments with monkeys, and also benzo(a)pyrene-6,12-dione (BP-6,12-quinone), as well as six more unidentified metabolites.

PELKONEN (1976) and PELKONEN and KARKI (1975) incubated BaP with tissue preparations from human fetal adrenal glands and liver, adult liver, and placenta. Thin-layer chromatography revealed the metabolites BP-9,10-diol, BP-7,8-diol, BP-4,5-diol, and (not-specified) hydroxymetabolites. When BaP was added to cultured fetal liver cells or to fibroblasts, quinones, conjugates, and covalently bound derivatives were found in addition to the metabolites mentioned above. The quantity of the diols and water-soluble metabolites was predominant in the preparations containing intact cells, whereas in the *in vitro* systems phenols prevailed.

The most important metabolites of carcinogenic PAH are supposedly the so-called "K-region" epoxides (Fig. 2), because they are believed to be directly responsible for the biological effects attributed to their parent compounds. In the case of BaA the K-region epoxide has been postulated to form at the 5- and 6-positions (BaA-5,6-epoxide) (GROVER *et al.*

06/05/2001 07:49 5054243202 JILL PODOLSKY, EHA PAGE 01

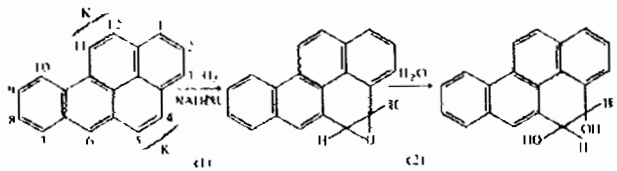


Fig. 2. *In vitro* metabolism of benzo(a)pyrene. Reaction (1) involves microsomal oxidase and NADPH; reaction (2) involves epoxide hydrase (GROVER *et al.* 1972).

1972). Under biological conditions (e.g., in rat liver homogenates) this epoxide may also be converted to phenol (probably 5-OH-BA) by spontaneous isomerization; it can be hydroxylated to form dihydrodiols (probably BA-5,6-diol), or conjugated with glutathione [S-(5,6-dihydro-6-hydroxy-5-benzanthracenyl) glutathione] (BOYLAND and SIMS 1964 b).

Other carcinogenic PAH which may form similar K-region epoxides include: DBahA (BOYLAND and SIMS 1965 a, GROVER *et al.* 1971 a, SELKIRK *et al.* 1971), MBaA (KEYSELL *et al.* 1973, SIMS 1970 a), DMBaA (KEYSELL *et al.* 1973), MCA (BURKI *et al.* 1972, SIMS 1966), and BeP and BaP (GROVER *et al.* 1972, SIMS 1970 a). Of these, perhaps the last one has been investigated most extensively. Until 1970 the K-region epoxide of BaP (BaP-4,5-epoxide) was still believed not to be present as a BaP metabolite, but now it is believed to be the first intermediary compound in the BaP degradation in animals (see Fig. 2). It is suspected to be the active carcinogenic metabolite of the metabolism of BaP (SELKIRK *et al.* 1975).

The enzyme system responsible for the metabolism of PAH is the microsomal cytochrome mixed-function oxygenase also known as aryl hydrocarbon hydroxylase (AHH) or benzpyrene hydroxylase (GELBOIN *et al.* 1972). This enzyme system is known to be present in rodent tissues (ARTO 1973), human liver (KUNTZMAN *et al.* 1966), human skin (LEVIN *et al.* 1972), human placenta (NEBERT *et al.* 1969), fetal liver (PELKONEN *et al.* 1972), macrophages (CANTRELL *et al.* 1973), lymphocytes (BUSBEE 1972, WHITLOCK *et al.* 1972), and monocytes (BAST *et al.* 1974). Studies with rodents revealed that this enzyme system can convert PAH to various hydroxylated derivatives including phenols, quinones, and epoxides (SELKIRK *et al.* 1974 a and 1975), and can also activate PAH to produce carcinogenic metabolites (GELBOIN *et al.* 1972).

The activity of this enzyme system can be induced by BaP (MASON 1965), MCA (RASMUSSEN and WANG 1974), DBacA, DBahA, BA, and DMBA (SLAGA *et al.* 1976). On the other hand, it can be inhibited by butylated hydroxyanisole and 7,8-benzoflavone (SPEIRS and WATTENBERG 1975, WIEBEL *et al.* 1971).

The specific enzyme which produces "K-region" oxides is called epoxide synthetase, and that which detoxifies it is epoxide hydrase

(OESCH *et al.* 1971, OESCH and DALY 1971); both enzymes are inducible in the rat by pretreatment with MCA or phenobarbitol (NEBERT *et al.* 1972). Epoxide hydrase has been shown to be present in adult human liver (OESCH 1974, OESCH *et al.* 1974), lungs (GROVER *et al.* 1973), placenta, fetal liver, and fetal adrenal gland (PELKONEN and KARKI 1975).

c) *Reactivity of intermediate metabolites*

In addition to metabolic pathways, considerable attention has been directed also to the study of chemico-cellular interactions that may explain the mechanism of PAH carcinogenesis. ABELL and HEIDELBERGER (1962) noted that carcinogenic PAH are covalently bound to proteins of mouse skin following topical application of the compounds. BROOKES and LAWLEY (1964) and GOSHMAN and HEIDELBERGER (1967) further found a rough correlation between carcinogenic activity and the amount of PAH bound to skin DNA. A similar correlation was also observed by SLAGA *et al.* (1976) in *in vitro* system where DMBaA, MCA, BaP, DBahA, and DBacA had been incubated with epidermal homogenates; however, there was no indication that the tumor-initiating ability of these PAH is correlated to their ability to induce aryl hydrocarbon hydroxylase.

Using mouse embryo cell cultures, DUNCAN *et al.* (1969) examined eight different PAH. It was found that these PAH can be classified in two distinct groups. One group with a high DNA-RNA binding index consisted of potent carcinogens while the other group had much lower (one-tenth) values for the binding index: with the exception of DBahA, this latter group consisted of noncarcinogens.

In a study with human cells (BROOKES and DUNCAN 1971) and with *in vivo* rat organ systems (JANSS *et al.* 1972, PRODI *et al.* 1970), the binding of BaP and DMBaA to DNA was also demonstrated.

Since the covalent binding properties of PAH are in sharp contrast to the fact that PAH are relatively inert chemically and cannot themselves undergo binding with DNA, RNA, or with proteins, it is supposed that metabolic transformation is needed before any such links are accomplished and carcinogenic effects are exerted. Two areas of research have attracted scientists most: determination of active forms (ultimate carcinogens) and the nature of the interaction between active forms and tissue constituents.

Although the ultimate carcinogenic mechanism is still not known, several types of metabolic products have been proposed as ultimate carcinogens derived from PAH. One type has been referred to as a carbonium ion by DIPPLE *et al.* (1968) and HULBERT (1975). Another type is that described by FRIED and SCHUMM (1967) and by WILK *et al.* (1968 and 1969), who postulated that the cation radical reacts with a cellular nucleophile group and then undergoes a final oxidation with a loss of a proton to complete the substitution reaction. Studies by UMANS *et al.* (1969) with BaP, by CAVALIERI *et al.* (1976) with BaP and BaA,

06/05/2001 07:49 5054243992 JILL PODOLSKY, PHA PAGE 02

and by CAVALIERI *et al.* (1975) with MCA seem to provide some support for this interpretation. In addition to the above-mentioned mechanisms, BOYLAND as early as 1950 postulated that epoxides are the activated intermediate metabolites. Since then more evidence to support this hypothesis has emerged. GROVER and SIMS (1971) demonstrated that K-region epoxides of PA and DBahA can react covalently *in vitro* with DNA and histone. The intermediate K-region epoxides of BA, DBahA, and MCA exhibit higher levels of biological activity leading to malignant transformation than their parent compounds, or the final products derived from them. This was confirmed in *in vitro* preparations by GROVER *et al.* (1971 b) and HUBERMAN *et al.* (1971 c), and in cell cultures by HARPER (1957), HUBERMAN *et al.* (1972), and MARQUARDT *et al.* (1972). In various tests for mutagenicity epoxides proved to be more active than their parent compounds (AMES *et al.* 1972, COOKSON *et al.* 1971, FAHMY and FAHMY 1973, HUBERMAN *et al.* (1971 b).

However, in biological assays on whole animals the K-region epoxides of BaA, MBaA, and MCA were found to exhibit less carcinogenic potency than their parent compounds, when applied either topically or parenterally (BOYLAND and SIMS 1967 b, MILLER and MILLER 1967, SIMS 1967 a). Similar observations were reported in more recent studies with PA, BaA, MBaA, DBahA, CH, and MCA in newborn mice (GROVER *et al.* 1975) and with DMBaA, DBahA, and BaP in *in vitro-in vivo* test systems, in which mouse lung tissue was pretreated with PAH and then implanted into isologous mice (FLAKS and SIMS 1975). Furthermore, LEVIN *et al.* (1976) found that BP-4,5-epoxide (a K-region epoxide) is less carcinogenic when applied topically to mouse skin than BaP or BP-7,8-epoxide (a non-K-region epoxide). The weak activity of BP-4,5-epoxide in this case was apparently due to its inactivation by enzymes in the mouse skin prior to reaching its site of action within the cell.

Some scientists, however, believe that the carcinogenic PAH are active *per se* and need no specific preliminary activation (FARBER 1968). In a recent study BURKI and BRESNICK (1975) applied MCA, its K-region epoxide (MCA-11,12-epoxide), and other MCA derivatives topically to mice and found that only MCA induced hyperplastic effects. This effect was also potentiated, under suitable conditions, by α-naphthaflavone, an inhibitor of PAH metabolism.

VII. Carcinogenicity

In 1915 YAMAGIVA and ICHIKAWA (1915) painted coal tar on the ears of rabbits and mice and found that tumors developed after repeated applications. Later experiments confirmed these results and included coal tar (TSUTSUI 1918, MURRAY 1921), soot extracts, crude shale oil, and blast furnace tar (BONSER 1932, LEITCH 1922, PASSEY 1922). The chemical basis of this "coal tar cancer" was then further investigated and the

active ingredients were identified as hydrocarbons (BLOCH and DREIFUSS 1921, KENNAWAY 1924). Soon some isolated compounds were identified as carcinogens, such as BaP, DBaiA, DMBaA, and MCA (KENNAWAY 1925, COOK *et al.* 1933).

Following these early studies many other PAH have been shown to have carcinogenic potential both in whole animals and in organ or tissue cultures.

a) In vitro bioassay

BERWALD and SACHS (1963) reported that hamster embryonic fibroblasts grown in culture with BaP or MCA developed characteristic morphologic alterations such as disorientation of cell arrangement and formation of criss-crossed, piled-up colonies. In another study BaP, MCA, DMBaA, and 10-methylbenz(a)anthracene (but not 8-methylbenz(a)-anthracene, CH and PR) induced *in vitro* transformation of normal cells to tumor cells (BERWALD and SACHS 1965). DIPAOLO *et al.* (1969 a and b) observed that such transformation can be induced with BaA, BeP, BaP, DBacA, DBahA, MCA, and DMBaA, but not with PA or P. Several cell lines derived from BaP treated colonies could produce fibrosarcomas when injected into hamsters.

LASNITZKI (1951, 1954, and 1963) and LASNITZKI and PELC (1957) reported that 20-methylcholanthrene (20-MCA) and MCA induce hyperplastic and preneoplastic changes in cultures derived from mouse ventral prostate. ROLLER and HEIDELBERGER (1967) also reported that DMBaA, MCA, and DBaA can induce hyperplasia, anaplasia, and abnormal mitosis in comparable cell cultures, but these did not prove to be transplantable into isologous mice; however, when DMBaA- or MCA-treated prostate pieces were dispersed with pronase and injected into C3H-mice, transplantable tumors developed (HEIDELBERGER and IYPE 1967).

In an attempt to develop cell lines for the study of PAH carcinogenesis *in vitro*, CHEN and HEIDELBERGER (1969 a, b, and c) obtained several B 1 prostate cell cultures derived from C3H mice. After treatment with MCA these cells injected into mice induced fibrosarcomas. Eight other PAH were also tested, five carcinogens among them, and there was a quantitative correlation between their carcinogenic activity and transformation-inducing capacity in cell cultures.

In other organ cell culture studies by LASNITZKI (1956), cultivated human fetal lung tissue was exposed to BaP at a concentration of 0.0 to 6.0 μg/ml. While the control explants showed outgrowth of bronchioli with one layer of columnar epithelium, the treated explants exhibited basal cell hyperplasia in the bronchiolar epithelium. In rodents CROCKER *et al.* (1965) observed epithelial metaplasia in respiratory tissues following continuous exposure of the tissues to DMBaA, BaP, or MCA at levels of 0.8 to 8.0 μg/ml for over seven days.

06/05/2001 07:49 5054243202 JILL PODOLSKY, FDA PAGE 03

b) Whole animal studies

Many data are available on the carcinogenicity of PAH in experimental animals: most of these are beyond the scope of this review. Data published during 1947–1969 has been reviewed by the *U.S. Public Health Service* (PHS) (HARTWELL 1951, SHUBIK and HARTWELL 1957 and 1969), and evaluated by the *U.S. NAS* (1972) and *EPA* (1975). A total of 20 PAH were identified as carcinogens: BaA, DMBaA, DBahA, BaP, BeP, MCA, indeno(1,2,3-cd)pyrene (IP), CH, dibenz(aj)anthracene (DBajA), DBacA, benzo(c)phenanthrene (BcPA), dibenzo(a,g)fluorene (DBagF), BbFL, benzo(j)fluroanthene (BjFL), 2-methylfluroanthene (2-MFL), benz(j)aceanthrylene (BjAC), dibenzo(ai)pyrene (DBaiP), dibenzo-(a,h)pyrene (DBahP), dibenzo(e,l)pyrene (DBelP), and dibenzeo(a,l)-pyrene (DBalP). The first eight in the list were also considered carcinogens by *WHO* (ANDELMAN and SUESS 1970).

In the *U.S. PHS* "Survey on Compounds which have been Tested for Carcinogenicity" (*TRACOR JITCO* 1973) it is indicated that 15 PAH, including nine known carcinogens, three known noncarcinogens, and three metabolites have been tested on various species of animals. Table XXI summarizes the carcinogenic data for these compounds derived from nonoral administration studies, and Table XXII the data resulting from experiments involving oral administration. While none of these tests identify new carcinogenic PAH not known prior to 1969, it is remarkable to note that only four PAH (DMBaA, BaP, DBahA, and MCA) and one metabolite (7-HM-DMBaA) have been proved to possess carcinogenic activity following oral intake. None of the previous issues of the *U.S. PHS* Surveys (1947–1969) has documented any evidence of tumor induction by PAH via the oral route.

Another comprehensive review (*IARC* 1973) listed data on the carcinogenicity of PAH and 13 of them are classified as proven carcinogens. The relevant data are presented in Tables XXIII and XXIV.

The data shown in these two reviews indicate that only a few of the proved carcinogenic PAH are capable of inducing cancer in experimental animals when administered orally. Moreover, the cancers induced orally are restricted mainly to leukemia, forestomach tumors, hepatoma, and pulmonary adenoma, whereas if nonoral routes of administration are employed skin tumors are prevalent. Mammary gland tumors are induced by both methods of administration.

VIII. Conclusions

Most foods contain very low levels of PAH, unless pyrolytic reactions contributed directly or indirectly to such compounds, as in cooking at high temperatures or in smoking processes. Notable exceptions are the shellfish which seem to concentrate and are unable to metabolize PAH.

Very little attention was paid to the problem of absorption, distribution, metabolism, and excretion of orally administered PAH; however, the few data available indicate that PAH are relatively effectively excreted in feces and urine and little is retained. Other studies, both *in vitro* and *in vivo*, show that PAH are converted in the animal body into phenols, dihydrodiols, quinones, and conjugated products by metabolizing enzymes in the microsomes. Currently there are about 40 different metabolites identified from eight known carcinogenic PAH, but no proper studies have been done yet on their conversion rates.

Among the PAH metabolites the so-called "K-region" epoxides, the precursors of dihydrodiols, seem to be the activated tumorigenic derivatives, at least in tissue culture and *in vitro* systems. Tested in whole animals the epoxides often show less carcinogenic activity than the parent compounds.

Since little or no work has been done on the possible teratogenic or reproductive effects of PAH, the appraisal of their potential hazards for health rests entirely on carcinogenicity studies. The current knowledge can be summarized as follows:

1. Of the 22 PAH identified in foods, 11 have been found to be carcinogenic in experimental animals. These are BaA, BaP, BeP, BbFL, CH, DBahA, DBajA, DBahP, DMBaA, IP, and MCA. Out of these only BaP, BaA, MCA, DBahA, and DMBA have been demonstrated to induce tumors following oral administration to rats and mice.

2. Of the above-mentioned 11 carcinogenic PAH, only very few have been demonstrated to exhibit positive dose-response relationship in chronic studies with mice: BaA, BajA, and BaP. The first two were tested by nonoral routes only, the latter by both nonoral and oral routes.

3. Of the 40 identified PAH metabolites only five have been tested for carcinogenicity in mice and rats, with negative results. Apart from the controversial results with K-region epoxides, which are tumorigenic in tissue culture but not in intact animals, the metabolites do not seem to be carcinogenic.

4. Until now there is no evidence to suggest that any of the 11 known carcinogenic PAH or their combinations can cause cancer in human beings via the oral route, especially in quantities likely to be present in foods. Neither are there any epidemiologic studies to link these PAH in foods to any specific disease, except some speculations that a high incidence of stomach cancer in Iceland could be caused by the high consumption of smoked fish and meat.

In summary it can be stated that although foods contain about 11 known carcinogenic PAH, there is no proof that at prevailing levels these contribute to the incidence of cancer. No realistic assessment of health hazards due to such PAH can be made, and the only recommendation one can formulate is that the level of PAH should be kept low in smoked or grilled meats and fish.

Table XXI. *Some tumorigenic effects of PAH via non-oral routes* (*TRACOR JITCO* 1973).

Compound	Animal	Route[a]; doses[b]; adjuvant[c]	Levels[d] causing no effects	Levels[d] and types of tumorigenic effects[e]
A	Mice	top.; 1; benzene	80 μg; observed for 42 d	—
	Rats	top.; 1; benzene	400 μg; observed for 37 d	—
	Guinea pigs	top.; 1; benzene	400 μg; observed for 27 d	—
BaA	Mice	s. c.; 1; tricap	—	500 μg; s. c. tumors
		top.; 1; benzene	1.0 mg	—
BcPA	Mice	top.; 1/d for 18 d; benzene	—	0.1 ml of 0.02 *M* sol.; skin tumors
DMBaA	Mice	top.; 1; acetone	20 μg	125–800 μg; skin pap, mamm adsarc, pulm. sq. cell carc.
		s. c.; 1; olive oil or others	—	0.5–40 μg; sarc., pulm. adeno. and pap.
	Pregnant mice	i. p.; 4 × 1.0 mg; sesame oil	—	skin pap. in offsprings
	Rats	i. v.; 3; adjuvant?	—	2 mg; skin tumors
		i. m.; 1; benzene	—	2 mg; soft tissue tumors
		s. c.; 1; dime. sulf.	—	2.5 mg; spindle cell sarc.
		i. vog.; 1/wk for 54 wk; acetone	—	1% sol; cerv.-vog. sarc. and pap.
		i. t.; 5; colloidal protein	—	2.5 mg; lung sq. cell carc.
	Rabbits	impl. (not specified); capsule	—	10 mg; osteosarcoma
	Hamsters	top.; 6 × 10 mg; paraffin	—	skin tumors
		i. t.; 2 × 500 μg; cellulose-saline	—	tracheal pap.
		s. c.; 1; tricap or sun oil	2.0 mg in sun oil	500 μg in tricap; s. c. fibrosarc.
		i. v.; 2; fat emulsion	—	3 mg; mamm. tumors, stom. carc.
	Guinea pigs	s. c.; 1; tricap	—	over 5 mg; inj. site tumors
		i. v.; 3; fat emulsion	—	3 mg; skin pap. and mamm. adeno.
7-HM-BaA	Mice	top.; 3/wk for 2 wk; dime. sulf.-acetone	—	230 μg; skin pap.
	Rats	s. c.; 1; dime. sulf.	—	2.5 mg; spinkle cell sarc.
7-HM-DMBaA	Rats	s. c.; 1; dime. sulf.	2.5 mg	—
P	Mice	top.; 1; benzene	160 μg	—
	Rats	top.; 1; benzene	800 μg	—

Table XXI. (*continued*)

Compound	Animal	Route[a]; doses[b]; adjuvant[c]	Levels[d] causing no effects	Levels[d] and types of tumorigenic effects[e]
	Guinea pigs	top.; 1; benzene	160 μg	—
BaP	Mice	top.; 3/wk for lifetime; acetone	1 μg	12–180 μg; skin pap.
		s. c.; 1; cott. oil or others	10 μg in cott. oil or 1.0 μg in olive oil	over 50 μg in cott. oil or over 8.0 μg in olive oil; fibrosarc.
	Pregnant mice	i. p.; 11 × 2 mg; sesame oil	—	lung adeno. in offspring
BeP	Mice	top.; 3/wk for 6 wk; benzene	0.1 ml of 0.01 *M*	—
DBacA	Mice	top.; 1; benzene	1.0 μg	—
		top.; 2/wk for 6 wk; acetone	—	85 μg; skin sq. cell carc.
DBahA	Mice	s. c.; 1; olive oil	—	0.1 mg; fibrosarc.
DBajA	Mice	top.; 2/wk for 60 wk; olive oil	39 μg	78 μg; skin sq. cell carc.
MCA derivative (11,12-diH-MCA)	Mice	top.; 2/wk for 57 wk; acetone	—	37 μg; skin sq. cell carc.
MCA	Mice	top.; 2/wk for 31 wk; acetone	—	0.5%; skin tumors
		top.; 1; benzene or acetone	—	125 μg; skin pap., carc. and sarc.
		s. c.; olive oil	—	over 0.2 mg; skin sarc., carc. and pulm. adeno.
	Rats	s. c.; 1; apricot oil	—	1.5 mg/100 g B W; s. c. tumors
	Hamsters	s. c.; 1; sun oil	—	2 mg; s. c. sarc.
BghiPR	Mice	top.; 1; benzene	0.8 mg	

[a] i. g. = intragastrically, i. t. = intratracheal administration, i. v. = intravenous injection, top. = topically or skin application, p. o. = oral, s. c. = subcutaneous injection, i. m. = intramuscular injection, i. vag. = intravaginal, i. p. = intraperitoneal administration, b. i. = bladder implantation, and impl. = implantation.

[b] 1, 2, 3, etc. = no. of applications or doses, 1/d, 2/d, 3/d, etc. = no. of applications or doses per day, 1/wk, 2/wk, 3/wk, etc. = no. of applications or doses per week, and d, wk, mo = day, week, month duration, respectively.

[c] Tricap = tricaprylin, sun. oil = sunflower oil, dime sulf = dimethyl sulfoxide, PEG = polyethylene glycol, and cott. oil = cottonseed oil.

[d] Amounts per application, per dose, per 100 g body weight, per g diet, or per ml drinking water.

[e] Stom. = stomach, mamm. = mammary, esoph. = esophagus, pulm. = pulmonary, adeno. = adenoma, pap. = papilloma, leuk. = leukemia, sq. cell = squamous cell, adsarc. = adenomasarcoma, carc. = carcinoma, adcarc. = adenocarcinoma, fibrad. = fibroadenoma, sarc. = sarcoma, and fibrosarc. = fibrosarcoma.

06/05/2001 07:49 5054243202 JILL PODOLSKY, ESQ PAGE 04

Table XXII. *Some tumorigenic effects of PAH via oral routes (TRACOR JITCO 1973).*

Compound	Animal	Route[a]; doses[b]; adjuvant[c]	Levels[d] causing no effects	Levels[d] and types of tumorigenic[e] effects	References
7,12-diHM-DMBaA	Mice	i. g.; 1; arachis oil	2 mg	—	JULL & RUSSELL (1970)
DMBaA	Mice	i. g.; 1/wk for 8 wk; almond oil	—	1 mg; mamm. tumors stom. sq. cell pap.	BIANCIFIORI *et al.* (1961)
		i. g.; 6; PEG	—	1 mg; lymphoma	CHEN (1970)
		i. g.; dose?; olive oil	—	0.04 mg/g B W tumors in ovary	KRARUP (1970 a)
		i. g.; 1; olive oil	—	over 0.25 mg/g B W; leuk., mamm. tumors and pulm. tumors	KRARUP (1970 b)
	Hamsters	i. g.; 1; "Labrafil 1944 CS"	—	1 mg; mamm. tumors	AUBERT *et al.* (1970)
	Rats	i. g.; 1; sesame oil	—	10 mg; mamm. tumors	ESKIN *et al.* (1968)
		p. o.; 3; apricot oil	—	10 mg; mamm. gland tumors	BESKROVNY *et al.* (1970)
		i. g.; 2; corn oil	—	10–50 mg; mamm. adcarc., mamm. fibrad. and mamm. adeno.	CARROLL & KHOR (1970)
		p. o.; 1; sesame oil	5 mg	over 10 mg; mamm. fibrad. and mamm. adcarc.	FLESHER & SYDNOR (1970)
		i. g.; 1; corn oil	—	40 mg; mamm. adcarc. and adeno.	KOVACS & SOMOGYI (1970)

Table XXII. (*continued*)

Compound	Animal	Route[a]; doses[b]; adjuvant[c]	Levels[d] causing no effects	Levels[d] and types of tumorigenic[e] effects	References
		i. g.; 1; sesame oil	—	10 mg; mamm. adcarc.	WEISBURGER *et al.* (1970)
	Rats	i. g.; 1; corn oil	—	30 mg; mamm. adcarc.	YOUNG *et al.* (1970)
7-HM-DMBaA	Rats	p. o.; 1; sesame oil	—	20 mg; mamm. fibrad.	FLESHER & SYDNOR (1970)
BaP	Mice	i. g.; 10 × 0.0-1 mg/wk; triethylene	—	over 0.1 mg; sq. cell carc. in stom.	FEDORENKO & YANYSHEVA (1967)
		p. o.; in diet for 98 d	0.25 mg/g for 7 d or 0.001 mg/g for 110 d	0.25 mg/g for 70 d; leuk.	RIGDON *et al.* (1967)
		p. o.; in diet for 155 d	25 mg/g for 100 d	25 mg/g for 100 d; leuk.	RIGDON *et al.* (1969)
		i. g.; 2; sesame oil	—	3 mg; pulm. adeno.	WATTENBERG & LEONG (1970)
	Rats	i. g.; 1/wk for 3 wk; various oils	2 mg; sun oil	2 mg in sun oil plus butter oil; polymorpho. cellular sarc. or spindle cell sarc.	TURKIYA *et al.* (1971)
DBahA	Mice	p. o.; in drinking water (aqueous-olive oil emulsion)	—	0.2 mg/ml; mamm. tumors pulm. adeno. and alveo. carc.	SNELL & STEWART (1962 and 1963)
MCA	Mice	i. g.; 1/wk for 8 wk; arachis oil	—	0.2 mg of 0.5% sol; mamm. tumors, stom. pap. or carc.	BIANCIFIORI *et al.* (1961)
	Rats	i. g.; 1/d for 4-8d; sesame oil	—	5–10 mg; leuk.	MOLONEY *et al.* (1971)

[a], [b], [c], [d], and [e] See footnotes for Table XXI.

06/05/2001 07:49 5054243202 JILL PODOLSKY, EHA PAGE 05

Table XXIII. *Some tumorigenic effects of PAH via non-oral routes* (*IARC* 1973).

Compound	Animal	Route[a]; doses[b]; adjuvant[c]	Levels[d] causing no effects	Levels[d] and types of tumorigenic effects[e]
BaA	Mice	top.; 2/wk for 68 wk; toluene	0.02% (amount?)	0.2% (amount?); pap.
		s. c.; 1; tricap.	—	5 mg; sarc.
	Rats	top.; 1/d for 5 mo; acetone	saturated (amount?)	—
		i. v.; 3 × 13 mg; adjuvant?	39 mg	
		b. i.; 1; paraffin	—	2 mg; pap.
BbFL	Mice	top.; 1; acetone	1 mg	—
		s. c.; 3 × 0.6 mg	—	sarc.
BjFL	Mice	top.; 3/wk for 9 mo; acetone	—	0.1% (amount?); pap. and carc.
BaP	Mice	top.; 1; toluene	94 μg	752 μg; pap.
		top.; 3/wk; acetone	0.0005% (amount?)	0.001% (amount?) pap.
		s. c.; 1; tricap.	0.03 mg	0.06 mg; sarc.
		i. p.; 1; water	—	2 mg; fibrosarc.
	Rats	top.; 1/wk for 21 wk; benzene	—	0.5%; pap.
		i. t.; 1/mo for 10 mo; India ink	0.002 mg	0.01 mg; lung tumors
		s. c. or i. m.; 1; olive oil	—	0.1 mg; tumors (not specified)
	Hamsters	top.; 1/2 wk; acetone	—	0.01% (amount?); skin tumors
		s. c.; 1; olive oil	0.01 mg	1 mg; sarc.
	Guinea pigs	s. c.; 1; lard	—	5 mg; tumors (not specified)
	Rabbits	top.; 5/wk; acetone	0.0001% (amount?)	0.005% (amount?); pap.
BeP	Mice	top.; 1 or repeated; acetone	single dose with 1.0 mg	repeated with 1.0 mg; pap.
		top.; 3/wk for ? period; acetone	—	0.1% (amount?); carc. and pap.

Table XXIII. (*continued*)

Compound	Animal	Route[a]; doses[b]; adjuvant[c]	Levels[d] causing no effects	Levels[d] and types of tumorigenic effects[e]
CH	Mice	top.; 2/d; various fats	7.5% (amount?)	—
		s. c.; 1; arachis oil	10 mg	—
DBahA	Mice	top.; 3/wk; acetone	0.001% (amount?)	over 0.1% (amount?); sarc.
		s. c.; 1; tricap.	—	over 0.0002 mg; sarc.
	Newborn mice	s. c.; 1; adjuvant?	0.01 μg	over 0.08 μg; sarc. and lung adeno.
	Rats	i. t.; 5 × 0—20 mg; India ink	0.5 mg	over 2 mg; lung sq. carc.
		s. c.; 1; olive oil	—	0.1 mg; sarc.
TBP	Mice	s. c.; 3 × 0.6 mg; olive oil	—	sarc.
DBaeP	Mice	top.; 3/wk; dioxane	—	0.1% (amount?); carc. and pap.
		s. c.; 1; olive oil	—	over 0.6 mg; sarc.
DBahP	Mice	top.; 3/wk; dioxane	—	0.05% (amount?); pap. and adeno.
		s. c.; 1; olive oil	—	6 mg; sarc.
	Rats	i. m.; 1; sun oil	—	0.5 mg; sarc.
DBaiP	Mice	top.; 2/wk; dioxane	—	0.05%; epidermoid carc. and pap.
		s. c.; 1; sesame oil	—	50 μg; sarc.
		s. c.; 3/wk; peanut oil	—	0.6 mg; sarc.
	Hamster	s. c.; 1; tricap.	—	over 250 μg; sarc.
DBalP	Mice	s. c.; 2 × 0.6 mg; olive oil	—	sarc.
I P	Mice	top.; 3/wk; dioxane	0.01–0.05% (amount?)	0.1% (amount?); sarc. pap.
		s. c.; 3 × 0.6 mg; olive oil	—	sarc.

[a, b, c, d, e] See footnotes for Table XXI.

06/05/2001 07:49 5054243202 JILL PODOLSKY, EHA PAGE 06

Table XXIV. *Some tumorigenic effects of PAH via oral routes* (*IARC* 1973).

Compound	Animal	Route[a]; doses[b]; adjuvant[c]	Levels[d] causing no effects	Levels[d] and types of tumorigenic effects[e]	References
BaA	Mice	i. g.; 1/3–7d for 16 mo; mineral oil	single dose of 0.5 mg	4.0 mg in 8 doses; pap. of forestomach	BOCK & KING (1959)
	Infant mice	i. g.; 15/5 wk; methocel aerosol	—	1.5 mg; lung adeno. hepatomas and stom. pap.	KLEIN (1963)
MCA	Mice	i. g.; 10 × 1.5 mg; methocel aerosol	—	hepatomas, lung adeno. and stom. pap.	KLEIN (1963)
BaP	Mice	i. g.; 1; PEG	0.05 mg	0.2 mg; forestom. tumors	HARTWELL 1951; SHUBIK & HARTWELL (1957 and 1969), *TRACOR/JITCO* (1973)
		p. o.; 30–250 ppm in diet for 110–197 d	30 ppm	over 50 ppm; forestom. tumors; at 250 ppm leuk. and lung adeno.	NEAL & RIGDON (1967), RIGDON & NEAL (1966 and 1969)
	Rats	i. g.; 1 or daily; adjuvant?	—	single dose of 100 mg or daily dose of 2.5 mg; pap. in esoph. and stom.	GELBOIN (1969), HUGGINS & YANG (1962)
	Hamsters	i. g.; 1/2 wk for 11 mo	—	2–5 mg; pap.	CHEN (1970)
		p. o.; 500 ppm in diet for 14 mo	—	tumors in esoph. and stom.	DONTENWILL & MOHR (1962)
DBahA	Mice	p. o.; in diet (level?) for 5–7 mo	—	total amount of 9–19 mg; forestom. tumors	LARIONOW & SOBOLEVA (1938)
		p. o.; in drinking water; mineral oil emulsion	—	0.4 mg/d; sq. cell carc., forestom. pap. and tumors in lung, heart, and intestine	LORENZ & STEWART (1948)
		p. o.; in drinking water; olive oil-water emulsion	—	0.76–0.85 mg/d; lung carc. and mamm. carc.	SNELL & STEWART (1962)
		i. g.; 15 mg over 15 wk; almond oil	—	mamm. carc.	BIANCIFIORI & CASCHERA (1962)

a, b, c, d, and e See footnotes for Table XXI.

Table XXV. *List of abbreviations.*

A	anthracene
ACP	acenaphthene
AC	acenaphthylene
ACT	acenaphthalene
AN	anthanthrene; dibenzo(cd,jk)pyrene
AT	benz(a)anthracene
BaA; BA	benzo(a)fluorene
BaF	benzo(a)pyrene; 1,2-benzpyrene
BaP; BP; 1,2-BP	benzo(e)pyrene; 3,4-benzpyrene
BeP; 3,4-BP	benzo(b)chrysene
BbCH	benzo(b)fluoranthene
BbFl	benzo(c)phenanthrene
BcFl	benzo(ghi)perylene
BcPA	benz(j)aceanthrylene; cholanthrene
BghiPR	benzo(k)fluoranthene
BjAC	benzo(l)fluoranthene
BkFl	1,12-benzperylene
BlFl	benz(a)anthracene-5,6-epoxide
BjFL	benzo(a)pyrene-4,5-epoxide
BPR; 1,12-BPR	benzo(a)pyrene-7,8-epoxide
BaA-5,6-epoxide	8,9-dihydro-8,9-dihydroxybenzanthracene
BaP-4,5-epoxide	5,6-dihydro-5,6-dihydroxybenzanthracene
BaP-7,8-epoxide	10,11-dihydro-10,11-dihydroxybenzanthracene
BA-8,9-diol	3,4-dihydro-3,4-dihydroxybenzanthracene
BA-5,6-diol	1,6-dihydro-1,6-dihydroxybenzo(a)pyrene
BA-10,11-diol	3,6-dihydro-3,6-dihydroxybenzo(a)pyrene
BA-3,4-diol	4,5-dihydro-4,5-dihydroxybenzo(a)pyrene
BP-1,6-diol	7,8-dihydro-7,8-dihydroxybenzo(a)pyrene
BP-3,6-diol	9,10-dihydro-9,10-dihydroxybenzo(a)pyrene
BP-4,5-diol	benza(a)pyrene-1,6-dione
BP-7,8-diol	benzo(a)pyrene-3,6-dione
BP-9,10-diol	benzo(a)pyrene-6,12-dione
BP-1,6-Quinone	2-hydroxybenzanthracene
BP-3,6-Quinone	3-hydroxybenzanthracene
BP-6,12-Quinone	4-hydroxybenzanthracene
2-OH-BA	5-hydroxybenzanthracene
3-OH-BA	8-hydroxybenzanthracene
4-OH-BA	9-hydroxybenzanthracene
5-OH-BA	3-hydroxybenzo(a)pyrene
8-OH-BA	6-hydroxybenzo(a)pyrene
9-OH-BA	9-hydroxybenzo(a)pyrene
3-OH-BP	10,11-benzofluoranthene
6-OH-BP	3,4-benzofluoranthene
9-OH-BP	11,12-benzofluoranthene
10,11-BFL	chrysene
3,4-BFL	coronene
11,12-BFL	dibenz(a,c)anthracene; 1,2,3,4-dibenzanthracene
CH	dibenzo(a,g)fluorene
CR	dibenz(a,h)anthracene; 1,2,5,6-dibenzanthracene
DBacA	dibenzo(a,h)pyrene
DBagF	dibenz(a,j)anthracene
DBahA; DBA	dibenz(a,i)anthracene
DBghP	dibenz(a,e)pyrene
DBaiA	
DBajA	
DBaeP	

06/05/2001 07:49 5054243202 JILL PODOLSKY, FHA PAGE 08

Table XXV. (*continued*)

DBaiP	dibenzo(a,i)pyrene
DBalP	dibenzo(a,l)pyrene
DBelP	dibenzo(e,l)pyrene
DB-9,10-AQ	1,2,5,6-dibenz-9,10-anthraquinone
DB-3,4-AQ	1,2,5,6-dibenz-3,4-anthraquinone
diOH-DB-9,10-AQ	dihydro-1,2,5,6-dibenz-9,10-anthraquinone
7-OH-DMBaA	7-OH-7,12-dimethylbenz(a)anthracene
7-HM-BaA	7-hydroxymethyl derivative of BaA
7-HM-DMBaA	7-hydroxymethyl derivative of DMBaA
7,12-diHM-DMBaA	7,12-dihydroxymethyl derivative of DMBaA
DMBaA; DMBA	7,12-dimethylbenz(a)anthracene
F	fluorene
FL	fluoranthene
IP	indeno(1,2,3-cd)pyrene
MP	methylpyrene
MBaA; MBA	methylbenz(a)anthracene
MBaP	methylbenzo(a)pyrene
4-MP	4-methylpyrene
MCA; 3-MCA	methylcholanthrene; 3-methylcholanthrene
MCA-11,12-epoxide	methylcholanthrene-11,12-epoxide
20-MCA	20-methylcholanthrene
MFL	methylfluoranthene
2-MFL	2-methylfluoranthene
3-MPA; MPA	3-methylphenanthrene
NA	naphthalene
TP	triphenylene
P	pyrene
PA	phenanthrene
PAH	polycyclic aromatic hydrocarbons
PR	perylene
TBP	tribenzo(a,e,i)pyrene

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06/05/2001 07:49 5054243202 JILL PODOLSKY, EPA PAGE 13