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**RECOMMENDED METHODS FOR THE
DETERMINATION OF POLYCYCLIC
AROMATIC HYDROCARBONS
IN FOODS**

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HYDROCARBONS IN FOODS

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PREFACE

The AOAC* and IUPAC have recognized the need for recommended methods for the determination of polycyclic aromatic hydrocarbons in foodstuffs. The carcinogenic properties of some of these substances which occur in the human environment as a result of air pollution, curing smokes, modes of cooking, food processing, etc., have been well documented. As a result of collaborative efforts by the AOAC and IUPAC, a method for the determination of benzo(a)pyrene in smoked foods was adopted as official by the AOAC in 1968 (1,2). The Food Section of the Applied Chemistry Division of IUPAC has accepted the procedure as a recommended method (3).

A general procedure for determining polycyclic aromatic hydrocarbons in smoked foods (4) was later shortened and applied to a variety of total diet composities including dairy products; meat, fish and poultry; root vegetables; oils, fats and shortenings; and beverages (5).

Since the former IUPAC Subcommittee on Smoke Constituents, Trace Substances Commission, was also seeking a method for determining such polycyclics in smoked foods, a joint IUPAC-AOAC collaborative study of the cited shortened procedure was undertaken. The results (6) obtained in the study of ham samples fortified with benzo(a)pyrene (carcinogenic), benzo(e)pyrene (non-carcinogenic), benz(a)anthracene (carcinogenic), and benzo(ghi)perylene (non-carcinogenic) at a level of 10 ppb ($\mu\text{g}/\text{kg}$), indicate that the method is reliable and reproducible. Statistical evaluation of the data from the 10 collaborators in Canada, England, the Federal Republic of Germany, and the USA show standard deviations between and within laboratories ranging from 7.4% to 12.7%. No laboratory was a statistical outlier for any of the hydrocarbons under study as tested by Youden's rank sum test (7). The procedure was adopted as an official method by the AOAC in 1972 (2), and as a recommended method by the Commission on Food Additives at its Madrid 1975 meeting. Formal publication of this action was deferred to permit the completion of on-going studies by Prof. G. Grimmer, Titular Member, on a different procedure of somewhat wider scope (8).

The latter, a gas chromatographic procedure, was applied in collaborative studies to meat and sunflower oil. The report of the results to the Commission has not been published. Consequently, Grimmer's (edited) report is included as an appendix with the present paper. Although the procedure (8) as presented and collaboratively studied (9) depends for identification of the contaminants solely on their (non-specific) relative retention times, it is more rapid than the other procedure described in (2). Moreover, it provides a profile of polycyclic aromatic hydrocarbons in food products which allows some judgement about the origin (food processing and/or air pollution) of these contaminants. Accordingly, the Commission considers the gas chromatographic procedure as a useful screening method for polycyclic aromatic hydrocarbons and at its Paris 1976 meeting recommended the method for that use. The grave implications of the occurrence of carcinogens in food make it imperative to regard the presence of polycyclic aromatic hydrocarbons when indicated by the screening method as tentative until it is confirmed by an independent and adequate identification technique such as mass spectrometry, ultraviolet spectrophotometry, spectrophotofluorometry, or the like. However, the indicated absence of polycyclic aromatic hydrocarbons is acceptable proof thereof within the limits of sensitivity of the method.

E. O. Haenni
Chairman, Commission on
Food Additives

*AOAC = Association of Official Analytical Chemists

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RECOMMENDED METHOD FOR THE DETERMINATION OF SOME POLYCYCLIC
AROMATIC HYDROCARBONS IN MEATS*

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PRINCIPLE

A comminuted or liquid food sample is saponified with alcoholic potassium hydroxide and the polycyclic aromatic hydrocarbons are extracted. They are purified by solvent partition and by column chromatography and separated by thin layer chromatography. The separated hydrocarbons are determined by spectrophotometry and the results are confirmed by spectrophotofluorometry.

METHOD

The procedure is described in detail in J. Assoc. Offic. Anal. Chemists 51, 122 (1978) and in Official Methods of Analysis, 12th Ed., p. 385, Association of Official Analytical Chemists, Washington, D.C. (1975).

*The applicability of the method to total diet composites including dairy products; oils, fats and shortenings; beverages; meats; fish and poultry; and root vegetables has been demonstrated by recovery studies at the 2 $\mu\text{g}/\text{kg}$ level. However, acceptance as an IUPAC recommended method was restricted to analysis of meats because only meat was used in the collaborative study.

RECOMMENDED SCREENING METHOD (PROFILE ANALYSIS) FOR
POLYCYCLIC AROMATIC HYDROCARBONS IN MEATS AND OILS*

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PRINCIPLE

Oil and fat samples are subjected to solvent partition. Other samples are comminuted, and saponified with aqueous methanolic potassium hydroxide. In either case the extracts are purified by column chromatography and separated in a second column chromatographic step into a fraction containing hydrocarbons with three rings (non-carcinogenic) and a fraction containing hydrocarbons with four to seven rings which includes the carcinogenic compounds. In each fraction the hydrocarbons are determined by high-performance gas-liquid chromatography utilizing internal standard(s) in conjunction with a flame ionization detector. In routine determinations of the carcinogenic hydrocarbons, only the fraction is used which contains the hydrocarbons with four to seven rings.

METHOD

The method is described in detail in Deut. Lebensm.-Rundschau 71(3) 93 (1975) and in J. Assoc. Offic. Anal. Chemists 58, 725 (1975).

*The authors have applied the method to other foods (poultry, fish, yeast). However, acceptance as a IUPAC recommended method was restricted to the analysis of meats and oils (fats) because only those commodities were used in the collaborative studies.

APPENDIX

IUPAC COLLABORATIVE STUDIES ON THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY GLC¹Part I: Determination of polycyclic aromatic hydrocarbons (PAH) in sunflower oil by GLC (July 1974)Material distributed to the participants (12):

- (1) Benzo(b)chrysene (internal standard, 10 mg)
- (2) Reference solution (PAH test, 12 July 1974), with N,N-dimethylformamide as solvent:

chrysene	74 mg/l (= ppm)
benzo(b)fluoranthene [B(b)f]	119
benzo(a)pyrene [B(a)p]	150
perylene	79
dibenz(a,j)anthracene [Db(a,j)a]	257
indeno(1,2,3-cd)pyrene [I(c,d)p]	252
benzo(b)chrysene	252
benzo(ghi)perylene [B(ghi)p]	238

- (3) Sunflower oil (2 x 100 g) for 2 analyses with the same PAH's added (5 to 12 µg/l) as were contained in the reference solution, but without benzo(b)chrysene.

Method:

"Polycyclic Aromatic Hydrocarbon Profile Analysis of High-Protein Foods, Oils, and Fats by Gas Chromatography", J. Assoc. Offic. Anal. chemists 58, 725-733 (1975).

Results:

The oil to be used as a sample was carefully refined, checked to be free of PAH, and fortified with 7 PAH's in the µg/l (ppb) range. The results are shown in Tables 1 and 2. Seven laboratories (A - G) analysed 13 samples of the sunflower oil (analyses Nos. 1 to 13).

Nine analyses of 13 are in the range of 59.3 to 77.5 ppb, that is 85.32 to 111.51% of the amount added. The mean value of these analyses is 68.96 ppb (0.78% below the amount added.) The variation coefficients (Table 2) range from 9.4 to 24.5% (because of the results from Lab D).

Thirteen analyses are in the range of 46.6 to 96.3 ppb, that is 67.05 to 138.7% of the amount added. The mean value is 66.15 ppb (4.82% below the amount added). Analysis No. 10 from Lab E is too high in most values (especially chrysene). Presumably the internal standard as well as benzo(a)pyrene and perylene were destroyed by heat or lost on an old GC-column by tailing. These 3 PAH's decompose more easily than the others.

Analyses No. 11 and No. 12 of Lab F are too low, but in good agreement between themselves. Probably more than the intended amount of the internal standard solution was added and therefore all PAH values are too low.

¹The collaborative studies were performed under the direction of Prof. G. Grimmer, Biochemisches Institut für Umweltcarcinogene, Sieker Landstrasse 19, D-2070 Hamburg-Ahrensburg, Federal Republic of Germany.

TABLE 1. Polycyclic aromatic hydrocarbons (PAH) found in 13 samples of 100 g each of sunflower oil by 7 laboratories. Samples were fortified with the PAH's listed in the first column.

Amount Added (ppb)	Amounts found (ppb)														Mean Value (ppb)
	Lab A		Lab B		Lab C		Lab D		Lab E		Lab F		Lab G		
	1	2	3	4	5	6	7	8	9	10	11	12	13		
Chrysene	11.2	10.5	11.9	11.9	9.6	9.6	8.5	10.5	11.30	22.69	11.3	10.1	15.0	11.88	
B(b)f	10.0	10.4	10.3	10.8	10.6	12.3	5.4	7.2	12.87	16.67	6.6	6.6	6.	9.80	
B(a)p	9.8	9.3	10.1	9.5	9.7	11.1	8.2	8.1	7.47	7.18	7.1	6.7	5.	8.41	
Perylene	7.6	6.9	7.0	7.2	6.5	6.3	4.4	6.5	7.98	5.00	4.7	4.6	3.	5.99	
Db(a)j/a	10.7	11.7	10.3	10.0	8.3	10.6	8.7	10.2	13.13	15.85	6.8	7.2	4.	9.94	
I(cd)p	9.8	10.9	9.6	9.5	10.4	10.1	14.8	12.3	12.18	13.65	5.4	5.7	6.	10.20	
B(ghi)p	10.4	10.0	10.0	9.8	10.6	10.5	9.3	8.9	12.08	15.32	5.3	5.7	10.	9.93	
Total(ppb)	69.5	69.7	77.5	68.7	65.7	70.5	59.3	63.7	77.01	96.36	47.2	46.6	49.0	66.15	
Total(%)	100.0	100.3	111.5	98.9	94.5	101.4	85.3	91.6	111.1	138.7	67.9	67.1	70.5		

TABLE 2. Mean values of analyses 1 to 9 from table 1.

	Amount added (ppb)	Mean values of 9 analyses (ppb)	Range max.-min. (ppb)	Standard deviation (ppb)	Variation coeff. (%)
Chrysene	11.2	10.58	3.4	± 1.19	11.3
B(b)f	10.0	10.16	6.9	± 2.40	24.5
B(a)p	9.8	9.27	3.0	± 1.05	11.0
Perylene	7.6	6.74	3.5	± 1.23	18.5
Db(a,j)a	10.7	10.58	4.0	± 1.40	13.6
I(c,d)p	9.8	11.32	5.3	± 1.85	16.5
B(g,h,i)p	10.4	10.31	2.7	± 0.95	9.4

Part II: Determination of PAH in meat by GLC (December 1974)Material distributed to the participants (12):

- (1) Benzo(b)chrysene (internal standard, 50 mg)
- (2) Reference solution (PAH test, 9 December 1974) with N,N-dimethylformamide as solvent:

chrysene	74 mg/l (ppm)
benzo(b)fluoranthene [B(b)f]	119
benzo(e)pyrene [B(e)p]	126
benzo(a)pyrene [B(a)p]	150
perylene	79
dibenz(a,j)anthracene [Db(a,j)a]	257
indeno(1,2,3-cd)pyrene [c,d)p]	252
benzo(b)chrysene	252
benzo(ghi)perylene [B(ghi)p]	238

- (3) Meat with PAH (2 x 100 g) for 2 analyses. Sample of minced meat (100 g) was weighed in a tin and injected with the PAH solution (2.00 ml). After soldering the tin was sterilized.
- (4) Silica gel WOELM (without water), 100 g.

Results:

Six laboratories (A, B, C, D, E, and H) analysed 12 samples. The results are given in Tables 3 and 4.

As in the case of sunflower oil, the deviation (in percent) from the total amount of PAH's added is considered the criterion of precision.

Eight analyses of 12 are in the range of 83.7 to 95.7 ppb, that is 92.08% to 105.30%. The mean value of these determinations is 88.02 ppb (= 96.8% of the total amount added). The variation coefficients are presented in Table 4.

Twelve analyses are in the range of 63.0 to 137 ppb (69.3 to 150.7%).

TABLE 3. Polycyclic aromatic hydrocarbons (PAH) found in 12 samples of 100 g each of meat by 6 laboratories. Samples were fortified with the PAH's listed in the first column.

	Amounts found (ppb)												Mean value (ppb)
	Lab A		Lab B		Lab C		Lab D		Lab E		Lab H		
	1	2	3	4	5	6	7	8	9	10	11	12	
Chrysene	13.4	14.5	13.1	11.7	8.1	9.1	12.1	n.d.	13.97	12.56	14.0	11.0	12.28
B(b)p	12.0	11.5	12.3	11.5	9.5	10.3	12.3	n.d.	12.54	12.14	22.	23.	13.58
B(e)p	7.5	12.2	7.8	7.4	5.0	4.7	10.9	7.6	n.d.	n.d.	16.	16.	9.73
B(a)p	11.7	9.0	10.5	10.6	7.9	8.6	9.9	n.d.	12.97	15.96	24.	19.	12.48
Perylene	9.2	7.0	8.8	8.4	3.3	5.2	6.9	6.2	11.10	6.55	16.	14.	8.40
Db(a,j)a	12.9	11.1	12.5	12.3	6.6	7.3	10.3	n.d.	12.00	12.59	17.	13.	11.53
(c,d)I(cd)p	11.8	11.1	11.1	11.1	14.2	12.5	11.6	11.6	12.50	11.19	14.	11.	12.09
B(ghi)p	12.4	11.0	11.5	11.5	8.4	9.8	5.7	12.0	13.14	11.45	14.	11.	11.26
Total(ppb)	90.9	87.4	87.5	84.5	63.0	67.5	83.7	---	95.72*	89.94*	137	118	91.35
Total(%)	100.0	96.2	96.3	92.9	69.3	74.3	92.1		105.3	98.9	150.7	129.8	

* Total with 7.5 ppb benzo(e)pyrene (for comparison only)

TABLE 4. Mean values of analyses 1 to 4 and 7 to 10 from table 3.

	Amount added (ppb)	Mean values of 8 analyses (ppb)	Range max.-min. (ppb)	Standard deviation (ppb)	Variation coeff. (%)
Chrysene	13.4	13.28	3.3	± 1.22	9.20
B(b)f	12.0	12.10	1.0	± 0.37	3.06
B(e)p	7.5	7.65	4.8	± 2.06	26.98
B(a)p	11.7	11.10	7.1	± 2.63	23.66
Perylene	9.2	8.89	5.5	± 2.02	22.68
Db(a,j)a	12.9	11.84	2.3	± 0.85	7.12
I(cd)p	11.8	11.67	2.5	± 0.92	7.93
B(ghi)p	12.4	11.49	3.4	± 1.26	10.95

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