Carcinogenic Polycyclic Aromatic Hydrocarbons in Norwegian Smoked Meat Sausages

Kristen Fretheim

Samples of a bologna type cooked sausage and a dry fermented type sausage made from mutton were analyzed for 12 polycyclic aromatic hydrocarbons. Cleanup was performed by digestion in methanolic potassium hydroxide, two sequences of liquid–liquid extraction, and chromatography on silica gel and Sephadex LH 20 columns. The final separation and quantification were done by gas–liquid chromatography on a high efficiency packed glass column. The amounts detected were generally low, strongly carcinogenic benzo[*a*]pyrene being present in a maximum concentration of 0.15 ppb.

The carcinogenic properties of certain polycyclic aromatic hydrocarbons (PAH) have been recognized for more than 40 years (Cook et al., 1932). Including derivatives, nearly 200 compounds of this type are considered carcinogenic, the examples of Figure 1 being among the most potent carcinogens known to experimental animals (Schmidt, 1969).

The occurrence of PAH in smoked food products is part of the general pollution problem caused by this class of substances. The compounds are formed during the incomplete combustion of all kinds of organic materials and dissipate via the atmosphere. Thus, foods of vegetable origin have been shown to contain several parts per billion of PAH, representing the main source of carcinogenic hydrocarbons ingested by humans (Grimmer and Hildebrandt, 1965a,b; Fritz, 1971).

Meat and fish are not subject to direct contamination by airborne pollutants, but the smoke utilized for curing inevitably contributes some PAH. It has been verified, however, that properly conducted industrial smoking of foods will result in a benzo[*a*]pyrene content of less than 1.0 ppb in the products (Tóth and Blaas, 1972a). Accordingly, in the Federal Republic of Germany, 1.0 ppb has recently been laid down as the legal maximum amount of benzo[*a*]pyrene in the edible part of smoked meat products.

Representative values for reported PAH contents of smoked foods are tabulated in Table I. The considerable variations observed can be related to a number of factors. With the smoldering wood smoke generators the supply of air is the most important parameter, determining to a large extent both the temperature and the rate of partial combustion. Tóth and Blaas (1972b) found that the relative amounts of PAH increased linearly with increasing smoldering temperature. Also, the smoldering of softwood from conifers results in higher relative amounts of PAH than does the use of hardwoods (Kersken, 1973). However, the amounts of PAH reaching the goods can be reduced significantly by cooling, washing, and/or filtration of the smoke (Tóth and Blaas, 1972b). Synthetic or cellulose casings reduce the diffusion of PAH into the emulsion to about 30% of the accessible amount (Filipović and Tóth, 1971). Furthermore, it should be noted that more recent equipment is advantageous in this regard. Smoke from the friction generator contains only one-third of the usual amount of PAH (Tilgner and Daun, 1969) and vaporous smoke contains only trace amounts (Reuter and Heinz, 1969).

This investigation was undertaken to determine whether the level of contamination from PAH in Norwegian smoked meat products calls for improvement measures during production. For this purpose the most commonly consumed product and the most heavily smoked product were chosen for analyses.

Norwegian Food Research Institute, N-1432 Ås-NLH, Norway.

EXPERIMENTAL PROCEDURE

Samples. Eight samples of a bologna type cooked sausage (commonly consumed, röykt kjottpölse) and five samples of a dry fermented sausage made from mutton (relatively heavily smoked, fårepölse) were collected from various parts of Norway. The sausages were stored frozen, and the bologna type was prepared in the usual household manner by heating in water for 20 min prior to analyses. The casings were removed from both types of sausage before grinding, and samples of 500 g with the bologna type and 300 g with the dry fermented type were analyzed. It should be noted that a difference in water content of samples must be adjusted for since rather delicate liquid–liquid extractions are involved.

Cleanup. The PAH extract was prepared in accordance with Grimmer and Böhnke (1975). The specific procedure used differs on four minor points only. (1) Sephadex LH 20 was purified by passing 150 ml of methanol followed by 100 ml of propan-2-ol through the column; (2) as internal standards benzo[*b*]chrysene and 2,2′-dinaphthyl in cyclohexane were employed simultaneously; (3) in the final step of the liquid–liquid extractions 240 ml (as opposed to 120 ml) of cyclohexane was used in accordance with Grimmer and Hildebrandt (1972); (4) two drops of dimethylformamide were added to the concentrated Sephadex column eluate, and the remainder of other solvents was evaporated in a stream of nitrogen at room temperature.

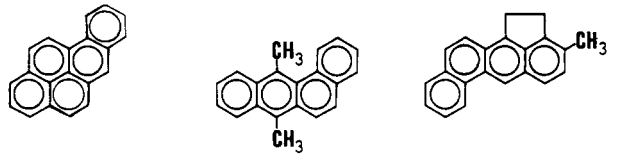
Gas–Liquid Chromatography (GLC). A Perkin-Elmer 900 gas chromatograph was employed. Impregnated Gas-Chrom Q column packing as well as the internal standard benzo[*b*]chrysene were provided by Professor Grimmer in connection with a collaborative study of Grimmer and Böhnke's (1975) procedure, and the 10 m × 2 mm i.d. glass column was packed accordingly. Our GLC parameters were the following: oven temperature isothermal at 260 °C; injection port and detector temperatures set at 325 and 375 °C, respectively; carrier gas (nitrogen) supplied with a head pressure of 5.6 kg/cm^2, giving a flow rate of 32 ml of gas of room temperature per min at an oven temperature of 260 °C. The retention time for the last reference PAH to elute was about 110 min.

Tentative identifications based on retention times were improved by duplicating the runs, a reference chromatogram being run in between. Figure 2 gives an example of a sample chromatogram. Quantification was performed manually by measuring peak heights and comparing the

Table I. Carcinogenic PAH's in Smoked Meat Products, ppb; Literature Data

	Benz[*a*]-anthracene	Chrysene	Benzo[*k*]-fluoranthene	Benzo[*a*]-pyrene	Dibenz[*a,h*]-anthracene	Benzo[*g,h,i*]-perylene	Literature
Bacon	0	0		0		Trace	Lijinsky and Shubik (1965)
Chipped beef	0.4			0		0	Howard et al. (1966)
Ham	2.8			3.2		1.4	Howard et al. (1966)
Ham[a]	1.3	0.5		0.7		0	Malanoski et al. (1968)
Ham[a]	9.6	2.6		0.7		0	Malanoski et al. (1968)
Hot sausage[a]	0.5	1.0		0.4		0	Malanoski et al. (1968)
Mutton, home smoked				1.3			Bailey and Dungal (1958)
Kochwurst (casing incl.)	0.5	0.3		0.26	0	0.1	Grimmer and Hildebrandt (1967)
Frankfurters	2.2	2.8	Qual.[b]	1.1		0.1	Filipovic and Tóth (1971)
Frankisch black smoked ham	12.0	16.8	Qual.[b]	3.8	Qual.[b]	2.4	Tóth (1971)
Ham black smoked in smoke from smoldering	6.1	12.0		7.1	Qual.[b]	5.8	Tóth and Blaas (1972a)

[a] These samples were picked out from a large selection since preliminary investigations disclosed a significant level of benzo[*a*]pyrene. [b] Qualitatively detected.



I II III

Figure 1. Strongly carcinogenic polycyclic aromatic hydrocarbons: (I) benzo[*a*]pyrene, (II) 7,12-dimethylbenz-[*a*]anthracene, (III) 3-methylcholanthrene.

values of peak height × retention time with those calculated from chromatograms of a reference solution. Reference compounds were generally obtained from commercial sources (Table II).

SAFETY PRECAUTIONS

As is evident from Table II, many of the reference compounds handled are potent carcinogens. Therefore, precautions were taken at all stages of the work to avoid inhalation of compound dust, spillage of solutions, and

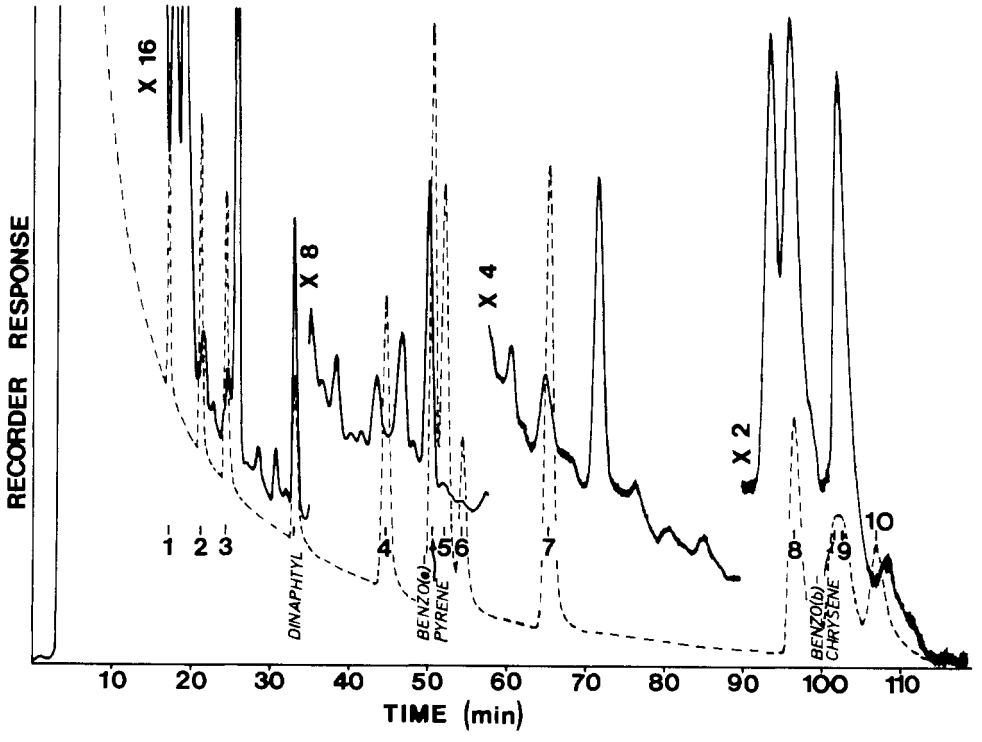


Figure 2. Reference (dashed line) and example of sample (solid line) chromatograms. The names of the reference compounds associated with the numbered reference peaks are given in Table III; the relative retention times were verified through chromatography of the individual compounds.

Table II. Carcinogenic Activities and Commercial Sources of PAH's

Compound	Carcinogenic act.[a,b]	Compd procured from
Benzo[*c*]phenanthrene	+++	Merck-Schuchardt
Benz[*a*]anthracene	+	Sigma
Chrysene	±	Koch-Light
Triphenylene	*c*	Fluka
Benzo[*k*]fluoranthene	–[d]	Schreiber[f]
7,12-Dimethylbenz[*a*]-anthracene	++++	Fluka
Benzo[*e*]pyrene	–	Merck-Schuchardt
Benzo[*a*]pyrene	+++	Koch-Light
Perylene	–	Koch-Light
3-Methylcholanthrene	++++	Koch-Light
Dibenz[*a,h*]anthracene	+++	Fluka
Picene	–[d]	Koch-Light
Benzo[*g,h,i*]perylene	–[e]	Koch-Light

[a] Unless otherwise stated, taken from: Committee on Biologic Effects of Atmospheric Pollutants (1972). [b] Code: –, not carcinogenic; ±, uncertain or weakly carcinogenic; +, carcinogenic; ++, +++, ++++, strongly carcinogenic. [c] The survey by Hartwell and Shubik (1951) indicates not carcinogenic for this compound. [d] Jones and Matthews (1974) give uncertain or weakly carcinogenic for this compound. [e] Souci (1968) gives strongly carcinogenic (++) for this compound. [f] Olaf Schreiber, D-2 Hamburg 50, Scheplerstr 1, Western Germany.

leaks from the GLC instrument.

RESULTS AND DISCUSSION

The compounds analyzed for have been identified by comparison of retention times and co-chromatography only; the retention times relative to perylene generally corresponded well with the values reported by Grimmer and Böhnke (1975). Due to the minute amounts present, attempts at systematic identification by mass spectrometry (MS) were unsuccessful. However, MS disclosed the presence of impurities in the dinaphthyl peak. For this reason, the product of height × retention time for the benzo[*b*]chrysene peak has consistently been used as the basis for quantification. With a few of the samples minor problems in estimating the peak height were caused by the presence of picene or an impurity with equivalent retention time; the compound(s) eluted partially overlapping the benzo[*b*]chrysene peak.

Mass spectrometry revealed that the major peak of nearly identical retention time to noncarcinogenic benzo[*e*]pyrene contained no detectable amount of this compound. The persistence of the impurity forced benzo[*e*]pyrene to be excluded from the analysis. The compound remained part of the reference solution, however, to allow regular checks on the somewhat difficult separation from highly potent benzo[*a*]pyrene (Figure 2). An estimation of the efficiency of the column indicated about 25 000 HETP.

The positive identification of compounds was no prerequisite under the scope of this work. If the PAH's in question were present in the analyzed material, the values reported in Tables III and IV are the maximum amounts. Beyond unsuccessful MS, no attempts were made at estimating the quantities of impurities possibly present in the peaks taken to represent the various PAH's. In view of these facts, the obtained quantitative data are to be considered as maximum contents of the respective compounds rather than absolute amounts. Therefore, it was felt appropriate to present the raw data as such, rather than presenting means and variation coefficients.

In general, quantification was subject to the greatest error with the first four PAH peaks to elute. The occurrence of impurities is the most frequent and their

Table III. Maximum Amounts of PAH's Present in Fermented, Smoked, and Dried Sausages Made from Mutton, ppb

Peak no.[a]		Samples 1	2	3	4	5
1	Benzo[*c*]phenanthrene	0.70	0.25	2.15	1.20	0.85
2	Benz[*a*]anthracene	0.30	0.05	0.20	0.15	0.10
3	Chrysene + triphenylene	1.95	0.35	4.50	0.25	0.07
4	Benzo[*k*]fluoranthene + 7,12-dimethylbenz[*a*]anthracene	1.35	0.15	0.25	0.20	0.05
5	Benzo[*a*]pyrene	0.15	0.07	0.08	0.07	ND[b]
6	Perylene	0.06	0.07	0.15	0.07	ND
7	3-Methylcholanthrene	0.06	0.07	0.15	0.04	0.15
8	Dibenz[*a,h*]anthracene	0.07	0.07	0.60	0.05	0.15
9	Picene	1.70	1.40	1.15	0.20	0.55
10	Benzo[*g,h,i*]perylene	0.35	0.25	0.50	0.40	0.35

[a] See Figure 2. [b] ND, not detected. To simplify tabulations a general detection limit of 0.04 ppb was adopted. Due to variations in response factors, chromatograms, etc., the actual detection limit was significantly lower in many instances.

Table IV. Maximum Amounts of PAH's Present in Bologna Type Cooked and Smoked Sausages, ppb

Compound	Samples 1 A[c]	1 B	2 A	2 B	3 A	3 B	4 A	4 B	5	6	7	8
Benzo[*c*]phenanthrene	0.70	0.55	0.15	0.50	0.35	0.35	0.15	0.40	0.45	0.65	1.05	0.40
Benz[*a*]anthracene	0.08	0.04	ND[b]	0.05	ND	ND	0.06	ND	0.20	0.15	0.55	0.08
Chrysene + triphenylene	0.25	0.20	0.35	0.30	0.15	0.15	0.55	0.15	0.85	0.40	1.20	0.55
Benzo[*k*]fluoranthene + 7,12-dimethylbenz[*a*]anthracene	1.25	0.30	2.15	0.20	0.40	0.06	0.40	0.07	0.20	0.10	0.05	0.15
Benzo[*a*]pyrene	ND	0.08	ND	ND	ND	ND	ND	ND	0.05	0.05	ND	ND
Perylene	0.07	ND	0.05	ND	ND	ND	0.04	ND	0.05	0.05	ND	ND
3-Methylcholanthrene	0.04	ND	ND	ND	ND	ND	ND	ND	0.10	0.15	ND	ND
Dibenz[*a,h*]anthracene	*a*	*a*	1.10	1.00	0.04	ND	ND	ND	0.05	0.05	0.05	0.15
Picene	0.18	0.05	0.30	0.60	0.35	0.60	0.60	0.60	0.65	0.05	0.75	0.60
Benzo[*g,h,i*]perylene	0.08	ND	0.06	ND	ND	0.05	ND	0.04	0.15	0.05	0.20	0.20

[a] Value unobtainable due to interfering impurities. [b] ND, not detected. See footnote *b* to Table III. [c] Duplicates.

concentrations the highest in this region, where compounds also are less easily separated. These are probably the reasons why considerable variations can be observed in the estimated concentrations of the first few PAH's to elute. Specifically, the reproducibility of the methods used, as judged from the results of the four duplicate analyses, clearly appears the least satisfactory for benzo[*k*]fluoranthene and 7,12-dimethylbenz[*a*]anthracene. Grimmer and Böhnke (1975), following the same procedure, reported a remarkable precision in the triplicate analysis of meat spiked with PAH's. However, the concentrations of PAH involved in their study were generally more than 100 times the ones reported here.

As far as two of the strongest carcinogens, benzo[*a*]pyrene and 3-methylcholanthrene, are concerned, the data leave no doubt that the amounts present, if any, are very low. With the majority of the bologna type sausages these compounds could not be detected at all, and in many cases the chromatograms were good enough to allow detection well below the practical limit of 0.04 ppb given.

It has been assumed in the present work that, for practical purposes, the recoveries of the various PAH's are the same in relation to the internal standard benzo[*b*]chrysene. This is in accordance with the reported findings of Grimmer and Böhnke (1975). In a study of a former version of the method, differing in the use of chromatography on aluminum oxide, and ultraviolet spectroscopy, recoveries in the range 75 to 88% were observed for various PAH's (Grimmer and Hildebrandt, 1972).

It seems evident from the results obtained that PAH's do not represent a serious contamination in the meat products investigated. This conclusion can probably be extended to include all Norwegian industrially smoked meat products since the dry, fermented sausage analyzed is the most heavily smoked commercially available product. It may be refuted that the casings of the sausages were not included in the analyzed material. However, neither these casings nor, in analogy, the rind of smoked ham are ordinarily eaten. Also, the very low levels detected leave a wide margin to, for example, the legally allowed content of 1.0 ppb of benzo[*a*]pyrene in the meat products of the Federal Republic of Germany.

The smoking equipment of Norwegian meat processing plants consists, almost without exception, of a smoldering wood generator and chamber of German or Austrian origin. Beechwood is the usual material used for burning, and the smoking process is carried out without any special measures against PAH contamination being taken. Thus, the explanation of the very low concentrations found probably lies with a tradition of light smoking. The black-smoked products of, for example, Germany do not have their counterparts in Norway, and it is generally assumed that Norwegian products would be judged lightly smoked by an international panel. As far as the potential health risk from PAH's is concerned, this is evidently an advantage.

ACKNOWLEDGMENT

The author thanks G. Grimmer, Biochemisches Institut für Umweltcarcinogene, Hamburg, Federal Republic of Germany, for providing the GLC column packing used. Thanks are also due to Grethe Enersen for skillful technical assistance.

LITERATURE CITED

Bailey, E. J., Dungal, N., *Br. J. Cancer* **12**, 348 (1958).
Committee on Biologic Effects of Atmospheric Pollutants, "Particulate Polycyclic Organic Matter", National Academy of Sciences, Washington, D.C., 1972, pp 5–11.
Cook, J. W., Hieger, I., Kennaway, E. L., Mayneord, W. V., *Proc. R. Soc. London, Ser. B* **111**, 455 (1932).
Filipović, J., Tóth, L., *Fleischwirtschaft* **51**, 1323 (1971).
Fritz, W., *Ernaehrungsforschung* **16**, 547 (1971).
Grimmer, G., Böhnke, H., *J. Assoc. Off. Anal. Chem.* **58**, 725 (1975).
Grimmer, G., Hildebrandt, A., *Dtsch. Lebensm.-Rundsch.* **61**, 237 (1965a).
Grimmer, G., Hildebrandt, A., *Z. Krebsforsch.* **67**, 272 (1965b).
Grimmer, G., Hildebrandt, A., *Z. Krebsforsch.* **69**, 223 (1967).
Grimmer, G., Hildebrandt, A., *J. Assoc. Off. Anal. Chem.* **55**, 631 (1972).
Hartwell, J. L., Shubik, P., "Survey of Compounds Which Have Been Tested for Carcinogenic Activity", Public Health Service Publication 149, 2nd ed, U.S. Government Printing Office, Washington, D.C., 1951, compound no. 765.
Howard, J. W., Teague, R. T., Jr., White, R. H., Fry, B. E., Jr., *J. Assoc. Off. Anal. Chem.* **49**, 595 (1966).
Jones, D. W., Matthews, R. S., *Prog. Med. Chem.* **10**, 159 (1974).
Kersken, H., *Fleischwirtschaft* **53**, 921 (1973).
Lao, R. C., Thomas, R. S., Oja, H., Dubois, L., *Anal. Chem.* **45**, 908 (1973).
Lijinsky, W., Shubik, P., *Ind. Med. Surg.* **34**, 152 (1965).
Malanoski, A. J., Greenfield, E. L., Barnes, C. J., Worthington, J. M., Joe, F. L., *J. Assoc. Off. Anal. Chem.* **51**, 114 (1968).
Reuter, H., Heinz, G., *J. Assoc. Off. Anal. Chem.* **49**, 169 (1969).
Schmidt, F., *Ernaehr.-Umsch.* **16**, 172 (1969).
Souci, S. W., *Dtsch. Lebensm.-Rundsch.* **64**, 235 (1968).
Tilgner, D. J., Daun, H., *Residue Rev.* **27**, 19 (1969).
Tóth, L., *Fleischwirtschaft* **51**, 1069 (1971).
Tóth, L., Blaas, W., *Fleischwirtschaft* **52**, 1121 (1972a).
Tóth, L., Blaas, W., *Fleischwirtschaft* **52**, 1419 (1972b).

Received for review December 11, 1975. Accepted May 18, 1976.