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Polycyclic Aromatic Hydrocarbons in Grilled Food

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The levels of 22 polycyclic aromatic hydrocarbons (PAH) were determined in 63 samples of grilled meat and meat products by capillary gas chromatography. The results reveal that the PAH levels are strongly dependent on the method of cooking and type of heat source used. The grilling of frankfurters in the flames of a log fire resulted in extremely high PAH levels, up to 212 $\mu\text{g}/\text{kg}$ benzo[a]pyrene (BaP). When the grilling was carried out over the embers, the average level of BaP was only 7.7 $\mu\text{g}/\text{kg}$. Relatively high PAH levels, an average of 17.6 μg of BaP/kg, were found in frankfurters grilled over smoldering spruce or pine cones. The BaP levels in charcoal-grilled frankfurters did not exceed 1 $\mu\text{g}/\text{kg}$, whereas charcoal-grilled whole meat samples contained 2.3-6.1 $\mu\text{g}/\text{kg}$. Frying or electric broiling of frankfurters did not lead to any appreciable increase of the original trace levels. Extracts from flame-grilled frankfurters were mutagenic to *Salmonella typhimurium* TA 100 after metabolic activation.

The formation of mutagenic and carcinogenic substances during the cooking of food is currently the subject of intensive research (Sugimura and Nagao, 1979, 1982; Powrie et al., 1982). It is well-known that grilling food can lead to the production and uptake of polycyclic aromatic hydrocarbons (PAH), some of which are potent carcinogens. The presence of PAH in smoked food is also well documented [Toth and Blaas (1972a,b), Fritz and Soós (1977), Potthast (1978), Larsson (1982a), and many others]. PAH, particularly benzo[a]pyrene (BaP), have been suggested as one, among other, etiological factor in large bowel cancer (Hecht and La Voie, 1981) and stomach cancer (Soós, 1980). Many of the studies on PAH in grilled and smoked food have concentrated on the determination of BaP only. This substance has served as an arbitrary indicator of the possible presence of other PAH and has often been used as a quantitative index of chemical carcinogens in foods. In the Federal Republic of Germany a 1 $\mu\text{g}/\text{kg}$ limit for BaP in smoked meat products has been in force since 1973.

Systematic investigations on PAH in grilled meat were first carried out by Lijinsky and Ross (1967). Their results

showed that the levels of PAH in charcoal-grilled meat was dependent on the fat content and the closeness of the meat to the heat source. They explained their findings by the theory that melted fat from the heated meat drips onto the hot coals and is pyrolyzed, giving rise to the formation of PAH, which are then deposited on the meat surface as the smoke rises. Several reports (Fritz, 1973; Toth and Blaas, 1973; Doremire et al., 1979) confirm that charcoal-grilled lean meat products contain lower levels of PAH (less than 1 μg of BaP/kg) than products with a high fat content. Lijinsky and Ross (1967) and Toth and Blaas (1973) reported that charcoal grilling of meat in a vertical grill, where the dripping of fat onto the heat source is prevented, or broiling with electricity and gas, where the heat source is above the food, resulted in only minimum contamination by PAH.

Extremely high levels of PAH (maximum 140 μg of BaP/kg) were found by Fritz (1973) in meat and bratwurst grilled over burning pine cones. The fat content did not seem to influence the PAH level in this case. Fritz stated that the contamination was instead due to incomplete combustion of the fuel itself. Work by Toth and Blaas (1973) and Binnemann (1979) have confirmed that grilling over pine or spruce cones results in serious PAH contamination of the grilled product.

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Another mode of cooking, in which contamination of food by PAH could be expected, is the grilling in the flames of an open log fire. This grilling method is customary in Sweden but to our knowledge PAH levels in food prepared in this way have not been studied before. The present study was undertaken to obtain information of the PAH levels in meat and meat products grilled under conditions which are customary in Sweden. Furthermore, the influence of different fuels on the accumulation of PAH in grilled foods was studied.

Because of the complexity of the PAH fraction, a more detailed characterization of PAH in the samples requires a method with high resolution and sensitivity. In this work capillary gas chromatography with flame ionization detection was used for the simultaneous determination of 22 PAH components, including BaP.

MATERIALS AND METHODS

Samples and Cooking Methods. Frankfurter-type sausages without casing (in this paper referred to as frankfurters) were cooked, generally to a medium or well-done state, with a variety of heat sources. (a) Log fire: Ten to fifteen logs of fir or birch, each approximately $40 \times 15 \times 10$ cm, were used. The frankfurters were kept in or just above the flames. (b) Log fire embers: The samples were placed on a rack on top of the dying embers. (c) Cone fire: A common-type grill (diameter and depth of the well 37 and 8 cm, respectively) was filled with spruce or pine cones. Approximately 50 mL of fire-lighting fluid was applied and ignited. After 5–10 min the frankfurters were placed on the rack positioned 15–20 cm above the cones. (d) Electric oven: The samples were placed on a rack positioned 5 cm below the heating coils. (e) Frying pan: The frankfurters were fried with a little margarine to a well-done or burnt state. Frankfurters, pork chops (thickness 2 cm), chicken halves, and T-bone steaks (thickness 2 cm) were charcoal grilled to a medium or well-done state. (f) Charcoal fire: Conditions were the same as in cone grilling, except that the cooking started 15–20 min after ignition.

The edible parts of the samples were blended in a high-speed mixer and kept at -20 °C until analyzed.

Analysis. All samples were analyzed in duplicate. The method of workup and extraction was based on the work of Grimmer and Böhnke (1975). After addition of an internal standard (β,β -binaphthyl), the sample (40 g) was saponified, extracted, and cleaned up as described previously for smoked fish (Larsson, 1982a). The purified sample solution was analyzed by capillary gas chromatography. The gas chromatograph was a PYE Unicam GCD with a $50 \text{ m} \times 0.30 \text{ mm}$ SE-54 glass capillary column. The flame ionization detector was connected to an electronic integrator. Sample volumes of 1–4 μL were injected by the falling needle technique. Conditions were as follows: carrier gas, hydrogen, 3 mL/min; makeup gas; nitrogen, 30, mL/min; injector temperature, 260 °C; detector temperature, 350 °C; oven, 165 °C initially for 6 min, programmed 4 °C/min to 255 °C, and held at 255 °C for 30 min. The peaks were identified by comparing the retention times with those of standards. The identified compounds were quantified by comparing the integrated peak areas with that of the internal standard. In a few samples with high PAH levels the peak identities were further confirmed by GC/MS analysis.

The detection limits for individual PAH components in grilled food samples of 40 g were 0.1–0.3 $\mu\text{g}/\text{kg}$.

The recoveries of added BaP from frankfurters were $97 \pm 10.2\%$ ($n = 4$) at the 1.6 $\mu\text{g}/\text{kg}$ level and $108 \pm 1.2\%$ ($n = 6$) at the 88 $\mu\text{g}/\text{kg}$ level, respectively. The accuracy

of the method has also been checked by analyzing different samples of smoked and grilled foods in two intercalibration exercises (Bjørseth and Olufsen, 1978; Larsson, 1982b). The PAH levels in these samples were not certified, but the result obtained by the present method showed good agreement with the calculated mean results.

Mutagenicity Tests. A few extracts obtained from flame-grilled frankfurters were subjected to the agar plate mutagenicity assay using *Salmonella typhimurium* strain TA 100, as described by Ames et al. (1975). Aroclor 1254 induced rat liver homogenates (S-9) were used for metabolic activation (Ames et al., 1975). The same amount of cofactors was added to the S-9 mix regardless of the amount of S-9 fraction. The PAH extracts were gently evaporated to dryness and the residues were dissolved in dimethyl sulfoxide. After addition of the test sample in 100- μL portions, the plates were incubated at 37 °C for 24–36 h. All tests were performed with triplicate plates.

RESULTS AND DISCUSSION

Frankfurters Grilled by Different Techniques. The PAH levels found in frankfurters cooked in a variety of ways are listed in Table I. The samples are grouped according to the heat source applied. Minimum, maximum, mean, and median values of individual compounds and of the sums of 22 PAHs (ΣPAH) are given for each set of samples. The concentrations of BaP and ΣPAH in the whole sample material varied between not detectable and 212 and 9–2930 $\mu\text{g}/\text{kg}$, respectively.

The PAH levels in fried or electrically broiled frankfurters did not differ significantly from the original trace levels. Slightly increased levels were found in charcoal-grilled frankfurters, but the BaP concentration did not exceed 1 $\mu\text{g}/\text{kg}$. When charcoal was replaced by spruce or pine cones, the PAH contamination was markedly increased, the mean concentrations of BaP and ΣPAH being 17.6 and 377 $\mu\text{g}/\text{kg}$. The results from these multicomponent analyses are in agreement with previously reported data on BaP and other PAHs in grilled Bratwurst (Fritz, 1973; Toth and Blaas, 1973; Binnemann, 1979).

Extremely high levels of PAH were found in frankfurters that were grilled in the flames of an open log fire. The mean concentrations of BaP and ΣPAH were 54.2 and 905 $\mu\text{g}/\text{kg}$. The maximum concentration of BaP, 212 $\mu\text{g}/\text{kg}$, was found in a well-done, but perfectly edible, sample. When grilling was carried out over the embers, i.e., when flames no longer emerged from the fire, the PAH contamination was significantly lower. The mean concentrations of BaP and ΣPAH in this case were 7.7 and 270 $\mu\text{g}/\text{kg}$. The PAH levels in two samples of herring that were flame-grilled and grilled over embers, respectively, were comparable with the levels in the corresponding frankfurter samples (Larsson, 1982c).

The distribution of BaP levels in the 53 samples of grilled frankfurters, shown in Table II, demonstrates the influence of the different heat sources. The elevated PAH levels found in samples that were exposed to fumes from the combustion of fuels (wood, cones, charcoal) indicate that the bulk of PAH in these samples is due to the uptake of PAH formed during incomplete fuel combustion. It seems logical that the combustion of charcoal, being an already pyrolyzed material, gives a relatively clean smoke and, accordingly, lower PAH levels in the grilled sample. The in situ formation of PAH in the food seems to be of minor significance at the air temperatures (150–400 °C) obtained at the surface of the food under normal grilling conditions.

The PAH profile, i.e., the relative abundance of individual PAH components in the sample, was also influenced

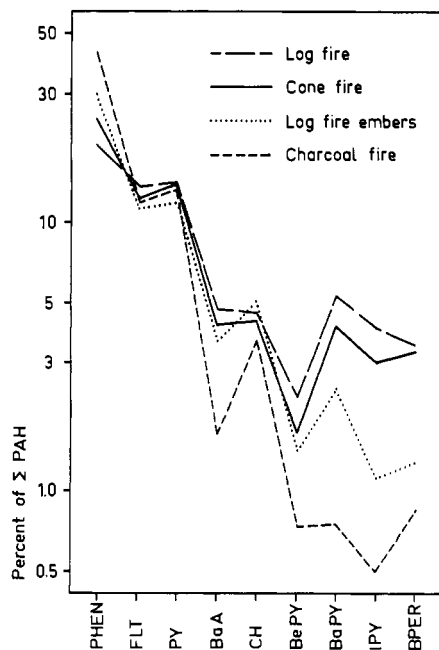


Figure 1. Profiles showing the percentage distribution of a number of PAH components (average ratios of PAH/ Σ PAH \times 100) in frankfurters grilled over various heat sources.

by the heat source. Figure 1 shows the percentage distribution of a number of PAH components (average ratios of PAH/ Σ PAH \times 100) in frankfurters grilled under various conditions. The proportion of high molecular PAHs showed a relative increase in the sample groups in the order charcoal fire, log fire embers, cone fire, and log fire.

Uptake of PAH in Flame-Grilled Frankfurters.

Samples of frankfurters were grilled simultaneously in the flames of a log fire at various distances from the burning wood. The samples, each consisting of five frankfurters, were positioned at 1, 6, 16, and 26 cm above the wood. The height of the flames was 20–35 cm during the experiment. Each sample was grilled to a well-done state. The levels of BaP and total PAH found in the grilled samples are plotted vs. the distance from the fuel in Figure 2a. In a second experiment, samples of frankfurters were positioned at 1, 7, 13, and 20 cm above the burning wood. The height of the flames was 15–20 cm. This time all samples were cooked for the same period of time (3.5 min), resulting in lightly grilled to burnt products, depending on the distance from the fuel. The change in concentrations of BaP and total PAH with increasing distance to the burning wood is illustrated in Figure 2b.

The results of these experiments show that the PAH levels in flame-grilled frankfurters are strongly dependent on the position of the samples in the flame during the grilling. Moderate levels were found in the samples grilled immediately above the fuel, whereas samples from the 6–7-cm zone showed dramatically increased PAH levels. Further up in the flames the PAH levels in the samples declined with increasing distance from the fuel. It seems probable that the presence of PAH in flame-grilled frankfurters is due to the uptake of PAH from the combustion fumes and that the change in PAH concentrations in the samples with the distance to the burning fuel reflects the distribution of PAH in the flame itself.

Another possibility regarding the formation of PAH is that PAH are formed in the food when the sample is subjected to elevated temperatures. However, the grilling of frankfurters in a propane flame of a Meker burner, when the measured flame temperature at the surface of the sample was ca. 600 °C, resulted in BaP levels of less than

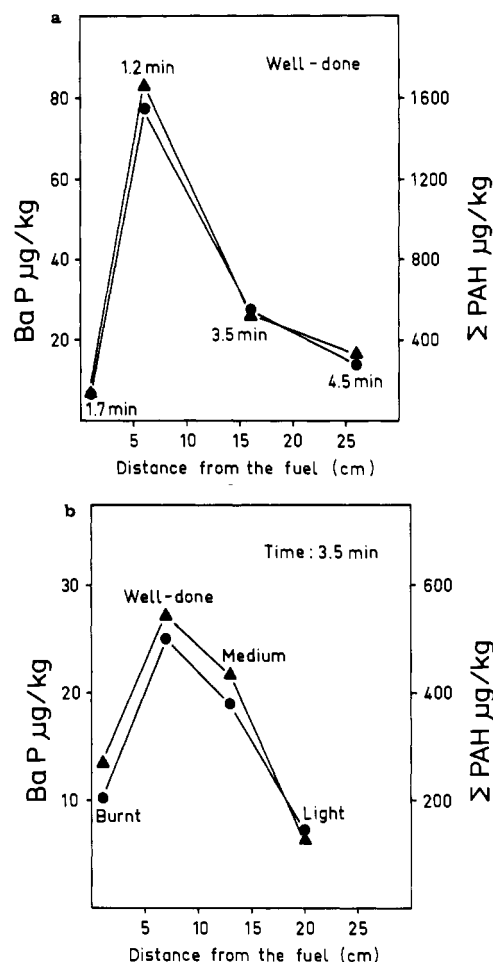


Figure 2. (a) Levels of BaP (▲) and Σ PAH (●) in samples of frankfurters simultaneously grilled in the flames of a log fire at various distances from the burning wood. All samples were grilled to a well-done state, which required the above given times. (b) Levels of BaP (▲) and Σ PAH (●) in samples of frankfurters simultaneously grilled in the flames of a log fire at various distances from the burning wood. All samples were grilled for the same period of time (3.5 min), resulting in lightly grilled to burnt products.

1 μ g/kg. When the experiment was repeated with the air inlet on the burner closed, to give a yellow reducing flame, and with the sample positioned so that the flame temperature at the surface was still around 600 °C, the concentration of BaP in the sample reached 15 μ g/kg. This implies that the in situ formation of PAH in flame-grilled frankfurters is of minor importance and that it is the process of fuel combustion that determines the levels of PAH in the grilled product.

Charcoal-Grilled Meats. The results of the analysis of PAH in charcoal-grilled meats are summarized in Figure 3. The results on charcoal-grilled frankfurters are included for comparison. The average levels of BaP and Σ PAH found in pork, T-bone steak and chicken were 3.6 and 221, 4.2 and 138, and 3.6 and 116 μ g/kg, respectively. These levels are significantly higher than those found in charcoal-grilled frankfurters, i.e., 0.3 and 51 μ g/kg. However, when frankfurters were grilled together with pork chops, the levels in the frankfurter samples were noticeably enhanced (Figure 3).

This confirms the theory, presented by Lijinsky and Ross (1967), that PAH in charcoal-grilled products originates from the pyrolysis of the fat that drips down on the hot coals during grilling. Comparatively small amounts of fat dripped from frankfurter samples during grilling, probably due to the fact that the fat in this product is

Table I. PAH Levels in Frankfurters Grilled by Different Techniques^a

		PAH, $\mu\text{g}/\text{kg}$					
		log fire, <i>n</i> = 17	log fire embers, <i>n</i> = 9	cone fire, <i>n</i> = 7	charcoal fire, <i>n</i> = 13	electric oven, <i>n</i> = 2	frying pan, <i>n</i> = 5
PHE	mean:	168	76.7	85.7	21.6	9.2	4.5
	median:	95.3	56.3	83.4	21.0	9.2	4.6
	max:	618	246	123	54.9	9.7	5.6
	min:	26.7	11.7	51.8	6.9	8.7	3.5
ANT	mean:	35.4	13.4	13.8	2.3	1.4	0.7
	median:	23.6	9.3	11.9	2.2	1.4	0.7
	max:	113	41.4	19.6	4.5	1.5	0.8
	min:	59.0	1.7	9.4	0.8	1.3	0.5
2-MPH	mean:	15.2	14.2	12.7	2.7	1.1	1.1
	median:	9.5	7.8	6.8	2.0	1.1	0.9
	max:	62.1	47.3	35.3	8.4	1.5	1.6
	min:	2.8	2.3	4.6	0.9	0.7	0.8
2-MAN	mean:	7.3	7.3	3.8	0.9	0.4	0.1
	median:	4.7	4.2	2.9	0.8	0.4	ND
	max:	34.6	26.2	9.4	2.3	0.6	0.4
	min:	1.4	1.7	1.6	ND	0.1	ND
1-MPH	mean:	14.4	11.5	18.8	2.6	0.9	0.7
	median:	11.6	6.5	8.7	2.0	0.9	0.6
	max:	55.1	40.3	57.6	10.3	1.2	1.4
	min:	2.9	1.8	5.6	0.7	0.6	0.5
9-MAN	mean:	2.1	1.6	0.4	0.3	ND	ND
	median:	1.8	1.1	0.2	ND	ND	ND
	max:	7.8	3.8	1.5	1.4	ND	ND
	min:	ND	ND	ND	ND	ND	ND
FLT	mean:	119	31.7	48.6	6.0	3.4	1.9
	median:	75.4	23.6	53.6	4.4	3.4	1.4
	max:	376	100	72.4	16.7	3.8	3.4
	min:	18.0	4.1	19.3	2.4	2.9	1.1
PY	mean:	127	32.5	55.4	6.5	3.4	1.8
	median:	75.5	20.5	63.6	5.6	3.4	1.4
	max:	452	101	84.1	16.0	3.8	3.1
	min:	19.6	5.5	20.6	2.4	2.9	1.2
BaFU	mean:	43.9	9.9	13.1	1.6	2.0	ND
	median:	21.8	4.4	15.2	1.2	2.0	ND
	max:	174	31.2	19.4	5.9	2.0	ND
	min:	4.3	1.5	4.4	ND	2.0	ND
BbFU	mean:	22.3	6.2	8.3	0.6	0.6	ND
	median:	12.4	3.1	8.8	0.5	0.6	ND
	max:	71.9	20.0	12.5	1.8	0.7	ND
	min:	3.1	0.6	2.8	ND	0.6	ND
1-MPY	mean:	16.2	6.8	6.3	0.7	0.4	ND
	median:	8.0	3.2	7.1	0.4	0.4	ND
	max:	55.0	23.5	9.9	2.7	0.5	ND
	min:	1.8	0.6	1.8	ND	0.3	ND
BaA	mean:	44.5	10.8	16.7	0.9	0.8	0.3
	median:	23.7	7.7	19.0	0.6	0.8	0.3
	max:	144	31.1	26.6	3.9	1.0	0.5
	min:	5.7	1.0	5.0	0.2	0.6	0.2
CH/TRI	mean:	44.1	15.1	16.8	2.0	1.1	0.6
	median:	22.3	9.9	18.5	1.4	1.1	0.6
	max:	140	48.0	25.6	6.3	1.3	1.2
	min:	5.5	1.4	6.1	0.4	0.9	0.3
BbF	mean:	29.8	6.4	8.9	0.4	0.1	ND
	median:	16.2	4.1	11.7	0.3	0.1	ND
	max:	92.3	19.8	16.2	1.2	0.2	ND
	min:	2.3	0.5	2.0	ND	ND	ND
Bj,kF	mean:	41.9	6.8	15.2	0.3	0.2	ND
	median:	21.9	4.4	19.2	0.1	0.2	ND
	max:	172	19.1	26.2	1.6	0.3	ND
	min:	1.1	0.6	2.0	ND	0.2	ND
BeP	mean:	21.8	4.3	7.1	0.4	0.1	ND
	median:	12.6	2.7	9.0	0.4	0.1	ND
	max:	80.9	14.6	12.4	1.0	0.2	ND
	min:	2.5	0.4	1.2	ND	ND	ND
BaP	mean:	54.2	7.7	17.6	0.3	0.2	0.1
	median:	27.9	5.2	22.8	0.3	0.2	0.1
	max:	212	25.5	30.8	1.0	0.3	0.2
	min:	5.7	0.6	2.1	ND	0.1	ND
PER	mean:	7.9	1.3	2.5	0.1	ND	ND
	median:	3.6	0.8	2.5	ND	ND	ND
	max:	27.9	4.0	4.0	0.4	ND	ND
	min:	0.5	0.1	0.6	ND	ND	ND

Table I (Continued)

		PAH, $\mu\text{g}/\text{kg}$					
		log fire, $n = 17$	log fire embers, $n = 9$	cone fire, $n = 7$	charcoal fire, $n = 13$	electric oven, $n = 2$	frying pan, $n = 5$
IPY	mean:	41.4	4.3	12.9	0.2	ND	ND
	median:	18.6	2.4	15.1	0.1	ND	ND
	max:	171	16.3	21.6	0.8	ND	ND
	min:	2.8	ND	2.2	ND	ND	ND
DBA	mean:	3.5	0.6	1.5	ND	ND	ND
	median:	2.6	0.6	1.6	ND	ND	ND
	max:	8.8	1.3	2.0	ND	ND	ND
	min:	0.2	ND	ND	ND	ND	ND
BghiP	mean:	35.5	4.0	14.3	0.4	ND	ND
	median:	18.1	1.8	17.8	0.2	ND	ND
	max:	153	14.1	23.7	1.3	ND	ND
	min:	2.5	ND	2.7	ND	ND	ND
AA	mean:	14.9	2.6	5.9	ND	ND	ND
	median:	7.0	2.7	6.7	ND	ND	ND
	max:	66.5	4.0	8.3	ND	ND	ND
	min:	1.1	ND	0.6	ND	ND	ND
Σ PAH	mean:	905	269	377	51	24	12
	median:	550	174	396	40	24	13
	max:	2930	878	536	141	27	15
	min:	124	40	172	17	22	9

^a ND = not detected; PHE = phenanthrene; ANT = anthracene; 2-MPH = 2-methylphenanthrene; 2-MAN = 2-methylanthracene; 1-MAN = 1-methylphenanthrene; 9-MAN = 9-methylanthracene; FLT = fluoranthene; PY = pyrene; BaFU = benzo[a]fluorene; BbFU = benzo[b]fluorene; 1-MPY = 1-methylpyrene; BaA = benz[a]anthracene; CH/TRI = chrysene and triphenylene; BbF = benzo[b]fluoranthene; Bj,kF = benzo[j]fluoranthene and benzo[k]fluoranthene; BeP = benzo[e]pyrene; BaP = benzo[a]pyrene; PER = perylene; IPY = indeno[1,2,3-cd]pyrene; DBA = dibenzanthracenes; BghiP = benzo[ghi]perylene; AA = anthanthrene; Σ PAH = sum of 22 PAHs.

Table II. Distribution of Benzo[a]pyrene Levels in 53 Samples of Frankfurters Grilled by Different Techniques

heat source	no. of samples	no. of samples with BaP levels in $\mu\text{g}/\text{kg}$							
		-0.1	0.1-0.5	0.5-1	1-5	5-10	10-50	50-100	>100
log fire	17					4	6	4	3
cone fire	7				2	1	4		
log fire embers	9			3	1	2	3		
charcoal fire	13	3	7	3					
frying pan	5	3	2						
electric oven	2	1	1						
"ungrilled"	2	1	1						

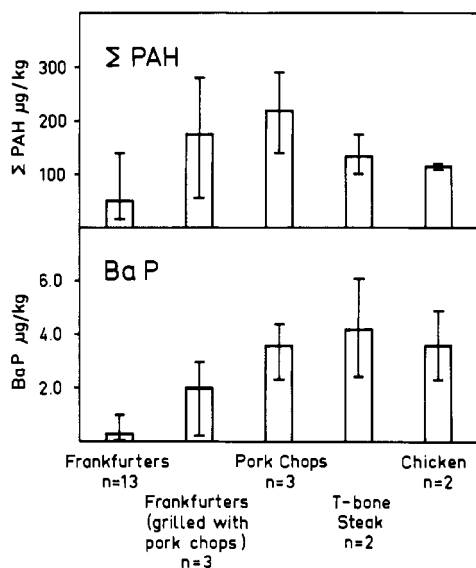


Figure 3. Levels of BaP and Σ PAH in samples of charcoal-grilled products.

bound up as a stable emulsion. This explains why charcoal-grilled frankfurters show low PAH levels compared to whole meat samples.

Mutagenicity of PAH Extracts from Flame-Grilled Frankfurters. Initial experiments showed that standard

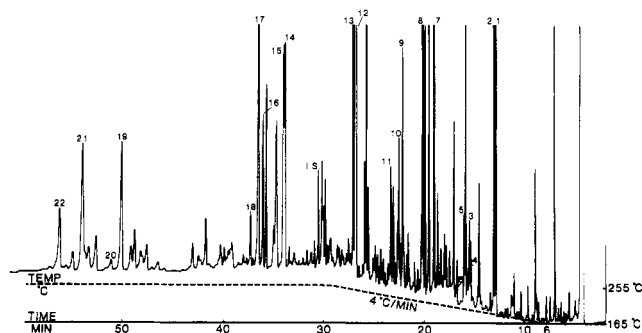


Figure 4. Gas chromatogram of a PAH extract from a sample of flame-grilled frankfurters. (1) Phenanthrene, (2) anthracene, (3) 2-methylphenanthrene, (4) 2-methylanthracene, (5) 1-methylphenanthrene, (6) 9-methylanthracene, (7) fluoranthene, (8) pyrene, (9) benzo[a]fluorene, (10) benzo[b]fluorene, (11) 1-methylpyrene, (12) benz[a]anthracene, (13) chrysene and triphenylene, (14) benzo[b]fluoranthene, (15) benzo[j]fluoranthene and benzo[k]fluoranthene, (16) benzo[e]pyrene, (17) benzo[a]pyrene, (18) perylene, (19) indeno[1,2,3-cd]pyrene, (20) dibenzanthracenes, (21) benzo[ghi]perylene, (22) antanthrene, and (I.S.) β,β -binaphthyl (internal standard).

mixtures of PAH were mutagenic toward *S. typhimurium* strains TA 98 and TA 100 in the presence of S-9 liver homogenate. Strain TA 100 showed the highest sensitivity and was used in the experiments reported here.

Figure 4 shows a gas chromatogram of a PAH extract

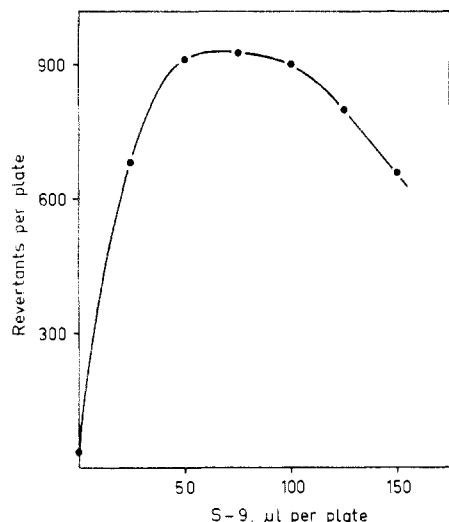


Figure 5. Effect of various amounts of S-9 on the mutagenicity of a PAH fraction isolated from flame-grilled frankfurters toward *S. typhimurium* TA 100.

Table III. Mutagenic Activity of PAH Extracts from Samples of Frankfurters Grilled in the Flames of a Log Fire

concn of BaP in sample, $\mu\text{g}/\text{kg}$	amount of BaP present in PAH extract, $\mu\text{g}/\text{plate}$	g equiv/plate ^a	TA 100 revertant ^b colonies
6.0	0.22	57	619
60	0.26	9	789
160	0.26	4	805

^a Equivalent weight in grams of grilled frankfurter.

^b Tested in the presence of 75 μL of S-9 and adjusted for spontaneous frequency.

from a sample of flame-grilled frankfurters with levels of BaP and ΣPAH of 160 and 2690 $\mu\text{g}/\text{kg}$. Aliquots of this extract were assayed for mutagenic activity toward TA 100. The effect of various amounts of S-9 on the response to an aliquot containing 0.5 μg of BaP is shown in Figure 5. With aliquots containing up to 0.26 μg of BaP, a linear dose-response curve was found, as illustrated in Figure 6, for the example extract in the presence of 75 μL of S-9.

The mutagenic activity found in extracts from three samples of flame-grilled frankfurters with the BaP levels 6.0, 60, and 160 $\mu\text{g}/\text{kg}$ are compared in Table III. The applied aliquots, each of such a size that it contained ca. 0.25 μg of BaP and equivalent to 4–57 g of grilled frankfurters, gave approximately the same number of revertants. It appears from the table that the mutagenic response is correlated to the amount of BaP in the extracts. The presence of varying amounts of coextracted matrix material did not seem to influence the response.

The results of the few experiments reported here show that PAH extracts from flame-grilled frankfurters are mutagenic toward strain TA 100 in the presence of S-9 liver homogenate. The mutagenicity observed is not necessarily due to PAH exclusively, as the extract may contain other compounds with mutagenic potential. Further studies will be necessary to determine the contribution of individual compounds to the mutagenic activity.

CONCLUSIONS

The present study on PAH in grilled foods comprises meat and meat products grilled under conditions that are customary in Sweden. The results reveal that the PAH levels in grilled foods are strongly dependent on the me-

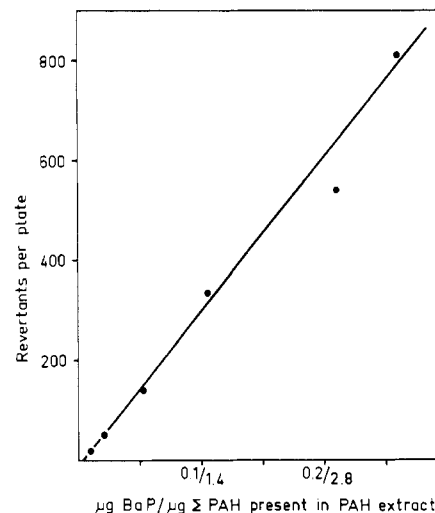


Figure 6. Yield of revertants with various amounts of a PAH-containing extract from flame-grilled frankfurters using 75 μL of S-9 mix.

thod of cooking and type of heat source used. Frying or electrical broiling does not lead to the production of PAH. The hydrocarbons seem to arise mainly from incomplete combustion of the fuel. Grilling over charcoal, the most frequently used fuel, results in low or somewhat elevated PAH levels, depending on the fat content of the food and which form the fat is present. The use of pine or spruce cones gives rise to high PAH levels in the grilled product. When food is grilled over a log fire, in direct contact with the flames, extremely high PAH levels can be expected. The levels of BaP in flame-grilled products may in fact exceed the present German 1 $\mu\text{g}/\text{kg}$ limit for smoked meat products by 2 orders of magnitude. Extracts from flame-grilled frankfurters have been shown to be mutagenic in the Ames test.

Whether the ingestion of grilled foods constitutes an increased risk to health has to be studied in suitable animal experiments. It should be noted that in one of the northern districts of Iceland, where the mortality rate from gastric cancer was found to be among the highest, the consumption of singed sea birds was particularly common (Thorsteinsson and Thordarson, 1968; Sigurjónsson, 1966). The process of singeing resembles that of flame grilling and gives also rise to high PAH levels in foods.

The grilling of food has become an increasingly popular outdoor activity during the summer in Sweden. On the basis of the results of the present study and with the intention of decreasing the intake of carcinogenic substances, the National Food Administration has issued the following recommendation concerning the grilling of foods: Avoid grilling over a burning log fire: wait until embers are formed and flames no longer emerge. Do not use pine or spruce cones as a fuel.

Registry No. PHE, 85-01-8; ANT, 120-12-7; 2-MPH, 2531-84-2; 2-MAN, 613-12-7; 1-MAN, 832-69-9; 9-MAN, 779-02-2; FLT, 206-44-0; PY, 129-00-0; BaFu, 238-84-6; BbFu, 243-17-4; 1-MPY, 2381-21-7; BaA, 56-55-3; CH, 218-01-9; TRI, 217-59-4; BbF, 205-99-2; Bj, 205-82-3; kF, 207-08-9; BeP, 192-97-2; BaP, 50-32-8; PER, 198-55-0; IPY, 193-39-5; DBA, 414-29-9; BghiP, 191-24-2; AA, 191-26-4.

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Guayule Byproduct Evaluation: Extract Characterization

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Composition profiles have been made of the water and acetone extracts of guayule woody tissue. Extractables from cultivars 593, N575, N576, 11634, 11635, and 12229 were surveyed. The major acetone extract components assayed were sesquiterpene esters (10-15%), triterpenoids (27%), and fatty acid triglycerides (7-19%). Sesquiterpene ester levels reflected processing heat history. All major extract triterpenoids were found to be C₃₀ compounds. Dilute acid hydrolysis of aqueous extract polysaccharides (63% of the extract) did not provide a good source of fermentable sugars.

As a renewable, native source of natural rubber (Campos-Lopez et al., 1979; Eagle, 1981), guayule [*Parthenium argentatum* (Gray)] is undergoing careful economic assessment. Current processing employs acetone to extract resinous components from the milled shrub or coagulated latex (Eagle, 1981). For every kilogram of rubber there can be one or more kilograms of resin (Buchanan et al., 1978; Burlett et al., 1981). Marketing high-value resin-based byproducts could significantly reduce guayule rubber manufacturing costs. Characterizing resin composition is necessary to determine optimum utilization. Consequently, byproduct evaluation should be consistent with commercially feasible processing.

A variety of secondary metabolites can be extracted from guayule. These include non-rubber isoprenoids such as terpenes and sesquiterpenes (Haagen-Smit and Siu, 1944), sesquiterpene esters (guayulins A and B) (Romo et al., 1970; Proksch et al., 1981), diterpene ketoalcohols (Dorado Bernal et al., 1962), phytosterols (Buchanan et al., 1978; Klein and Pirschle, 1923; Schmid and Stoehr, 1926), and triterpene ketoalcohols (argentatins A, B, and C) (Rodriguez-Hahn et al., 1970). Fatty acid triglycerides have been saponified and the acids characterized (Buchanan et al., 1978; Keller et al., 1981; Belmares et al., 1980). Polyphenolics (Buchanan et al., 1978), including leaf flavonoids and flavonoid glycosides (Mears, 1980), have been identified. In addition, there exists a wide range of water extractables including proteins (Banigan et al., 1982) and mono- and polysaccharides (Banigan et al., 1982; Traub and Slattery, 1946).

We report here the quantitation of some major components and component classes in the acetone and water

Table I. Shrub Procurement and Woody Tissue Extraction

cultivar	source ^a	time of harvest	age at harvest, year ^b	extract yield, wt %	
				aqueous	organic
593	UA	3/81	3	15.0	6.5
593	LA	4/81	4	14.5	7.4
593	LA	9/81	4		
N575	UA	8/81	3.25		
N576	UA	8/81	4		
11634	LA	9/81	4	9.5	11.0
11635	LA	9/81	4		
12229	UA	8/81	4		

^a UA: University of Arizona, Tucson, AZ; LA: Los Angeles State and County Arboretum, Arcadia, CA. ^b Includes 1 year in greenhouse prior to field transplant.

extracts of guayule woody tissue. Non-rubber isoprenoid component compositions are detailed. While our primary emphasis was on characterizing cultivar 593 extractables, survey analyses were also run for cross comparison of extracts from several other cultivars.

EXPERIMENTAL SECTION

Shrub Procurement. Guayule shrub samples were provided from cultivated plots in Arizona and California. Table I summarizes procurement data.

Shrub Processing and Extraction. Shrub was manually defoliated, mechanically chipped, and then passed through an 8-in. single disk attrition mill. Resin samples were obtained by exhaustive extraction of milled woody tissue. Typically, a 450-g sample of milled guayule was extracted with four 1-L aliquots of water previously heated to boiling. The combined extracts, usually 2-3% solids, were desolventized at 40 °C under vacuum to give a tan, resinous residue. Anal. Found: C, 41.59; H, 5.97; N, 3.85; P, 1.45; Ca, 2.79; Na, 1.36; Mg, 0.62. Acetone extractions

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