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Identification of Polycyclic Aromatic Hydrocarbons (PAHs) in Five Different Cigarettes

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Cigarette smoking is one of the most damaging health hazard for human beings due to PAH emissions for the active and passive smokers and some other constituents of tobacco and cigarette smoke. In this study PAH contact of two Turkish and three foreign cigarettes are determined for tobacco and cigarette under different forms of smoking. Four kinds of smoking programmes are applied to each cigarette, namely direct smoking and smoking two, four and five cigarettes using a cigarette holder. For PAH analysis tobacco, collected cigarette butts and cigarette holders were extracted using toluene-methanol mixture (3:1 v/v) until exhaustion. The yield of tars are determined. The extracted tars were fractionated into aliphatic, neutral aromatic and polar fractions using column chromatography. The aromatic fractions were subjected to capillary column gas chromatography with FID detector on a 25 $m^*0.22$ mm i.d. capillary column. The compounds were identified using Lee Retention Indices and internal standard of benzene, naphthalene, phenantrene, chrycene and piecene. The compounds identified in the aromatic fraction from tobacco and smoked cigarettes show distinctive differences. This is believed to be the consumption of original PAH compounds during combustion and pyrosynthesis of some new compounds during smoking

Keywords: Tobacco, polycyclic aromatic hydrocarbons, cigarette tar

1. Introduction

Cigarette smoking is a well proven health hazard for the mainstream and sidestream smokers. The sources of the hazardous chemicals are some of the original products present in the tobacco, some of which are decomposed and pyrosynthesised during combustion of the tobacco.

Tobacco smoking has various harmful effects on human health, among which carcinogenic activity for the lungs, larynx, oral cavity, oesophagus and bladder [1] is of considerable concern. The tobacco's share in all cancer deaths is estimated to be 30% and 85% of the total and lung cases [2]. Furthermore, over half of esophageal cancer deaths, between $30-40\%$ of bladder cancer deaths and up to 30% of the deaths from pancreatic cancer are attributed to smoking [2]. PAH compounds are the largest group of chemical cancinogens known today [3]. It is well known that they are present in tobacco and in the smoke of burning tobacco. The yield of polycyclic aromatic compounds in tobacco smoke depends on a number of variables such as frequency and duration of puffs, type of tobacco and the type and the permeability of cigarette paper and filter.

Women who smoke are more likely to undergo menopause earlier than non-smokers are, furthermore exposure to PAH's during pregnancy is sufficient to kill over 80% of the developing female germ cells [4].

In a different study when given to mice PAH-toxic chemicals related into environment by fossil fuel combustion and also present in tobacco smoke cause like destruction of eggs in the ovaries [5].

Andrew *et al.* report that their study indicate that genetic damage reflecting individual exposure and susceptibility to PAH may play a role in breast cancer; but more research is needed to determine whether the findings are relevant to causation or progression of breast cancer [6].

In this study, the PAH distribution of unburned cigarettes, the condensates at the cigarette tips smoked by mainstream smokers and condensates collected in the expansion filter apparatus smoked

Table 1
The amount of tar in tobacco, cigarette butts and cigarette-holders (mg/cig)

	T	Smoking Program		
		1-B	2-B	2-H
M	287.1	39.7	51.0	65.0
SG	278.0	64.3	53.5	84.5
ML	274.5	32.7	41.5	35.5
C	215.5	38.7	53.5	80.5
P	184,.2	81.4	64.0	—
		3-B	3-H	4-B
M	287.1	43.3	35.3	55.4
SG	278.0	33.0	41.0	40.6
ML	274.5	34.3	36.5	47.4
C	215.5	31.8	41.3	44.2
P	184.2	54.0	31.5	66.3
		4-H		
M	287.1	43.3		
SG	278.0	48.7		
ML	274.5	22.2		
C	215.5	34.8		
P	184.2	32.2		

Smoking program 1: Normal smoking, Smoking program 2: Two cigarette smoked with a cigarette-holder, Smoking program 3: Four cigarette smoked with a cigarette-holder, Smoking program 4: Five cigarette smoked with a cigarette-holder, Smokking programme

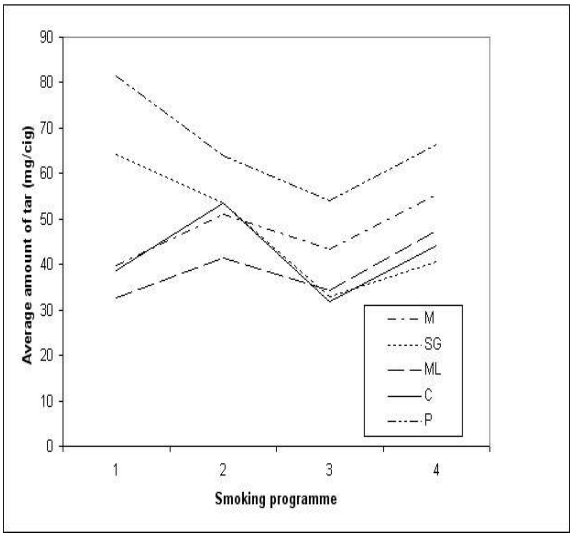

Figure 1. Average amount of tar content of cigarette butts obtained from different smoking programme.

by smokers were analysed on five different brands of cigarettes two of which are produced in Turkey.

2. Related Literature

The structure of tobaccos, show considerable differences in chemical composition which encouraged manufacturers to blend various tobaccos to enhance certain flavors [7]. For example high nicotine percentage is an important criterion for the qualification of the tobacco [7]. Other components that goes into the manufacture of cigarette are the rapping paper, the sticker (glue) and the kind and the length of filter. All these components have got an important role in distribution of the combustion products of the tobacco[8]. The texture and length of the filter may be used to lower the emissions from tobacco combustion to the smokers. For example, active carbon in the filter reduces the harmful compounds considerably [8].

The cigarette smoke consists of combustion of decomposition, evaporation and condensation products diluted with air which is introduced to provide oxygen to the tip core. The smoke mixture consists of solid-liquid mixture particulate phase and a gas vapour mixture gaseous phase. The health hazard is mainly the particulate phase which contains nicotine and other pyridine derivatives, PAH, nitrosoamines, phenols, lactones, pesticide residues, radioactive materials and N-alkyl heterocyclics. The gaseous phase contains CO, NH_3, and NOx [8].

Tobacco is reported to contain around 3000 volatile and non-volatile organic compounds. These may be classified into hydrocarbons, acids, phenols, nitroso compounds, sulphur compounds, lactones, alcohols, aldehydes, ketones, ethers and esters [9]. PAH content of tobacco smoke was investigated and over 150 compounds were identified. The quantity of PAH in the smoke was found to be a function of the frequency and duration of puffs, tobacco type, moisture of tobacco, cigarette paper and filter type [10].

Lee *et al.* [11] reports the daily intake of PAH from 20 cigarettes. The amounts of fluranthene pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene were reported to be 1.7, 1.4, 0.5, 0.1, 0.1 and $0.3\mu g/20$ cigarettes respectively.

Table 2
Filter Characteristics
[1]Long [2]Filtered
[3]Filter containing 10mm. charcoal part

Cigarette	Filter Length (mm.)
$M^{1,2}$	27
SG^{2}	25
ML^{2}	15
$C^{1,2}$	27
P^{3}	25

3. Experimental

In these experiments two Turkish ML, SG and three foreign M, C, P, cigarettes were investigated. All of the five cigarettes were filter tipped and one of which utilised a charcoal layer as an extra filtering aids.

The cigarettes were at first Soxhlet extracted to exhaustion using 3:1 (V/V) toluene-methanol mixture. The five different brands of cigarettes were smoked by five different smokers, who were advised to adopt similar smoking trends. The tips of the smoked cigarettes were collected and were subjected to toluene-methanol Soxhlet extraction. Same smokers were asked to smoke cigarettes using cigarette holders that condense the tar at the expansion section. The collected tar in these holders was also Soxhlet extracted using toluene-methanol mixture.

The solvent extracted tars were fractionated using silica gel column chromatography into aliphatic, neutral aromatic and polar fractions using hexane toluene and methanol respectively. The aromatic fractions obtained in each case were analysed by a Hewlett Packard 5890 GC capillary column chromatography using FID detector and 25 m. long, 0.2 mm. i. d. SB-Biphenyl 30 capillary column. The heating programme was 60 ^{o}C initial for 2 minutes, with a ramp of 4 ^{o}C/min. up to final temperature of 300 ^{o}C held for 10 minutes. Nitrogen was used as the carrier gas. The peaks were identified using Lee's Linear Retention Indices and for the internal standards for one, two, three, four and five rings benzene, naphthalene, phenanthrene, chrysene and picene were used.

4. Results And Discussion

The tar contents (T) determined for the unsmoked cigarettes, tar condensed at the tip of smoked cigarettes (B) and tar collected at the holder (H) are compiled in Table 1. for the five different cigarette brands.

Using these values Fig. 1 can be drawn in which change of tar content of cigarette butts are shown with changing smoking program where normal smoking, cigarette-holder smoking with two, four and five cigarettes are numbered as 1, 2, 3 and 4 respectively.

From the results presented at Fig. 1 it is clear among the five cigarettes M contained the biggest amount of inherent tar. The tar contents decreased in decreasing order for SG, ML, C, and P.

Under normal smoking conditions tar condensed per tip was found to be increasing on the order ML, C, M, SG, and P.

The total amount of tar compounds detected at the tip and holder of each cigarette smoked is less than the tar content found for a single cigarette. There is not a distinct preference of PAH to accumulate at either tip or filter of the cigarettes.

Preference of tar at the tip or filter also does not show a systematic behaviour for the number of smoked cigarettes. One possible explanation may be effect of smoking behaviour of the smokers.

Investigation of the results show that P had the highest tar condensation in the butt under normal smoking conditions even though it did not have the highest tar content in its tobacco. This is attributed to the 10 mm charcoal filter of the cigarette in addition the 15 mm celulosic filter. On the contrary in all other forms of smoking the lowest amount of tar were collected at the holders used with cigarette P compared to the holders used with other four brands.

The filter characteristics of the cigarettes are shown in Table 2

Examining the filter length and tar condensed at the tip apart from the obvious filtering efficiency of the charcoal containing cigarette P there is not a direct relationship between the length of filter and tar condensed at the tip.

The fractionation of the tar obtained from unsmoked cigarettes, normally smoked cigarette tips and cigarette holders into aliphatics, aromatics and polar fractions are shown in Table 3.

Table 3
Fractionation results of the tars obtained from different smoking program

	n-hexane eluate (%) (aliphatics)	Toluene eluate (%) (neutral aromatics)	Methanol eluate (%) (polar compounds)
M	65.2	8.1	26.7
C	69.0	12.8	18.2
P	68.4	3.1	28.5
ML	62.6	7.3	30.1
SG	55.4	8.6	36.0
M(2-B)	73.0	9.5	17.5
C(2-B)	88.3	3.5	8.2
P(2-B)	54.5	4.4	41.1
ML(2-B)	69.5	4.7	25.8
SG(2-B)	81.5	6.3	12.2
M(4-B)	84.0	5.9	10.1
C(4-B)	90.7	3.5	5.8
P(4-B)	60.1	36.1	3.8
ML(4-B)	73.5	11.4	15.1
SG(4-B)	84.4	9.5	6.1
M(4-H)	57.1	38.1	4.8
C(4-H)	40.2	54.1	5.7
P(4-H)	61.2	32.8	6.0
ML(4-H)	71.2	8.8	20.0
SG(4-H)	77.7	13.9	8.4

Fractionation results show that generally for all tobacco types unsmoked tobacco tars were 55−69% aliphatic, 18−36% polar and 8−13% aromatic. However for normal smoking the tars condensed at the cigarette tip were 73−90% aliphatic and 8−25% polar for M, C, ML, and SG and 55%. aliphatic and 40% polar for P.

From these results it is safe to conclude that the tars condensed from the cigarette smoke are differed drastically from the original tar extracted from the tobacco. This change may be attributed to pyrosynthesis [7] and thermal cracking reactions [7] at the tip temperature reaching up to 880 ^{o}C [7].

The distribution of the holder tar into paraffinic, aromatic and polar fraction showed some difference to the distribution of tar collected at the tip. For example the paraffinic, aromatic and polar fractions changed between $40-75\%$., $14-54\%$, and $5-20\%$. The difference between these results were attributed to the condensation of lower molecular weight molecules comparatively earlier at the tips compared to the holder.

5. Capillary Column Chromatographic Analyses Of Neutral Aromatic Fraction Of Tobacco Tars

The toluene eluates from the silica gel column was fed to capillary column chromatography. For the internal standards for one, two, three, four and five rings benzene, naphthalene, phenanthrene, chrysene, and picene were used. The linear retention indices for all the peaks were calculated using these standards and identification was made using Lee's Linear Retention Indices [9], Of course the detection was limited by the number of available indices in literature. The results for the unsmoked tobacco and tar collected at the filter tips are shown in Table 4.

Total number of peaks from gas chromatographic analysis of unsmoked cigarette of M, C, P, ML, and SG are 110, 62, 36, 59 and 63. And also for neutral aromatic fraction of tar collected at the filter tip of cigarettes of M, C, P, ML, and SG are 42, 24, 49, 25, and 47 compounds are found.

From the results it is observed that for unsmoked cigarette the number of identified peaks are 57,19,12,30, and 21 compounds for M,C, P, ML, and SG respectively. Among these com-

Table 4
The results for the unsmoked tobacco and tar collected at the filter tips

			Unsmoked tobacco			Filter Tips				Carsio genic Activity
Compoud	Lee Ret. Ind.	M	C	P	M L	S G	M	C	P	M L	S G	
2-Methylpyridine	** 125.11	+										
3-Methylpyridine	** 133.54	+										
2,6-Dimethylpyridine	** 136.27	+				+						
Indane	** 168.87						+					
Indene	** 170.83	+										
1,2,3,4-Tetrahydronaphtelene	195.47			+								
Naphtelene	200.00	+										-
1-Aminoindan	** 207.63		+									
Quinoline	** 210.26	+										+
Isoquinoline	** 214.14	+										-
7-Methylbenzol[b]thiophene	* 219.16		+									
2-Methylbenzol[b]thiophene	* 220.76	+										
5-Methylbenzol[b]thiophene	* 222.09		+									
Indole	** 222.66	+					+					-,+
Azulene	223.74					+						
2-Methylquinoline	** 224.13	+										+
1-Methylisoquinoline	** 229.21	+	+	+								
3-Methylquinoline	** 232.47	+										
3-Phenylpyridine	** 250.03		+									
4-Azabiphenyl	** 252.35	+			+							
Acetanaphtane	253.14	+					+					-
Dibenzofuran	258.77	+										
Bibenzyl	260.49	+	+									
2-Cyanonaphtalene	** 260.88				+							
5-Nitroindan	** 261.55	+										
1-Aminonaphtalene	** 262.98	+										
2,3,6-Trimethylnaphtalene	265.09	+										
2-Aminonaphtalene	** 265.53							+				
1-Methylacenaphtalene	266.57	+	+									
2,3,5-Trimethylnaphtalene	267.54	+										
1,2,3,4,4a,4b-Hexadihydrodibenzothiophene	* 271.69							+				
9-Methylfluorene	273.79				+							
4-Azafluorene	** 279.85					+						
2-Nitronapytelene	** 280.63							+				
3-Methyl-2-aminonaphtalene	** 283.73		+									
2-Methylfluorene	288.42	+										
2-Nitrobiphenyl	** 290.25	+										
4-Aminobiphenyl	** 298.05	+										
Naphtol[2,1-b]thiophene	* 300.00	+										
Naphtol[2,3-b]thiophene	* 304.47		+		+							
2-Methyldibenzothiophene	* 316.19						+					
4-Methylnaphtol[1,2-b]thiophene	* 317.19					+						
o-Terphenyl	317.43		+									
4-Methylnaphtol[2,1-b]thiophene	* 318.12					+						
9- or 4-Methylphenantren	322.81	+						+				
3-Methylcarbazol	** 328.34									+		
3-Ethyldibenzothiophene	* 328.81				+							

			Unsmoked tobacco					Filter Tips					Carsio genic Activity
Compoud	Lee	Ret. Ind.	M	C	P	ML	SG	M	C	P	ML	SG	
3-Aminofluorene	**	329.08						+					
2,6-Dimethyldibenzothiophene	*	335.90	+										
6-Phenylquinoline	**	340.84					+						
1,4-Dimethylcarbazole	**	343.16	+										
Fluorathene		344.51	+										
Acephananthrylene		347.67	+										
1-Azafluoranthene	**	348.17	+										
Pyrene		351.51	+										
9-Cyanophenantrene	**	351.84	+						+				
2-Nitrofluorene	**	353.06				+		+					
Phenalenol[6,7-bc]thiophene	*	353.45	+										
4-aminophenantrene	**	353.97						+				+	
m-Terphenyl	**	356.12	+										
p-Terphenyl	**	362.29					+						
Methylfluorantrene		362.76						+			+	+	
Methylacephenanthrylene		362.76						+			+	+	
9-Aminoanthracene	**	363.91					+			+			
Triptycene		365.02				+							
3-Aminophenantrene	**	365.60					+						
Benzo[a]fluorene		366.72						+					-
2-Aminoanthracene	**	367.45				+							
Benzo[b]fluorene		369.17						+					
4-methylpyrene		369.40					+						
3,5-Diphenylpyridine	**	372.84								+			
1-Methylpyrene		373.40					+						-
9-Phenylcarbozole	**	381.51		+				+					
1,1-Binaphthyl		385.23				+							
Benzo[c]phenentrene		391.24			+	+	+						+
Bezo[b]naphtol[1,2-d]thiophene	*	392.59					+					+	
Benz[c]acridine	**	392.60				+				+			
Phenantrol[9,10-b]thiophene	*	394 96	+		+			+				+	
Cyclopental[c,d]thiophene	*	396.55				+							
4-Azachrysene	**	401.16				+							
Phenantro[3,2-b]thiophene	*	401.89	+										
Phenantro[2,3-b]thiophene	**	402.19	+										
1-Methylbenzo[b]naphtol[1,2-d]thiophene	*	402.59				+	+						
11-Methylbenzo[b]naphtol[1,2-d]thiophene	*	404.15					+	+					
10-Methylbenzo[b]naphtol[2,1-d]thiophene	*	404.28						+		+			
3-Methylbenzo[b]naphtol[2,1-d]thiophene	*	407.55				+			+		+		
Anthra[2,3-b]thiophene	*	407.57	+									+	
2-Methylbenzo[b]naphtol[2,1-d]thiophene	*	407.63			+								
8-Methylbenzo[b]naphtol[2,1-d]thiophene	*	407.69		+				+		+			
10-Methylbenzo[b]naphtol[1,2-d]thiophene	*	409.04	+										
3-Methylbenzo[b]naphtol[1,2-d]thiophene	*	411.48	+										
Benzo[c]carbozole	**	411.89		+						+			
4-Aminopyrene	**	412.31			+	+	+						
2-Aminopyrene	**	413.83	+	+		+	+						

			Unsmoked tobacco					Filter Tips					Carsio genic Activity	
Compoud	Lee Ret.	Ind.	M	C	P	ML	SG	M	C	P	ML	SG		
6-Methylbenzo[b]naphtol[2,3-d]thiophene	*	415.02	+											
4-Methylbenzo[b]naphtol[1,2-d]thiophene	*	415.41								+				
1-Methylbenzo[b]naphtol[2,3-d]thiophene	*	415.54				+								
7-Methylbenzo[b]naphtol[2,3-d]thiophene	*	417.07				+	+				+	+		
Benz[a]anthracene-7,12-dione		417.26	+		+				+	+				
3-Methylphenantrol[9,10-b]thiophene	*	417.70		+				+						
2-Methylcrysene		419.6 6			+	+							+	
12-Methyl[a]anthracene		419.66			+	+							++	
4-Methyl[a]anthracene		419.66			+	+							-,+	
1-Nitropyrene	**	421.48				+								
2,2-Binaphtyl		421.81	+				+							
2,2-Biquinoline	**	422.56	+											
11-Methylbenzo[b]naphtol[2,3-d]thiophene	*	422.85	+											
3-Methylphenantrol[2,1-b]thiophene	*	423.48			+	+	+							
2-(2-Naphtyl)benzo[b]thiophene	*	430.65				+								
7,9-Dimethylbenz[a]acridine	**	438.32										+		
7,10-Dimethylbenz[a]acridine	**	439.46										+		
7,12-Dimethylbenz[a]acridine		440.30				+							++	
Benzo[j]fluoranthene		443.13							+		+		++	(**)
Benzo[b]fluoranthene		443.13							+		+		++	
Benzo[2,3]phenantrol[4,5-bcd]thiophene	*	443.29				+				+				
Benzo[k]fluoranthene		444.02			+		+		+		+	+	++	
Triphenylenol[4,5-bcd]thiophene	*	448.45				+		+						
Pyrenol[1,2-b]thiophene	*	449.30								+				
Benzo[e]pyrene		452.29				+	+						-,+	
Pyrenol[2,1-b]thiophene	*	455.01		+										
Perylene		457.17						+					-	
3-Methylchlanthrene		462.09						+	+				++	
Dinaphtol[2,1-b:1,2-d]thiophene	*	472.62								+				
Dinaphtol[1,2-b:1,2-d]thiophene	*	486.58		+										
Benzo[b]phenantrol[9,10-d]thiophene	*	487.32	+											
Anthra[1,2-b]benzo[d]thiophene	*	488.45	+											
Dibenz[a,h]acridine	**	488.55	+										+	
Benzo[b]phenantrol[2,1-d]thiophene	*	488.89	+											
Dinaphtol[1,2-b:2,3-d]thiophene	*	489.14	+											
9,13-H-Triphenylenol[2,3-b]thiophene	*	489.81	+											
Dibenzo[a,j]carbazole	**	490.57								+			+	
Benzo[b]phenantrol[1,2-d]thiophene	*	492.31									+			
Triphenylenol[1,2-b]thiophene	*	494.41	+											
Dinaphtol[2,3-b:2,3-d]thiophene	*	495.17	+											
Dibenz[a,h]anthracene		496.20		+									+	

PASH, (*) N-PAH (++) Highly active
(+) Active
(-) Passive

pounds 5, 1, 5, 6 and 3 are reported to be carcinogenic, respectively [9]. But the numbers of compounds with carcinogenic activities are not complete due to lack of respective information.

The analysis of neutral aromatic fraction of tar collected at the filter tip of the cigarettes showed that for M, C, P, ML, and SG 19, 8, 16, 8, and 11 compounds are detected and 3, 4, 1, 4 and 2 carcinogenic compounds were obtained.

Similar to the conclusion reached after the fractionation results, identification of individual compounds from unsmoked tobacco and from filter tip of smoked cigarette, it has been realised that the structure of tar changes drastically during the smoking process. The number of PAC carcinogenic compounds reduced greatly during the smoking process. This may indicate the level of health hazard associated with tobacco chewing. Also it has been observed that some of the compounds identified in the condensate tar were not present at the original tobacco.

6. Conclusions

It has been found that the tar condensate at the tip and original tar content did not have a direct relationship for the five different cigarettes investigated. Similarly the length of the filter tip did not have a definite linear relationship. However, charcoal application in the filter increases the tar condensation. The structure of tar changes drastically during smoking period. In neutral aromatic fraction of all cigarettes carcinogenic compounds are detected either in tobacco or condensate at the tip.

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