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Chemical Characterization of Mutagenic Fractions of Particles from Indoor Coal Combustion: A Study of Lung Cancer in Xuan Wei, China

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■ In the rural Xuan Wei County, Yunnan Province, lung cancer mortality rates for women are among the highest in China. Most of these women are nonsmokers, and studies have shown that lung cancer in Xuan Wei is associated with domestic use of smoky coal under unvented conditions. The objective of this study is to determine the chemical constituents that may be linked to the high lung cancer rates in Xuan Wei using the bioassay-directed fractionation method. Ten high-volume filter samples (<10 μm) collected from the home inhabited by a person with lung cancer during cooking periods on four consecutive days were subjected to Soxhlet extraction. This composite sample extract was fractionated on a normal-phase column into seven fractions. The second fraction was the most active in the bioassay, containing mainly polycyclic aromatic hydrocarbon (PAH) and alkylated PAH. The two polar fractions 6 and 7 were the next most active. The most active PAH fraction was further separated into 11 subfractions, based on the number of aromatic carbons. The results indicated that the presence of three to four-ring alkylated PAHs in the sample extract is a significant factor that may be linked to the high incidence of lung cancer in Xuan Wei, China.

Introduction

The U.S. Environmental Protection Agency is conducting a collaborative program with the People's Republic of China to investigate the etiology of unusually high lung cancer mortality rates among the residents of a rural county in Xuan Wei, China. In this county, the lung cancer mortality rates for women are among the highest in China, and the rates for men are also among the highest (*1*). The unadjusted lung cancer mortality rate in the Cheng Ghan (CG) commune was 151.8 per 100 000, whereas the average rate in China was 5 per 100 000 during 1973–1975 (*1*). For generations, Xuan Wei residents, particularly women, have been exposed to unvented smoke emissions from indoor burning of coal and wood for cooking and heating. Most women in Xuan Wei are nonsmokers, who spend more time cooking indoors than men, and have lung cancer mortality rates similar to or even higher than the rates of men who are mostly smokers.

Three types of fuel are used by Xuan Wei residents: smoky coal, smokeless coal, and wood. Results from previous studies (*2–5*) have suggested that the high lung cancer mortality rates in Xuan Wei are associated with exposure to unvented indoor smoky coal emissions. The indoor air from homes using smoky coal as fuel showed the highest indoor levels of inhalable particles (<10 μm), extractable organic materials, polycyclic aromatic hydrocarbons (PAHs), mutagenicity, and carcinogenicity. The fuel survey also showed that smoky coal use in homes is highly associated with lung cancer mortality rates. A mouse skin tumor initiation study showed that the organic extract from the indoor particles of one home using smoky coal is the most active, followed by sample extracts from homes using smokeless coal and wood (*4*).

In this study we have performed a detailed chemical characterization of the same composite organic extract of the indoor air particles from the home in the CG commune used in the mouse skin tumor initiation study. This organic extract sample has shown high skin tumor initiation activity and is a potent complete carcinogen in Sencar mouse skin assays (*4*). The extract was fractionated into seven fractions by using high-performance liquid chromatography (HPLC) fractionation with solvents of increasing polarity. The most active fraction (PAH) was further fractionated into 11 fractions based on the number of aromatic carbons. The primary and secondary fractions were analyzed by gas chromatography/mass spectrometry (GC/MS) to determine PAH and other organic components and were assayed for genotoxicity with the Kado assay. The goal of the overall study is to determine the chemical constituents that may be linked to the high lung cancer rates in Xuan Wei using the bioassay-directed fractionation method.

Materials and Methods

Sample Collection and Extraction. Indoor air particles (<10 μm) were collected from one home in the CG commune in Xuan Wei. The overall sampling design and procedure have been discussed in detail elsewhere (*2, 3*). Ten high-volume filters with total particulate weights of 5563 mg collected from the CG home during cooking periods were combined. The particulate concentrations in individual filters were from 16.64 to 61.28 mg/m^3, and the average particulate concentration was 29.65 mg/m^3. The filters were extracted with dichloromethane (DCM) for 12 h and further extracted with acetone for 12 h. The DCM and acetone were distilled-in-glass grade from Burdick and Jackson.

HPLC Fractionation. The composite extract was fractionated on a semipreparative (10 mm × 300 mm) HPLC column packed with Adsorbosphere silica. The HPLC system used consisted of a Kratos Model 430 gradient programmer with Model 400 pumps and Kratos 757 ultraviolet (UV) absorbance detector with a preparative (3-mm path length) flow cell. The eluate was monitored

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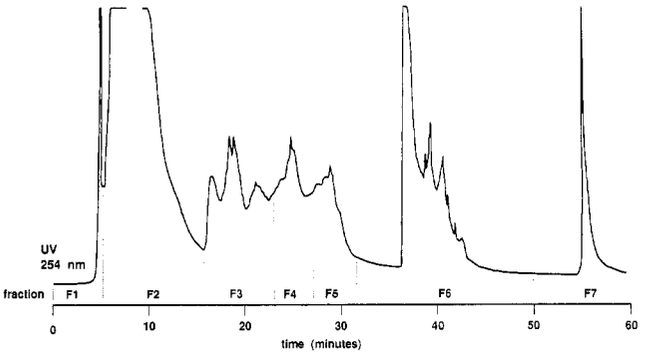

Figure 1. HPLC chromatogram of the primary HPLC fractionation.

for UV absorbance at 254 nm. The LC flow rate was 3.6 mL/min, and aliquots of the extract (7.5 mg in 150 μL) were injected. Eight duplicate injections were performed, and seven fractions were collected in clean jars using a Gibson Model 201 fraction collector. The HPLC chromatogram with the fraction cut points is shown in Figure 1. The gradient condition started with hexane/DCM (95:5) for 10 min, followed by a gradient to 100% DCM in 10 min and a 10-min hold, then to 100% acetonitrile in 10 min and a 10-min hold, and finally to 100% methanol in 1 min and a 20-min hold. All solvents (Burdick–Jackson distilled-in-glass) used were degassed and filtered. Selected standard solutions containing PAHs (Supelco) were eluted from the HPLC column, using the same gradient conditions before and after the large-scale fractionation to determine the fraction cut points and the column performance. Each of the eight duplicate fractions was combined and concentrated to 5 mL by Kuderna–Danish evaporation. The concentrated fractions were used for residue weights, bioassay, secondary fractionation, and GC/MS analyses.

The fraction that showed the most mutagenicity (fraction 2; F2) was combined with F1 (F_{1+2}) and further fractionated into 11 subfractions using an aminosilane HPLC column (μBondapack-NH2, 30 cm by 9 mm, Waters Associates) with UV detection at 295 nm (*6*, *7*). Fractions A–H were collected at 5 mL/min with a mobile phase of 1% DCM in *n*-hexane (*n*-C_6), fraction I with 10% DCM in *n*-C_6, fraction J with 40% DCM in *n*-C_6, and fraction K with 100% DCM in a back-flush mode. Triplicate injections of a total of 23.5 mg of F_{1+2} were made. The respective subfractions were combined and concentrated for residue weights, bioassay, and GC/MS analyses.

GC/MS Analysis. The unfractionated extract, the primary fractions, and the secondary fractions were analyzed by 70-eV electron impact GC/MS. A Finnigan 4500 GC/MS with 2300 INCOS data system was used. The GC column was an Ultra No. 2 fused-silica capillary column (50 m × 0.31 mm, 0.17-μm film thickness; Hewlett-Packard Co.). Helium was used as the carrier gas. The column temperature was set at 60 °C for 2 min and then programmed to 290 °C at 8 °C/min. The MS was set to scan from m/z 45 to 450 amu at 1 s/scan. Identification of the target PAH was based on a comparison of the mass spectra and retention times relative to the internal standard (9-phenylanthracene). Tentative identification of nontarget compounds was accomplished by manual interpretation of background-corrected spectra together with an on-line computerized library search. The library used was the most currently available EPA/NIH mass spectral database containing 42 197 unique reference spectra.

Mutagenicity Testing Method. The unfractionated DCM and acetone extracts, and the primary and secondary HPLC fractions, were solvent-exchanged into dimethyl sulfoxide. These bulk sample extracts and HPLC fractions were tested for mutagenicity in a microsuspension reverse-mutation assay using *Salmonella typhimurium* strain TA98 with metabolic activation of S9 from Aroclor-pretreated rats (a supernatant of rat liver homogenate containing microsomes) to detect indirect-acting, frameshift mutagens (*8*, *9*). Each sample was tested in six doses with two replicates per dose over a dose range of 5–75 μg of organics. A sample was considered to be positive if it showed a dose–response relationship. The slope values of the linear portion of the dose–response curves were determined by least-squares linear regression and used to obtain the mutagenic activity.

Table I. Organic Extractable Mass of the Composite Filter Samples Sequentially Extracted by Dichloromethane and Acetone

sample code	extractable organic mass[a] mg	extractable organic mass[a] mg/m³	% extractable organic mass
DCM extract	4495	23.96	81
acetone extract	92.0	0.49	1.7

[a] A total of 10 filter samples during four sampling days was used for extraction. The particulate concentrations in individual filters ranged from 16.64 to 61.28 mg/m³ of air sampled. The total particle weight in these filters was 5563 mg, and the average particulate concentration was 29.65 mg/m³.

Results and Discussion

Unfractionated Composite Sample Extract. The particle mass and extractable organic materials from the consecutive DCM and acetone extractions of 10 composite air particulate filters (<10 μm) collected in one home in CG are summarized in Table I. Extremely high particle mass concentration (29.65 mg/m³) and extractable organic mass (>80%) were present in the indoor particulate matter relative to the typical values for U.S. residential indoor air in the presence of environmental tobacco smoke (*10*). The CG results also agree with previous studies (*3*, *4*) which found that indoor smoky coal emissions gave the highest indoor concentrations of fine particles (<10 μm), associated with high extractable organic mass, followed by wood emissions and smokeless coal emissions. As shown in Table I, most of the organic materials are removed in the DCM extraction, and the subsequent acetone extraction yields about 2% of the total extractable mass. The acetone extract also accounted for less than 5% of the total mutagenicity. This finding indicates that most of the bioactive components are removed by DCM extraction. Results obtained in previous work have similarly indicated that the DCM extract of ambient air particulate matter from the United States comprises the majority of the mutagenic components, while the subsequent methanol extract contains mainly nonmutagenic components (*11*, *12*). Thus, the bulk DCM extract was used for studies of the bioassay-directed fractionation and chemical characterization discussed below. The DCM extract is referred to as the bulk extract or the unfractionated extract in this paper. The GC/MS results indicate that the major components of the bulk extract are PAH and alkylated PAH. The extract is very complex because of the presence of alkylated PAH and numerous isomeric parent compounds. In order to further characterize mutagenic compounds in the extract, we fractionated the extract into seven fractions for mutagenicity testing and chemical analysis.

Primary HPLC Fractionation and Chemical Characterization. The HPLC chromatogram of the primary fractionation with the fraction cut points is presented in

Table II. Organic Mass and Mutagenicity Distribution of the Composite DCM Extract after the Primary HPLC Fractionation

fraction	organic fraction mass[a] mass, mg	organic fraction mass[a] % total mass	mutagenicity, revertants/ μg	weighted mutagenicity of fraction[b] revertants/ μg	weighted mutagenicity of fraction[b] % total mutagenicity
F1	2.4	4.1	0	0	0
F2	25	43	5.5	2.4	61
F3	5.0	8.6	0.80	0.069	1.8
F4	4.0	6.8	0.91	0.062	1.6
F5	5.0	8.6	1.50	0.13	3.3
F6	10	17	4.4	0.75	19
F7	7.0	12	4.2	0.50	13

[a] A total of 97% of mass was recovered from the HPLC column. The percent of total mass of each fraction was based on the total recovered mass from HPLC fractionation. [b] Weighted mutagenicity of the fraction equals % of fraction mass × measured mutagenicity (revertants/μg). The percent of total mutagenicity was based on the sum of the weighted mutagenicity.

Figure 1. The distributions of organic mass and mutagenicity among the fractions are summarized in Table II. An aliquot (60 mg) of the bulk extract was fractionated into seven fractions from eight duplicate injections onto the semipreparative silica column. Excellent mass recovery (97%) was obtained from the primary fractionation, demonstrating that there is no loss of organic material in the HPLC column or in the concentration process. We also showed from the method blank that no inadverent contamination occurred. The HPLC chromatograms for each duplicate fractionation are identical, and the retention times of the PAH standards are essentially the same before and after fractionating the extract. These findings suggest that the surface chemistry of this column did not change significantly during the fractionation, thus ensuring good reproducibility of each duplicate fractionation. This allowed us to combine each corresponding fraction for further analysis.

As shown in Table II, most of the mass (43% of total) and mutagenicity (61% of total) were present in the second fraction (F2). Based on the elution time data for the PAH standards, this fraction consists mainly of PAH and alkylated PAH, as confirmed by GC/MS analysis. By contrast, for ambient air particulate matter from the Washington, DC, and Philadelphia areas, the polar fractions are found to contain the most organic mass and mutagenicity (*11*). The outdoor PAH concentrations were, at least, 1 order of magnitude lower than the indoor levels in Xuan Wei (*3*); thus, outdoor air intrusion is not a significant factor for the high indoor levels. The high PAH content of the Xuan Wei extract is mainly due to the indoor unvented smoky coal emissions. The two most polar fractions (F6 and F7) account for 17% and 12% of the total mass and 19% and 13% of the total mutagenicity, respectively.

Tables III–V summarize the compounds found in the three most active fractions, F2, F6, and F7, respectively. Examination of the data in Table III indicates that two- to seven-ring parent and alkylated PAHs are present in the second fraction (F2). Many identified PAHs are known animal carcinogens such as benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and dibenzo[*a*,*e*]pyrene (*13–15*). This fraction also contains more alkylated PAHs than their parent PAHs. Some alkylated PAHs, such as 5-methylchrysene, are known to be more carcinogenic than their parent compound (*14*, *15*). These high levels of PAH and alkylated PAH may contribute to the high lung cancer rates in the CG commune. We found two- and three-ring nitrogen heterocyclic compounds and their alkylated derivatives in F6. In this fraction we also found quinoline and alkylated quinolines with one to four carbons. Most C4-alkylquinolines and some C2- and C3-alkylquinolines are present in F7. Dibenzo[*a*,*i*]carbazole is also identified in F7. These nitrogen heterocyclic compounds in F6 and F7 account for some of the mutagenicity in these fractions. Because most of the mutagenicity is in F2, this fraction was subfractionated by HPLC for further mutagenicity assay and chemical analysis.

Table III. Major Components of the Second Fraction (F2) from the Primary HPLC Fractionation

compound[a]	mol wt	isomers present[b]
*naphthalene	128	1
*methylnaphthalenes	142	2
C2-naphthalenes	156	6
C3-naphthalenes	170	7
C4-naphthalenes	184	7
*biphenyl	154	1
methylbiphenyl	168	3
C2-biphenyl	182	6
C3-biphenyl	196	8
C4-biphenyl	210	6
*acenaphthylene	152	1
*dihydroacenaphthylene	154	1
*fluorene	166	1
methylfluorene	180	1
C2-fluorene	194	6
C3-fluorene	208	4
*phenanthrene	178	1
*anthracene	178	1
methylphenanthrene/anthracene	192	5
C2-phenanthrene/anthracene	206	10
C3-phenanthrene/anthracene	220	7
C4-phenanthrene/anthracene	234	3
phenylnaphthalene	204	1
C2–4H-cyclopenta[*def*]phenanthrene	218	6
C3–4H-cyclopenta[*def*]phenanthrene	232	2
C4–4H-cyclopenta[*def*]phenanthrene	246	5
C5–4H-cyclopenta[*def*]phenanthrene	260	1
*fluoranthene	202	1
*pyrene	202	1
aceanthrylene	202	1
acephenanthrylene	202	1
methylpyrene/fluoranthene	216	3
C2-pyrene/fluoranthene	230	7
C3-pyrene/fluoranthene	244	4
C4-pyrene/fluoranthene	258	2
*cyclopenta[*cd*]pyrene	226	1
*benz[*a*]anthracene	228	1
*chrysene	228	1
methylbenz[*a*]anthracene/chrysene	242	10
C2-benz[*a*]anthracene/chrysene	256	8
benzofluoranthenes	252	3
*benzo[*e*]pyrene	252	1
*benzo[*a*]pyrene	252	1
methylbenzo[*a*]pyrene isomer	266	8
*indeno[1,2,3-*cd*]pyrene	276	1
*benzo[*ghi*]perylene	276	1
methylindeno[1,2,3-*cd*]pyrene isomer	290	2
*dibenz[*a*,*h*]anthracene	278	1
*dibenzo[*a*,*e*]pyrene	302	1
dibenzopyrene	302	3
*coronene	300	1

[a] * means these compounds confirmed from the retention times and mass spectra of the standard solution. The identification of alkylated PAHs was based on the mass spectra with characteristic fragment ions and molecular ions. The terms C*n* refer to *n* carbon atoms present in groups on the parent compound. [b] This is the number of resolvable isomers observed.

Secondary HPLC Fractionation and Chemical Characterization. On the basis of the GC/MS analysis, the major components in F1 are aliphatic compounds, along with trace amounts of naphthalene and methyl-

Table IV. Major Components of the Sixth Fraction F6 from the Primary HPLC Fractionation

compound[a]	mol wt	isomers present[b]
*quinoline	129	1
methylquinoline	143	2
C2-quinoline	157	8
C3-quinoline	171	7
C4-quinoline	185	1
tetrachloroethane	166	1
*acridine	179	1
methylacridine	193	2
methylacridinone	209	3
C2-acridinone	223	9
nitrogen-containing compds	229	2
benzonaphthothiophene	234	1

[a] * means these are compounds confirmed from the retention times and mass spectra of the standard solution. The identification of alkylated nitrogen heterocyclic compounds was based on the mass spectra with characteristic fragment ions and molecular ions. [b] This is the number of resolvable isomers observed.

Table V. Major Components of the Seventh Fraction F7 from the Primary HPLC Fractionation

compound[a]	mol wt	isomers present[b]
C2-quinoline	157	2
C3-quinoline	171	4
C4-quinoline	185	5
unknown aliphatic compounds		7
nitrogen-containing compounds	229	2
fluoranthenamine isomer	217	1
methylfluoranthenamine	231	3
*dibenzo[*a*,*i*]carbazole	267	1

[a] * means this is a compound confirmed from the retention times and mass spectra of the standard solution. The identification of alkylated nitrogen heterocyclic compounds was based on the mass spectra with characteristic fragment ions and molecular ions. [b] This is the number of resolvable isomers observed.

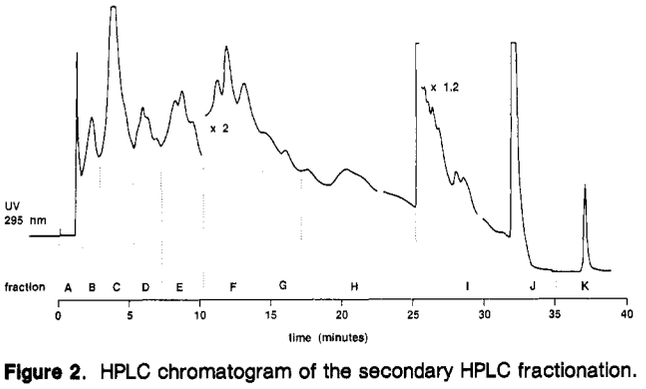

Figure 2. HPLC chromatogram of the secondary HPLC fractionation.

naphthalene. We therefore combined the F1 and F2 fractions, designated as F_{1+2} for the secondary HPLC fractionation, to separate F_{1+2} into 11 subfractions and to investigate the relationship between mutagenicity and PAHs with different ring sizes. The HPLC chromatogram of the secondary fractionation is given in Figure 2. The resulting mass and mutagenicity distribution of the secondary HPLC fractionation of F_{1+2} are given in Table VI. The major components tentatively identified in each subfraction are summarized in Table VII. Subfraction 1 ($F_{1+2}A$) contains only aliphatic hydrocarbons, mainly alkanes, and no PAHs are detected. The amount of aliphatic hydrocarbons present in $F_{1+2}A$ (2.1 mg) is in good agreement with that found in F1 (2.4 mg). The small difference may be due to the trace amounts of naphthalene found in F1 and the residue weight measurement variations. The estimated precision of residue weight measurement is 5%. As expected, this fraction was not mutagenic.

Table VI. Mass and Mutagenicity Distribution of the PAH Fraction after the Secondary HPLC Fractionation

	organic fraction mass[b]		mutagenicity,	weighted mutagenicity of fraction[c]	
fraction[a]	mass, mg	% total mass	revertants/ μg	revertants/ μg	% total mutagenicity
$F_{1+2}A$	2.1	9.2	0	0	0
$F_{1+2}B$	1.2	5.3	16	0.85	2.7
$F_{1+2}C$	3.3	15	43	6.5	21
$F_{1+2}D$	1.4	6.2	106	6.6	21
$F_{1+2}E$	1.7	7.5	108	8.1	26
$F_{1+2}F$	1.4	6.2	73	4.6	15
$F_{1+2}G$	0.72	3.2	70	2.2	7.1
$F_{1+2}H$	0.98	4.3	22	0.95	3.1
$F_{1+2}I$	2.8	12	3.8	0.46	1.5
$F_{1+2}J$	6.3	28	0	0	0
$F_{1+2}K$	0.76	3.3	32	1.1	3.5

[a] F_{1+2} denotes the combined F1 and F2 fractions from the primary HPLC fractionation; A–K denote the fractions from the secondary HPLC fractionation. [b] A total of 97% of mass was recovered from the HPLC column. The percent of total mass of each fraction was based on the total recovered mass from the column. The F_{1+2} fractions account for 47% of the bulk extract. [c] Weighted mutagenicity of the fraction equals % fraction mass × measured mutagenicity (revertants/μg). The percent of total mutagenicity was based on the sum of the weighted mutagenicity. The F_{1+2} fractions account for 61% of the mutagenicity found in the bulk extract.

Table VII. Summary of Tentative Identified Major Components in HPLC Subfractions of F_{1+2}

fraction code[a]	major components[b]
$F_{1+2}A$	alkanes
$F_{1+2}B$	C1–C4-naphthalenes, C1–C5 biphenyls, acenaphthylene, fluorene, C1-fluorenes
$F_{1+2}C$	C1–C3-fluorenes, phenanthrenes, anthracene, C1–C4-anthracenes/phenanthrenes, fluoranthene, C1–C2-fluoranthenes
$F_{1+2}D$	fluoranthene, pyrene, C1–C4-fluoranthenes/pyrenes, cyclopenta[*cd*]pyrene, C1–C2-benzonaphthothiophene
$F_{1+2}E$	B[*a*]A, chrysene, C1–C3-B[*a*]As/chrysenes
$F_{1+2}F$	benzofluoranthenes, B[*e*]P, B[*a*]P, methyl-252
$F_{1+2}G$	benzofluoranthene C1–C3-252, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene
$F_{1+2}H$	indeno[1,2,3-*cd*]pyrene, dibenzanthracenes, C1–C2-dibenzanthracene, benzocyclopentachrysenes, coronene, methylcoronene
$F_{1+2}I$	dibenzopyrenes, methyldibenzopyrenes, C1–C2-dibenzanthracenes, aliphatic alcohols, phthalate
$F_{1+2}J$	diisooctyl phthalate
$F_{1+2}K$	fatty acids, fatty acid esters, aliphatic amides

[a] F_{1+2} denotes the combined F1 and F2 fractions from the primary HPLC fractionation; A–K denote the fractions from the secondary HPLC fractionation.' [b] B[*a*]A = benz[*a*]anthracene; B[*e*]P = benzo[*e*]pyrene; B[*a*]P = benzo[*a*]pyrene; alkyl-252 = alkylated PAH with parent PAH having a molecular weight of 252.

Subfraction 2 ($F_{1+2}B$) consisted of alkylated naphthalenes, biphenyls, and fluorenes, as well as their corresponding parent compounds. Alkyl homologues of naphthalene with as many as four carbons (C4) were detected. We also found acenaphthylene in this subfraction but not its alkylated derivative. Only methylfluorenes were found in this subfraction; most alkylfluorenes with up to three carbons (C3) were detected in the following subfraction ($F_{1+2}C$). There was relatively little mutagenicity (2.7% of total) present in subfraction 2, mainly because most two-ring PAHs have little or no mutagenicity.

Subfraction 3 ($F_{1+2}C$) contained a large amount of alkylated three-ring PAHs, mainly C1–C4 phenanthrene/anthracene isomers, which make up about 80% of the mass of this subfraction and account for 21% of the mutagenicity. Both phenanthrene and anthracene are nonmutagenic compounds (*13*). However, for these three-ring PAHs, alkylation increases the biological activity of the parent PAH. For example, 9,10-dimethylanthracene is mutagenic in TA-98 and is active as a complete carcinogen and tumor initiator (*15*). We expect that most of the activity in this subfraction is from the alkylated three-ring PAHs. Note that about 50% of the fluoranthene detected is present in this subfraction, and the remaining 50% is in the following subfraction 4 ($F_{1+2}D$). All of the isomeric four-ring compound pyrene is present in subfraction 4.

Subfraction 4 ($F_{1+2}D$) is the second most active subfraction based on the unit mass (106 revertants/μg). This subfraction has only 6.2% of the total fraction mass, but has the same weighted mutagenicity (21%) as found in subfraction 3. Higher amounts of the C1–C4 pyrene/fluoranthene isomers were found in subfraction 4 than those of their parent PAHs. The alkylated four-ring PAH accounts for more than 90% of the total mass. We also found cyclopenta[*cd*]pyrene in this subfraction, which is a mutagenic compound, but only represents less than 1% of the total subfraction mass according to the peak height. This compound produces about 354 revertants/μg of mutagenicity in TA-98 (*16*), so if it would represent 1% of total mass, it could contribute about 3% of the total mutagenicity of this fraction. Neither pyrene nor fluoranthene are considered to be mutagenic. We expect that most of the mutagenicity present in subfraction 4 is from the alkylated pyrene and fluoranthene isomers.

Subfraction 5 ($F_{1+2}E$) is the most active subfraction (108 revertants/μg), in terms of unit fraction mass and weighted mutagenicity, which accounts for 26% of the total mutagenicity. The high activity of subfraction 5 was expected, since C1–C3 benz[*a*]anthracene (B[*a*]A) and chrysene isomers make up more than 90% of this subfraction. Many of the methyl and dimethyl isomers of these two compounds such as 2-, 3-, 4-, 5-, and 6-methylchrysene (MC) are carcinogens and have mutagenic activity (*13–15*). The carcinogenic 5-MC and 7,12-dimethylbenz[*a*]-anthracene (7,12-DMBA) were tentatively identified in this subfraction, based only on the GC elution times and mass spectra. However, we do not have all the possible methylated and dimethylated standard compounds. It is conceivable that other methylated or dimethylated isomers may coelute with 5-MC or 7,12-DMBA under the GC conditions used. Especially for 7,12-DMBA, this compound has not been reported to be present in environmental samples. Most of the mutagenicity of subfraction 5 is expected from the C1–C3 alkylated B[*a*]A and chrysene isomers.

Subfraction 6 ($F_{1+2}F$) is the third most active subfraction (73 revertants/μg) based on unit mass. Because this subfraction only contains about 6% of the fraction mass, the weighted mutagenicity (15%) for this subfraction is even less than that for subfraction 3 (21%). This subfraction contains mainly the known carcinogen benzo[*a*]-pyrene (B[*a*]P), several carcinogenic benzofluoranthenes, and their methyl derivatives. Note that small amounts of methyl and dimethyl B[*a*]A and chrysene isomers are also present in this subfraction. A significant difference between this subfraction and the previous two subfractions is the relatively low content of alkylated PAH; for example, only methylbenzofluoranthenes/benzopyrenes are present, and none of the dimethyl or higher methylated derivatives are detected in subfraction 6.

Subfraction 7 ($F_{1+2}G$) shows about the same mutagenicity (70 revertants/μg) per unit of fraction mass as subfraction 6, but only about 3% of the fraction mass, resulting in 7.1% of the total mutagenicity. This subfraction consists of C1–C3 benzofluoranthenes/benzopyrenes, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene. These compounds can also account for the activity found in the subfraction.

Subfraction 8 ($F_{1+2}H$) shows relatively weak mutagenic activity compared to subfractions 3–7. The major components present in this subfraction are dibenzoanthracene isomers, coronene, and their methylated derivatives. Note that we found about 25% of indeno[1,2,3-*cd*]pyrene in the previous subfraction 7, and the remaining 75% of this compound is present in the subfraction 8. All the benzo-[*ghi*]perylene is present in subfraction 7.

Subfraction 9 ($F_{1+2}I$) has even less mutagenicity than subfraction 8 and only accounts for about 2% of the total mutagenicity. We identified the carcinogenic compound dibenzo[*a*,*e*]pyrene in subfraction 9, according to the GC elution time and the mass spectrum of the standard solution. A series of other dibenzopyrenes were also found in this subfraction. The low mutagenic activity is probably due to the low concentrations of these compounds. We also tentatively identified some nonmutagenic compounds, such as aliphatic alcohol and phthalates, in this subfraction.

The three most mass abundant subfractions are $F_{1+2}J$ (28%), $F_{1+2}C$ (15%), and $F_{1+2}I$ (12%). Among these three subfractions, $F_{1+2}C$ contributes significantly to the mutagenicity (21%), and the other two subfractions are either less mutagenic ($F_{1+2}I$) or nonmutagenic ($F_{1+2}J$). There is only one nonmutagenic component (diisooctyl phthalate) present in subfraction 10 ($F_{1+2}J$). This compound accounts for 28% of the mass in F_{1+2}, is equivalent to 12% of the bulk extract mass, and shows no mutagenicity. Only trace amounts of diisooctyl phthalate are found in the method blank, and phthalates are common contaminants in environmental samples. This compound may therefore arise from sampling artifacts and the sample particles themselves.

Subfraction 11 ($F_{1+2}K$) contains only aliphatic compounds and no PAH. However, this subfraction still shows some mutagenic activity (32 revertants/μg) and accounts for 3.5% of the total mutagenic activity. A homologous series of tetradecanoic, hexadecanoic, and octadecanoic acids and their acid esters were tentatively identified. We would not expect these compounds to show any mutagenic activity. We also found hexadecanamide and octadecanamide, based on their mass spectra. It is possible that these aliphatic amides may contribute to some of the observed mutagenicity.

Conclusions

The PAH fraction from a smoky coal sample extract was isolated by semipreparative silica HPLC fractionation. The PAH fraction accounts for 43% (or 31% when diisooctyl phthalate is excluded) of the organic mass and 61% of the total mutagenicity. This fraction was further separated into 11 subfractions, based on aromatic carbon number, in a secondary HPLC fractionation. We found that most of the mutagenicity in the PAH fraction is from the subfractions containing alkylated three- and four-ring PAHs, and the subfraction containing B[*a*]P is not the most active fraction. The concentrations of alkylated PAHs are also higher than their parent compounds.

In the mouse skin tumorigenicity study (*4*), there was no correlation between the B[*a*]P concentration and the

tumorigenic activity in the unfractionated smoky coal extract (the same extract used in this study) and the smokeless coal extract. In comparisons of smoky coal and smokeless coal sample extracts, higher concentrations of alkylated four-ring PAH were observed in the smoky coal sample extract, which exhibits higher tumorigenicity, compared to the smokeless coal sample extract. These findings suggest that the presence of the alkylated three- and four-ring PAHs in the smoky coal sample extract may be a significant factor that can be linked to the high lung cancer mortality rates in the CG commune at Xuan Wei, China.

The two polar fractions (F6 and F7), when combined, contributed about 30% to the mutagenicity. The nitrogen heterocyclic compounds may account for some of the mutagenicity; other classes of polar compounds may also contribute to the activity. To date, very little information is available in the literature about the mutagenic polar compounds present in environmental or air samples. Further investigations need to be carried out in this area. Further studies on bioassay-directed fractionation and chemical characterization of unknown polar components (F6 and F7) are in progress in an attempt to identify unknown mutagens or carcinogens from indoor smoky coal emissions associated with high lung cancer mortality rates in Xuan Wei, China.

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Registry No. Naphthalene, 91-20-3; methylnaphthalene, 1321-94-4; biphenyl, 92-52-4; methylbiphenyl, 28652-72-4; acenaphthylene, 208-96-8; dihydroacenaphthylene, 83-32-9; fluorene, 86-73-7; methylfluorene, 26914-17-0; phenanthrene, 120-12-7; methylphenanthrene, 31711-53-2; phenylnaphthalene, 35465-71-5; fluoranthene, 206-44-0; pyrene, 129-00-0; aceanthrylene, 202-03-9; acephenanthrylene, 201-06-9; methylpyrene, 27577-90-8; cyclopenta[*cd*]pyrene, 27208-37-3; benz[*a*]anthracene, 56-55-3; chrysene, 218-01-9; methylbenz[*a*]anthracene, 43178-22-9; benz[*a*]anthracene, 56-55-3; benzofluoranthene, 56832-73-6; benzo[*e*]pyrene, 192-97-2; benzo[*a*]pyrene, 50-32-8; methylbenzo[*a*]pyrene, 25167-89-9; indeno[1,2,3-*cd*]pyrene, 193-39-5; benzo[*ghi*]perylene, 191-24-2; methylindeno[1,2,3-*cd*]pyrene, 64158-98-1; dibenz[*a*,*h*]anthracene, 53-70-3; dibenzo[*a*,*e*]pyrene, 192-65-4; dibenzopyrene, 58615-36-4; coronene, 191-07-1; quinoline, 91-22-5; methylquinoline, 27601-00-9; tetrachloroethane, 25322-20-7; acridine, 260-94-6; methylacridine, 54116-90-4; methylacridinone, 139584-03-5; acridone, 139584-05-7; benzonaphthothiophene, 61523-34-0; fluoranthenamine, 78020-40-3; methylfluoranthenamine, 139584-06-8; dibenzo[*a*,*i*]carbazole, 239-64-5.

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