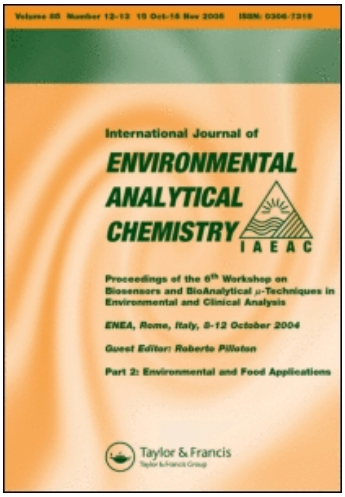

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BIOASSAY-DIRECTED FRACTIONATION OF THE ORGANIC EXTRACT OF SRM 1649 URBAN AIR PARTICULATE MATTER

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Separation of 2 grams of the methylene chloride extract of NIST SRM 1649 (Washington, D.C. urban air particulate matter) into six compound class fractions by acid-base partitioning and silica gel column chromatography is demonstrated here. Recoveries of organic mass and *Salmonella typhimurium* TA98 mutagenic activity were greater than 80%. The fractionation method showed reproducible distribution of both mass and mutagenic activity; the compound class mass distribution results were similar to those obtained for more typical analytical fractionation of a milligram quantity of the extract mass. The most polar compound class fractions contained the greatest proportions of mutagenic activity.

KEY WORDS: SRM 1649, urban particles, ambient air, bioassay-directed, fractionation, mutagenicity.

INTRODUCTION

Urban air particles contain extractable organic matter which has both mutagenic[1] and carcinogenic[2,3] activity. Since the extremely complex chemical nature of these extracts prohibits ready detection and unambiguous identification of individual components, fractionation of an extract according to class or type is essential. Separation of an extract to isolate a specific compound class, followed by a microbial mutagenicity assay has been effective in identifying known or suspected mutagens in cigarette smoke,[4] kersene flames,[5] synfuels,[6] xerographic toners,[7] and diesel exhaust emissions.[8,9] However, to detect the heretofore unknown mutagenic components of an extract, a different approach is needed. The combination of sequential fractionation techniques and mutagenicity assays, termed bioassay-directed fractionation, has been proposed as the most direct method for detecting previously unknown mutagenic species.[10] The vastly simplified fractions which result from sequential separations permit identification of components; more importantly, the bioassays limit the identification effort to only significantly

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mutagenic fractions. To ensure analysis of only significantly mutagenic fractions, full recovery of mutagenicity must be achieved with each fractionation step.

The sequential fractionations inherent in bioassay-directed fractionation require initially much larger quantities of material than are required for chemical analysis and/or mutagenicity characterization preceded by a single separation step. This requirement for large sample sizes has meant that collection of particles and initial fractionation of the particulate extract be performed on relatively large scale. Several methods have been developed and employed for collection of gram quantities of air particles. The Massive Air Volume Sampler (MAVS) was designed and used successfully to collect gram quantities of size-fractionated air particles over relatively short periods of time, 1–2 weeks.[11] Very large baghouses have been used to collect kilogram quantities of urban air particulate matter over long periods, 1–2 years.[12]

In contrast to these sampling innovations, a fractionation method for gram quantities of an urban air particulate extract that successfully recovers mass and mutagenicity has not been reported. Relatively low recoveries [46% recovery of mass, 21% recovery of direct-acting mutagenic activity (TA98, −S9), and 58% recovery of indirect-acting mutagenic activity (TA98, +S9)] have been reported for separation of 1.4 g of an urban air particulate extract using the solvent partitioning method developed for fractionation of cigarette smoke condensate.[13,14] This paper reports greater than 80% recovery of mass and mutagenicity for fractionation of 2 grams of a representative urban air particulate extract. This method provided reproducible distribution of both mass and mutagenicity and a compound class mass distribution similar to that obtained for analytical separation of milligram quantities of the extract mass. In addition, the distribution of mutagenic activity demonstrated that the most polar compound class fractions, in particular the organic acids, contained the greatest proportions of the mutagenic activity.

EXPERIMENTAL

Collection of Air Particles

The urban air particulate sample used is available as Standard Reference Material (SRM) 1649 "Urban Dust/Organics" from the National Institute of Standards and Technology (NIST, formerly NBS) and contains certified concentrations of several polycyclic aromatic hydrocarbons (PAH).[12] Although the NIST preparation of this SRM was performed prior to and independently from the present work, some details of its preparation are offered here as a convenience to the reader. The air particulate sample was collected in the Washington, D.C. area using a baghouse designed for this purpose. This material was collected over a period exceeding 12 months. The particulate material was removed from the canvas filter bags by a specially designed vacuum cleaner, combined into a single lot, and screened through a 200 mesh sieve (125 μm) to remove bag fibers and other extraneous material. The sieved material was thoroughly mixed in a V-blender and bottled.

The particulate sample was stored at room temperature after collection and blending. Once bottled, the samples were stored at −4 °C.

Extraction of Air Particles

For this study the SRM 1649 air particles were extracted using the Soxhlet technique under yellow fluorescent lights. All solvents used in this study were Burdick and Jackson Distilled-In-Glass quality. A 0.61 g particle sample was extracted with 20 mL of methylene chloride for 24 hours. The resulting extract, 35 mg (5.7% extractable organics), was concentrated to 1 mL by rotary film and vortex evaporation prior to separation. A second particle sample, 70.6 g, was extracted with 800 mL of methylene chloride for 72 hours. The resulting organic extract, 4.25 g (6.0% extractable organics), was concentrated by rotary film evaporation to 150 mL; two sample extracts, each containing approximately 2.1 g (2.10 g and 2.06 g), were removed for replicate fractionation and bioassay analyses.

Acid-Base Partitioning of Particulate Extracts

An overview of the fractionation scheme used is shown in Figure 1. The organic bases were removed from the extract first by partitioning multiple times with 5% aqueous H_2SO_4. The partitioned aqueous phases were combined and the organic bases were recovered in methylene chloride after adjusting the pH to 12–13 with 40% aqueous KOH.

The organic acids were separated from the remaining extract by partitioning multiple times with 5% aqueous NaOH, until yellow color was no longer visible in the extracted aqueous phase. A 10 min centrifugation at 1500 rpm was used to break the emulsion formed during aqueous NaOH partitioning (Model K centrifuge, International Equipment Co.). The separated phases of the emulsion were combined with previously generated layers. Organic acids were recovered in methylene chloride after adjusting the aqueous phase pH to 1–2 with 6M H_2SO_4. The quantity of each reagent, the number of repeats of partitioning for aqueous extraction and organic back extraction for separation of both the milligram (35 mg) and gram (2.1 g) quantities of extract mass are given in Figure 1. The organic acid and organic base fractions were concentrated 200 fold by rotary film and vortex evaporation. The neutral component fraction was concentrated approximately 20 fold.

Silica Gel Column Chromatography of Neutral Components

Silica (70–150 mesh, ICN Nutritional Biochemicals) was activated for 18 hours at 160 °C and deactivated with distilled water (5% w:w). The water was added dropwise to the silica which was then sealed and allowed to equilibrate for several hours while mechanically shaken at very low speed.

For separation of the smaller quantity of neutral component material (28 mg), 8 g of deactivated silica gel was packed as a hexane slurry into an open bed gravity flow chromatography column (10 mm i.d. × 250 mm length column, Kontes Co.). For separation of the larger quantity of neutral material (1.8 g), 200 g of

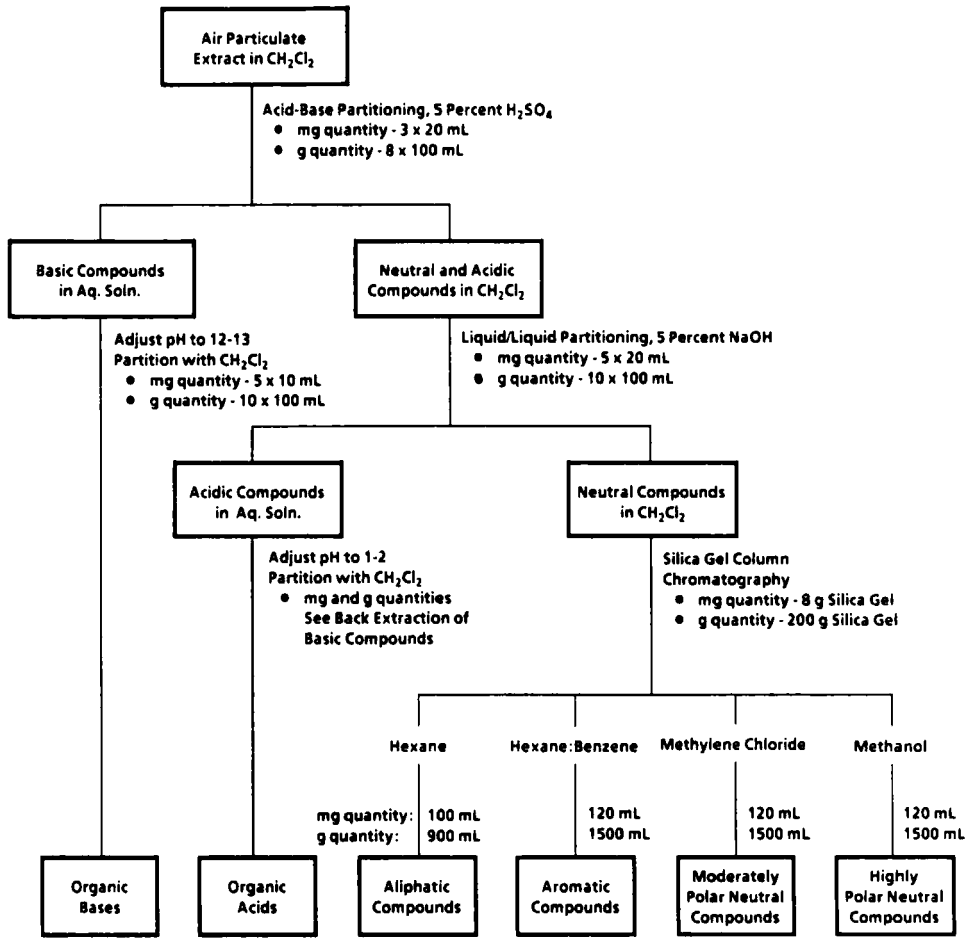

Figure 1 Fractionation scheme for milligram and gram quantities of air particulate extracts.

deactivated silica gel was packed as a hexane slurry into a closed bed chromatography column (25 mm i.d. × 750 mm length column, GPC Autoprep column, Analytical Biochemistry Labs, Inc.). With solvent flowing, the screw-type end fittings of the column were adjusted to compress the silica gel bed until air bubbles were no longer visible. For both column preparation and sample fractionation the solvent flow was introduced at the bottom of the column and collected at the top. The solvent flow rate was controlled by an HPLC pump (Model 110, Altex-Beckman Co.). The 200 g silica gel beds were rinsed sequentially with 350 mL hexane, 500 mL methylene chloride, 500 mL methanol, 500 mL methylene chloride, and 600 mL of hexane at a flow rate of 5 mL/min. The column was equilibrated with hexane at a flow rate of 1 mL/min for 24 hours prior to sample introduction.

Prior to separation of 1.8 g of neutral component material, the methylene chloride solvent of that fraction was exchanged, using vortex evaporation, with

50 mL of the hexane column elution volume. Soluble sample material was injected using an HPLC injector (5 mL loop, Rheodyne Co.). After injection of the hexane-soluble material, 50 mL of the next column elution solvent (hexane:benzene) was added to the hexane-insoluble material. This new solution was reserved for injection with initiation of the second elution solvent. This procedure to solubilize material was repeated with each succeeding elution solvent. For separation of the smaller quantity of neutral component material, 28 mg, the concentrated methylene chloride solution was added directly to the head to the silica gel bed.

Neutral component material was separated by sequential elution with solvents of increasing polarity: hexane, hexane:benzene (1:1 v:v), methylene chloride and methanol. The quantities of silical gel and elution solvents are shown in Figure 1. The appropriate quantity of silica gel for the larger extract mass was scaled from analytical separation of 25–75 mg of extract on 8 g of silica gel. Four column volumes each of the latter three elution solvents were used. Arrival of the polycyclic aromatic hydrocarbon (PAH) elution front at the end of the silica gel bed, as monitored by fluorescence of those compounds under long wavelength UV light (Model UVSL-58, Ultra-Violet Products, Inc.), determined the exact point (typically 2.5 column volumes) for switching from hexane to hexane:benzene.

Materials and Methods Blanks

The following three types of blanks were prepared:

Solvent Blanks: A 750 mL aliquot of each silica gel elution solvent was concentrated by rotary film and vortex evaporation to 200 μL. Half of the material was used to determine residual mass; the other half was analyzed for microbial mutagenicity.

Silica Gel Column Chromatography Blanks: An open bed chromatography column (18 mm i.d. × 200 mm length), containing 30 g of deactivated silica gel, was eluted sequentially with 250 mL of each elution solvent. The four fractions were collected, concentrated, divided and analyzed as above. The column solvent was returned to hexane and the same sequence of solvents was applied a second time. Each collected fraction was processed as above.

Fractionation Method Blanks: A 50 mL aliquot of methylene chloride was carried through the entire fractionation scheme (Figure 1) using reagent quantities and methods identical to those used for separation of the 2.1 g quantities of extract. The resulting fraction blanks were concentrated, divided and analyzed as above.

Microbial Mutagenicity Methods

Organic extracts and fractions were assayed for mutagenicity toward *Salmonella typhimurium* strain TA98 with and without exogenous metabolic activation (+S9, −S9) in the Ames plate incorporation assay.[15] The specific protocol used, which included minimal histidine in the base agar and incubation of the plates for

Table 1 Comparison of distribution and recovery of extract mass for separation of milligram and gram quantities of extract

Fraction	*Percent distribution of recovered mass for extract quantity*		
	Milligram[a]	*Gram*[b]	
		Mean ± DM	*(% RDM)*
Organic acid	24	15 ± 0	(0)
Organic base	1.0	0.50 ± 0.04	(8)
Hexane	21	17 ± 0[c]	(0)
Hexane:Benzene	3.9	11 ± 1[c]	(9)
Methylene chloride	3.5	5.5 ± 0.2	(4)
Methanol	47	51 ± 1	(2)
Procedure	*Percent recovery of mass for extract quantity*		
	Milligram	*Gram*	
		Mean ± DM	
Acid/base/neutral partitioning	94	100 ± 0	
Column chromatography	83	79 ± 1	
Overall scheme	81	81 ± 1[d]	

[a]Single sample.

[b]Average values for two replicate samples (Mean) ± deviation from the mean (DM) and percent relative deviation from the mean (% RDM).

[c]After correction for aliphatic hydrocarbons recovered in Hexane:Benzene fraction, the values of percent mass for Hexane and Hexane:Benzene fractions would be 20% and 7%, respectively. See text for discussion of this correction.

[d]Mass of fractionation blanks: mg and (% of SRM 1649 fraction mass); Acid <0.01 mg, (<0.01%); Base <0.01 mg, (0.1%); Hexane 0.01 mg, (<0.01%); Hexane:Benzene 0.04 mg, (0.02%); Methylene chloride 0.02 mg, (0.02%); Methanol 2.6 mg, (0.3%).

72 hours, is described elsewhere in detail.[16] Individual fractions and extracts were assayed at seven doses with triplicate plates at each dose. Positive controls for TA98 included 2-aminoanthracene (+S9) and 2-nitrofluorene (−S9). The plates were counted electronically using an automatic colony counter (Model 880, Artek). Mutagenicity was determined for the dose reponse data using the Stead non-linear model[17,18] and Bernstein linear model.[19] The materials and methods blanks were tested in the presence of S9 at a single dose. Results of these tests were recorded as either active or non-active.

RESULTS AND DISCUSSION

Reproducibility of Mass Distribution

The percent distribution of mass by compound class fraction for separation of both milligram and gram quantities of extract, as shown in Table 1, demonstrated the ability to scale acid-base partitioning and silica gel column chromatography proportionally. The mass distributions were similar with exception of the Organic Acid and Hexane:Benzene fractions. Secondary fractionation of the Hexane:Benzene fraction on silica gel showing 30% by weight of that fraction to be aliphatic

hydrocarbons indicated some bandspreading from injection (see Table 1, footnote c). Although the *in situ* cleaning of the large silica gel bed with solvents as polar as methanol might have altered the degree of water deactivation, the effect was not major. The percentages of mass in the two polar neutral compound class fractions were very similar for large and small extract quantities.

The difference in mass percentages observed for the Acid fractions may be due to the presence of a sampling artifact, Dichlorophen, at relatively high levels in the extract.[20] Dichlorophen (2,2'-methylene-bis-4-chlorophenol), a commercial fungicide presumed to have been coated on the canvas filter/particle collection bags, is weakly acidic and its distribution during acid-base partitioning could be affected by the degree of emulsion formation. Data presented elsewhere using this method for acid-base partitioning of milligram and gram quantities of an urban air particulate extract which does not contain Dichlorophen show much closer agreement of mass distribution, less than 1% deviation from the mean.[21]

Also as shown in Table 1, separation of the two 2.1 g particulate extracts resulted in essentially identical mass recovery and compound class mass distribution. Mass measurements before and after acid-base partitioning and after silica gel column chromatography indicated that the majority of sample loss occurred during chromatographic separation of the neutral compounds. Dark colored organic material remaining at the top of the silica gel column at the end of the fractionation procedure indicated either the presence of extremely polar neutral compounds in the extract or decomposition of material on the silica gel. As shown in a subsequent study, an additional elution solvent, methanol acidified with HCl, removed these extremely polar neutral compounds from the silica gel bed; very little mutagenicity, less than 1% of the total, was found in this fraction.[21]

Mass and Mutagenicity of Blanks

The large volumes of solvent used in fractionating gram quantities of extract did not contribute to either mass or mutagenic activity of fractions. The hexane, hexane:benzene, and methylene chloride solvent blanks contained no more than 0.01 mg of residual mass; this mass contributes less than 0.05% to the mass of each corresponding fraction when separating a 2.1 g extract of SRM 1649. The methanol solvent blank, containing 0.35 mg of residual mass, contributes less than 0.1% to mass of the Methanol fraction obtained in fractionating 2.1 g. The residual mass of each concentrated solvent blank produced no mutagenic activity.

The methanol fraction of the silica gel column blank contained 2 mg of mass and indirect-acting mutagenic activity. The prior hexane, hexane:benzene, and methylene chloride eluate column blanks had less than 0.02 mg of residual mass and no measured mutagenicity. The second elution of the silica gel bed with the four solvents produced fractions of minimal mass with no mutagenic activity. These results led to subsequent cleaning of silica with solvent prior to activation.

The fractionation method blanks demonstrated that methods described here will not significantly affect mutagenicity and mass measurements of extract fractions. The fractionation method blanks were not mutagenic and the residual mass of each was extremely low (see footnote d, Table 1). The quantity of mass was

Table 2 Evaluation of mutagenicity of replicate samples from separation of gram quantities of extract mass

Fraction	*Mutagenicity (rev/μg)[a] toward*			
	TA98 − S9		*TA98 + S9*	
	Mean[b] ± DM	*(% RDM)*	*Mean ± DM*	*(% RDM)*
Organic acid	15 ± 1	(7)	12 ± 0.5	(4)
Organic base	2.7 ± 0.7	(26)	9.1 ± 0.9	(10)
Hexane	0.0 ± 0	(0)	0.0 ± 0	(0)
Hexane:Benzene	2.0 ± 0.05	(2)	2.9 ± 0.04	(1)
Methylene chloride	9.1 ± 4.9	(54)	7.2 ± 1.1	(15)
Methanol	0.96 ± 0.24	(25)	2.1 ± 0.3	(14)
Unfractionated extract	4.3 ± 0.3	(7)	4.1 ± 0.1	(2)

[a]Revertants/μg. Stead non-linear model slope.
[b]Average value for two replicate samples.

greatest in the methanol fraction blank, 2.6 mg; however, this quantity represented less than 0.5% of the mass in the Methanol fraction from separating a 2.1 g extract of SRM 1649.

Reproducibility of Mutagenic Activity

The average mutagenic activity, with and without S9 activation, for replicate samples (that portion of each 2.1 g extract assayed prior to fractionation and the six resulting fractions of each extract) showed very close agreement. As shown in Table 2, the percent deviation from the mean for replicates was less than 30% in all but one case: the −S9 Methylene Choride fractions. In this case, lower mutagenicity and higher toxicity were found for one of the replicates. At the lower doses the average revertants per plate of these samples compared extremely well; however, at the highest dose the variation due to very high toxicity accounted for the difference in Stead model slope treatment of the data. The −S9 mutagenicities of these Methylene Chloride replicates as determined by the Bernstein linear model method were very similar in value but substantially lower than those obtained with the Stead non-linear model method.

The mutagenicity values for replicate samples fell within the 95% confidence limits of each other with exception of the Methylene Chloride fractions, as noted above. The reproducibility of the mutagenicity values may be due not only to the repeatability of the fractionation but also to the non-linear model slope treatment of mutation plate count data where toxicity is a significant factor. The mutagenicity in the Bernstein linear model for each fraction was consistently lower than mutagenicity in the Stead non-linear model with exception of the Hexane:Benzene fraction. Mutagenicity values of this fraction were essentially identical for the two methods, as would be expected, because toxicity was not a significant factor. Since the Stead non-linear model slope method uses all data points, even those indicative of toxicity, a more accurate evaluation of mutagenicity can be expected than could be obtained from a linear regression method in which toxic doses are eliminated from the curve.[17]

Table 3 Distribution and recovery of weighted mutagenic activity for separation of gram quantities of extract mass

Fraction	*Weighted mutagenic activity*[a], *rev/μg*		*Distribution of mutagenic activity*[b], %	
	−S9	*+S9*	*−S9*	*+S9*
Organic acid	2.25	1.80	65	50
Organic base	0.01	0.05	<1	1
Hexane	0.00	0.00	0	0
Hexane:Benzene	0.22	0.32	6	9
Methylene chloride	0.50	0.40	14	11
Methanol	0.49	1.07	14	29
Sum of fractions	3.47	3.64		
Unfractionated extract	4.3	4.1		
Mutagenicity recovery	81%[c]	89%[c]		

[a]Percent mass for gram quantity fractionation (Table 1) multiplied by the mutagenic activity shown in Table 2.
[b](Weighted mutagenic activity $i / \sum_{i=1}^{i}$ weighted activity) × 100%.
[c]($\sum_{i=1}^{i}$ weighted activity/activity of unfractionated extract) × 100%.

Recovery and Distribution of Mutagenicity

The weighted mutagenic activity of each fraction, as defined in Table 3, footnote (a), was used to calculate recovery and distribution of mutagenicity. The sum of the weighted mutagenicities, equal to the recovered activity, assumes the additivity of compound activity without major synergistic or antagonistic effects. As shown in Table 3, recoveries of mutagenic activity averaged 81 and 89% for direct- (−S9) and indirect-acting (+S9) mutagenic activity, respectively. These mutagenicity recoveries represent an improvement over recoveries of 30–50% reported elsewhere for fractionation of urban air particulate extracts.[14,22] Because mutagenicity recoveries are high, we assume that synergistic and antagonistic effects of mutagenic compounds are minimal in this extract.

The distribution of recovered mutagenic activity among the compound classes, weighted according to the amount of recovered mass, is also shown in Table 3. The Organic Acid fraction contained the greatest proportion of the mutagenic activity, 65% of the direct-acting and 50% of the indirect-acting activity. The polar neutral fractions, Methylene Chloride and Methanol fractions, together contributed 30–40% of the activity. In contrast to these polar species, the PAH and mono-nitro-PAH, present in the Hexane:Benzene fraction, contributed relatively little to overall mutagenicity. The PAH contributed at most 8% to the +S9 activity of the extract; the mono-nitro-PAH contributed at most 6% to the −S9 activity.

Advantage of SRM 1649 for Analytical Methods Development

The sampling method for SRM 1649 precludes consideration of this sample as a representative respirable (<2.5 μm diameter) urban ambient air particulate sample. However, availability of SRM 1649 in such large quantities facilitates the development and comparison of analytical methods to be applied to the characterization and identification of mutagenic compounds in more representative size-

Table 4 Comparison of distribution of mutagenic activity for various urban sites

Activity	*Fraction*	*Distribution of recovered mutagenic activity, %*					
		Urban street canyon		*Urban industrial*		*Urban non-specific*	
		I[a]	*II*[b]	*I*[c]	*II*[d]	*I*[e]	*SRM 1649*[f]
TA98, −S9	Organic acid	23	18	55	38	64	65
	PAH/Nitro-PAH	54	55	22	8	4	6
	Polar neutral	23	18	4	53	31	28
	Mutagenicity recovery	11[g]	30	21[g]	84	46	81
TA98, +S9	Organic acid	2	18	23	21	31	50
	PAH/Nitro-PAH	80	68	38	12	7	9
	Polar neutral	18	11	12	66	61	40
	Mutagenicity recovery	26[g]	48	58	88	27	89

[a]New York City sampling location, see Ref. 23.
[b]Oslo, Norway sampling location, see Ref. 22.
[c]Ohmuta, Japan sampling location, see Ref. 14.
[d]Philadelphia sampling location, see Ref. 21.
[e]Los Angeles sampling location, see Ref. 24.
[f]Washington, D.C. sampling location, as presented here.
[g]Recovery not stated in reference; calculated from data presented.

fractionated air particles. Detection of Dichlorophen suggests that the collection method resulted in this artifact. The long collection period and temporary storage of the particles at room temperature may have resulted in the artifactual formation and/or degradation of mutagenic compounds. Despite these concerns, a comparison of the distribution of mutagenic activity among compound classes indicates that the SRM 1649 extract falls in the range of mutagenicity of other urban air particulate extracts. The distribution of both direct- and indirect-acting mutagenicity in three general compound class fractions for six different urban air particulate samples is shown in Table 4. Although these data were collected by different laboratories, comparisons made here may be valid because the fractionation schemes used were roughly equivalent and the mutagenicity tester strain was identical in all cases.

As seen in Table 4, the percentages of mutagenic activity in organic acid and PAH/nitro-PAH fractions for the particulate extracts from the two urban industrial sites[14,21] are intermediate between those values for two urban street canyon samples[22,23] and urban sites distant from specific emission sources. For each street canyon particulate extract, the greatest percentage of activity was found in the PAH/nitro-PAH fraction. Since relatively high PAH and nitro-PAH levels are associated with gasoline and diesel engine emissions, the indirect- and direct-acting activity of this fraction may be due to these compound classes, respectively. Certainly, higher recovery of activity, presumably in the more difficult to recover polar compound class fractions, would shift the distribution of activity, with higher percentages in the polar fractions of these two samples.

The two particulate samples from urban sites not directly affected by point sources show high percentages of activity in the organic acid fraction and relatively little activity in the PAH/nitro-PAH fraction. Atmospheric transformation of PAH to hydroxylated-nitro-PAH has been proposed as a mechanism to account for increases of mass and activity in organic acid fractions of urban air samples.[24,25] Such reactions may be responsible for the phenomena observed here, as numerous hydroxylated-nitro aromatic and hydroxylated-nitro polycyclic aromatic compounds have been identified recently in another urban air particulate extract.[26,27] The percentage of recovered activity in the polar neutral fraction shows the least apparent correlation with sampling location. In general, though, a higher percentage of recovered activity in the polar neutral fraction is observed here for samples in which overall recovery of mutagenicity is high.

The advantage, then, to using SRM 1649 for methods development in bioassay-directed fractionation lies in the fact that relatively large proportions of mass and mutagenic activity are found in the acidic and polar neutral fractions. Because full recovery of polar molecules through fractionation procedures is relatively more difficult to achieve than for non-polar molecules, the SRM 1649 extract provides a significant challenge to any separation procedure developed.

Disclaimer

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tradenames or commercial products does not constitute endorsement or recommendation for use. The research at Battelle Columbus Laboratories was supported by U.S. EPA Contract 68-02-3169.

References

1. J. L. Huisingh, In: *Short-term Bioassays in the Analysis of Complex Environmental Mixtures II* (M. D. Waters, S. S. Sandhu, J. L. Huisingh, L. Claxton and S. Nesnow, eds) (Plenum Press, New York, 1981), pp. 9–19.
2. J. Leiter, M. B. Shimkin and J. J. Shear, *J. Natl. Cancer Inst.* **3,** 155 (1942).
3. W. C. Heuper, P. Kotin and E. C. Tabor, *Arch. Pathol.* **74,** 89 (1962).
4. S. Mizusaki, T. Takashima and K. Tomaru, *Mutat. Res.* **48,** 29 (1977).
5. W. G. Thilly, J. Longwell and B. M. Andon, *Environ. Health Perspect.* **48,** 129 (1983).
6. D. W. Later, M. L. Lee, R. A. Pelroy and B. A. Wilson, In: *Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry* (M. Cooke, A. J. Dennis, G. L. Fisher, eds) (Battelle Press, Columbus, Ohio, 1982), pp. 427–438.
7. H. S. Rosenkranz, E. C. McCoy, D. R. Sanders, M. Butler, D. K. Kiriazides and R. Mermelstein, *Science* **209,** 1039 (1980).
8. M. G. Nishioka, B. A. Petersen and J. Lewtas, In: *Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry* (M. Cooke, A. J. Dennis and G. L. Fisher, eds) (Battelle Press, Columbus, Ohio, 1982), pp. 603–614.
9. I. Salmeen, R. A. Gorse, T. Riley and D. Schuetzle, In: *Polynuclear Aromatic Hydrocarbons: Formation, Metabolism and Measurement* (M. Cooke and A. J. Dennis, eds) (Battelle Press, Columbus, Ohio, 1983), pp. 1057–1066.
10. L. D. Claxton, In: *Genotoxic Effects of Airborne Agents* (R. R. Tice, D. L. Costa and K. M. Schaich, eds) (Plenum Press, New York, 1982), pp. 19–33.
11. R. Jungers, R. Barton, L. Claxton and J. L. Huisingh, In: *Short-term Bioassays in the Analysis of Complex Environmental Mixtures II* (M. D. Waters, S. S. Sandhu, J. L. Huisingh, L. Claxton and S. Nesnow, eds) (Plenum Press, New York, 1981), pp. 45–65.
12. Certificate of Analysis; Standard Reference Material 1649, Urban Dust/Organics; National Bureau of Standards, Washington, D.C. 1982.
13. A. P. Swain, J. E. Cooper and R. L. Steadman, *Cancer Res.* **29,** 579 (1969).
14. J. Tokiwa, S. Kitamori, K. Takahashi, Y. Ohnishi, *Mutat. Res.* **77,** 99 (1980).
15. B. N. Ames, J. McCann and E. Yamasaki, *Mutat. Res.* **31,** 347 (1975).
16. L. D. Claxton, M. Kohan, A. C. Austin and C. Evanc, U.S. Environ. Prot. Agency, Off. Res. Dev.; EPA 1982, EPA-600/2-82-008, 1–148.
17. A. G. Stead, V. Hasselblad, J. P. Creason and L. Claxton, *Mutat. Res.* **85,** 13 (1981).
18. L. E. Myers, N. H. Adams, T. J. Hughes, L. R. Williams and L. D. Claxton, *Mutat. Res.* **182,** 121 (1987).
19. L. Bernstein, J. Caldor, J. McCann and M. C. Pike, *Mutat. Res.* **97,** 267 (1982).
20. C. M. Sparacino, F. E. Frazier, M. G. Nishioka and J. Lewtas, this issue.
21. M. G. Nishioka, C. C. Chuang, B. A. Petersen, A. Austin and J. Lewtas, *Environ. Int.* **11,** 137 (1985).
22. M. Moller, I. Alfheim, S. Larssen and A. Mikalsen, *Environ. Sci. Technol.* **16,** 221 (1982).
23. J. Lewtas, A. Austin, L. Claxton, R. Burton, R. Jungers, In: *Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry* (M. Cooke, A. J. Dennis and G. L. Fisher, eds) (Battelle Press, Columbus, Ohio, 1982), pp. 449–459.
24. B. Zielinska, J. Arey, R. Atkinson, T. Ramdahl, A. M. Winer and J. N. Pitts, Jr., *J. Am. Chem. Soc.* **108,** 4126 (1986).
25. R. Atkinson, J. Arey, B. Zielinska and S. M. Aschmann, *Environ. Sci. Technol.* **21,** 1014 (1987).
26. M. G. Nishioka, C. C. Howard, D. A. Contos, L. M. Ball and J. Lewtas, *Environ. Sci. Technol.* **22,** 908 (1988).
27. M. G. Nishioka, C. C. Howard, D. A. Contos, L. M. Ball and J. Lewtas, *Environ. Sci. Technol.* **23,** 248 (1989).