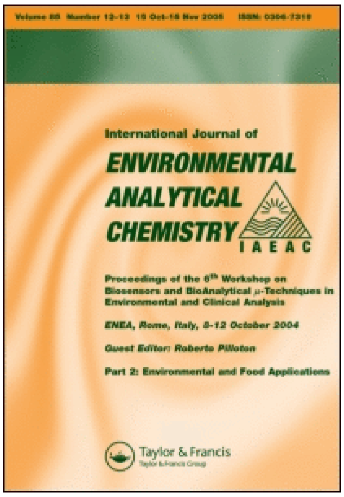

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Use of Cyanopropyl-Bonded HPLC Column for Bioassay-Directed Fractionation of Organic Extracts from Incinerator Emissions

D. M. Demarini[a]; R. W. Williams[b]; L. R. Brooks[b]; M. S. Taylor[b]
[a] Genetic Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC, U.S.A. [b] Environmental Health Research and Testing, Inc., NC, U.S.A.

To cite this Article Demarini, D. M. , Williams, R. W. , Brooks, L. R. and Taylor, M. S.(1992) 'Use of Cyanopropyl-Bonded HPLC Column for Bioassay-Directed Fractionation of Organic Extracts from Incinerator Emissions', International Journal of Environmental Analytical Chemistry, 48: 3, 187 — 199
To link to this Article: DOI: 10.1080/03067319208027399
URL: http://dx.doi.org/10.1080/03067319208027399

Intern. J. Environ. Anal. Chem., Vol. 48, pp. 187–199
Reprints available directly from the publisher
Photocopying permitted by license only

USE OF CYANOPROPYL-BONDED HPLC COLUMN FOR BIOASSAY-DIRECTED FRACTIONATION OF ORGANIC EXTRACTS FROM INCINERATOR EMISSIONS

D. M. DeMARINI

Genetic Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (U.S.A.)

R. W. WILLIAMS, L. R. BROOKS and M. S. TAYLOR

Environmental Health Research and Testing, Inc., P.O. Box 12199, Research Triangle Park, NC 27709 (U.S.A.)

(Received, 25 July 1991; in final form, 5 November 1991)

The present study has shown that cyanopropyl- (CN) bonded silica may be applicable for the fractionation by high pressure liquid chromatography (HPLC) of mass and mutagenic activity of organic extracts from some incinerator emissions. Dichloromethane-extractable organics from particles emitted by two different municipal waste incinerators and by a pilot-scale rotary kiln incinerator that was combusting polyethylene plastic were fractionated by HPLC, and the mutagenicity of the collected fractions was determined by means of a microsuspension mutagenicity assay with Salmonella TA98. The CN-bonded silica column provided high (80–100%) mass and mutagenicity recoveries for most emission extracts, and it fractionated the mutagenic activity. The results suggest that the emissions from municipal waste incinerators contain a high amount of direct-acting (-S9) mutagenic activity that is resolvable by HPLC using CN-bonded silica. Sub-fractionation of selected mutagenic HPLC fractions and subsequent analysis by gas chromatography-mass spectroscopy can be used to identify mutagenic species within complex incinerator emissions. The coupling of microsuspension bioassays to HPLC fractionation should be a useful tool for this type of analysis.

KEY WORDS: Municipal waste incineration, HPLC, complex mixtures, mutagrams, mutagenicity

INTRODUCTION

Incineration as a means of managing hazardous and municipal waste is gaining increased attention as the availability of land for waste disposal diminishes[1,2]. However, little health effects data exist for most incinerators beyond that necessary for the issuance of a permit/license for operation. These data, which include the determination of carbon monoxide levels, particle emission levels, and destruction efficiencies for selected compounds, reveal only a limited amount of information regarding the nature of the products of incomplete combustion (PICs) that are formed during incineration.

PIC's which are present to some extent in emissions from all combustion processes, have been found to be carcinogenic in humans and rodents and to be mutagenic in bacteria and mammalian cells[3]. A comprehensive review[4] has shown the usefulness of mutagenicity bioassays for evaluating the health effects of airborne mutagens and potential carcinogens present in the PICs from a variety of combustion emissions. One approach to identifying the hazardous components of the organic PICs present in complex combustion emissions involves the use of bioassay-directed chemical analysis[5]. This involves the identification of mutagenic chemical fractions by means of the Salmonella (Ames) mutagenicity assay[6], followed by chemical analysis of these mutagenic fractions.

Bioassay-directed fractionation has been used successfully to separate mutagenic from nonmutagenic mass for a variety of combustion emissions, including diesel exhaust[7], kerosene heaters[8], PIC-impacted urban air[9], and woodsmoke[10]. Although there are a few studies on the mutagenicity of incinerator emissions[11–15], none have employed bioassay-directed fractionation. Compared to other emissions, incinerator emissions pose several problems (discussed below) that will complicate the design of a fractionation procedure suitable for routine use with a variety of incinerator emissions.

Unlike diesel, kerosene, or woodsmoke emissions, incinerator emissions may be less homogeneous as a class of combustion emissions than the other above-mentioned emissions. This is because there is a wide variety of types, sizes, and operating conditions of municipal, hazardous, and medical/pathological waste incinerators. In addition, as the name of these incinerators implies, such combustors use a wide range of feed stocks that vary tremendously in chemical composition. An additional problem is that the emissions from some incinerators, especially municipal waste incinerators, contain significant amounts of water and are highly acidic, thereby complicating extraction/fractionation procedures (unpublished observations).

Various analytical or preparative liquid chromatography techniques have been used to fractionate complex combustion emissions for bioassay[4,6,9,16,17]. Most of these have used normal-phase silica-gel affinity chromatography, which has provided separation of nonpolar to polar species (usually by class) with moderate to good mass/mutagenicity recovery. However, silica gel also can absorb irreversibly some highly polar compounds, many of which may be present in incinerator emissions. Thus, we have begun to examine bonded-phase HPLC columns for possible use in the bioassay-directed fractionation of various incinerator emissions.

The present paper reports on our initial studies using cyanopropyl- (CN) bonded silica, which was chosen because (a) of its ability to tolerate wet extracts when used in a normal-phase mode, (b) it equilibrates rapidly in the mobile phase, and (c) it is less reactive than silica with some sample components, resulting in better mass recoveries[18]. This silica-bonded phase has given excellent recoveries of many polycyclic aromatic hydrocarbons (PAHs) and sulfur heterocycles, and recoveries of 50–100% have been obtained for hydroxylated and/or nitrogen-containing aromatics[19], all of which may be important components of incinerator emissions. In addition, the selectivity, separation mechanisms, and solubility parameters for CN-bonded silica are known[19–21].

Thus, we have used CN-bonded silica to fractionate the organic extracts of emission particles from: (a) a municipal waste incinerator designated Incinerator A, (b) a municipal waste incinerator that also burns some medical/pathology waste, which is designated Incinerator B, and (c) a pilot-scale incinerator with an afterburner that combusted polyethylene plastic during upset conditions; this sample is called PE-After. The three samples were chosen because they represent a range of incinerator types, combustion conditions, and feed stocks.

Mass recoveries, mutagenicity recoveries, HPLC chromatograms, and the resulting mutagenicity profiles (mutagrams)[22] were determined for all extracts except for the PE-After extract, for which there was no determination of mutagenicity recovery due to limited amount of sample. However, the PE-After extract was fractionated by HPLC on a silica column, and the resulting chromatogram and mutagram were compared to those produced by fractionation by HPLC on CN-bonded silica.

EXPERIMENTAL

Combustion emission samples

The incineration emissions evaluated included three types of feed stocks, three types of combustion processes, and two types of collection devices. Particles were collected on filters, extracted by sonication with dichloromethane (DCM), and the percent extractable organic mass (EOM) for each sample was determined as described previously[23].

The polyethylene (PE) emissions were generated as described[24] by combusting polyethylene rods under upset conditions in a pilot-scale rotary kiln with a secondary combustion chamber (afterburner). The particles were collected on filters by means of a dilution tunnel/baghouse designed specifically for collection of large samples for bioassay[25].

Incinerator A was a sample collected from a municipal waste incinerator composed of two refuse-fired boilers with a total capacity of 200 tons/day that was equipped with a reciprocating stoker, an economizer, and an electrostatic precipitator (ESP)[26]. Combustion emissions were vented into a common stack. A 10-cfm (0.28 m^3 min^{-1}) Source Dilution Sampler (SDS)[27] was used to collect particles, and the sampling probe was inserted into the emission gases from one boiler just prior to the entrance of the gases at the base of the stack. Incinerator B consisted of two starved-air Consumat combustors with a common ESP and stack[26]. The unit has a capacity of 50 tons day^{-1} and burns primarily municipal waste and $\sim$3–5 tons day^{-1} of hospital waste. The SDS sampler was used to collect particles in the stack just downstream from the ESP outlet[27]. Extracts from incinerators A and B were first fractionated into base/neutral, polar/weak acid, weak acid, and strong acid fractions on a solid-phase extraction column using a nonaqueous ion-exchange technique prior to fractionation by HPLC[18] (Thompson *et al.*, in preparation). Because most of the mutagenicity of these municipal waste combustion emission samples resided in the base/neutral fraction[18], only this fraction was fractionated further by HPLC.

High pressure liquid chromatography

Combustion extracts (~150–200 μg of EOM injected/column) were fractionated using either a DuPont Zorbax 5-μm cyanopropyl column (25 cm × 4.6 mm, cat #880952705) or an Alltech 3-μm silica-gel column (15 cm × 4.6 mm, cat. #27000). An Alltech Direct-Connect guard column (cat. #27000) containing either CN (cat. #28553) or silica (cat. #28550) replacement packing was placed in line with the analytical column. A Varian 5560 LC equipped with a Varian UV-200 detector and having ternary solvent-gradient delivery was used throughout the study. LC fractions were captured by an ISCO Foxy fraction collector, and data were captured by a Varian 604 data station.

Solvents (spectrometry grade) were purchased from Burdick and Jackson, Muskegon, MI; only one lot of each solvent was used per HPLC run. The following PAHs (Cas. No.) were obtained from commercial suppliers: acridine (260-94-6), 1-aminopyrene (1606-67-3), anthraquinone (84-65-1), benzo(a)pyrene (50-32-8), carbazole (86-7-8), dibenzo(a, j)acridine (108321-82-0), 1,3-dihydronaphthalene (132-86-5), naphthalene (91-20-3), 1-nitropyrene (5522-43-0), and pyrene (129-00-0). The structure and/or purity of each was confirmed by HPLC-mass spectroscopy.

PAH calibration standards, emission extracts, and blanks were processed as follows using ternary-gradient elutions of *n*-pentane, dichloromethane, and methanol. Condition #1 consisted of a 30-min step-gradient run. UV absorption was monitored at 254 nm, and one fraction was collected every 0.5 min. A final fraction (5 min) was also collected. The step-gradient conditions started at 100% *n*-pentane, which was held for 15 min, followed by a 2-min gradient to 100% DCM, which was held for 3 min, followed by a 10-min gradient to 100% methanol, which was held for an additional 5 min. All gradients were linear, and all flows were 1 ml min^{-1} except for the pentane-elution step, which was 0.5 ml min^{-1}.

Condition #2 consisted of 100% *n*-pentane held for 20 min (1 ml min^{-1}) followed by a 20-min gradient to 100% DCM. A final 20-min gradient to 100% methanol ended the program. UV absorption was monitored at 325 nm with one fraction collected per minute.

Sample preparation for bioassay

HPLC fractions were captured in 2-dram (7.4-ml) borosilicate vials. These vials were used empty unless fractions were to be used for bioassay analysis, in which case the vials contained 2 μl of dimethyl sulfoxide (DMSO). DMSO serves as a solvent that is compatible with the bacteria used in the bioassay. The HPLC solvents are toxic to the bacteria and were exchanged after fraction collection as described[28].

Reconstituted samples were prepared using the same LC procedures as described previously for capture of individual fractions. This was performed to compare the mutagenic potencies of extracts before and after fractionation. Reconstituted samples were prepared by capturing all of the eluate from an injection and then concentrating it to a known gravimetric value. Concentration was performed using rotary evaporation (35°C and 500 torr vacuum using a Brinkman RE-121 rotary evaporator) until the volume was reduced adequately to permit quantitative transfer of the sample to a 10-ml volumetric flask. Individual doses of the reconstituted extracts as well as unfractionated whole extracts were prepared by transferring known volumes of stocks into 2-dram vials containing 2 μl of DMSO and solvent exchanging them to a final volume of 2 μl using a dry stream of nitrogen.

Gravimetric mass determinations were performed on samples prior to HPLC fractionation by transferring replicate volumes (10–250 μl) of sample using gas-tight syringes into individual tared aluminum weigh cups by procedures described previously[28]. Data collected from the mass residue trials were then used to determine mass concentration/total mass recovered. A Sartorious 4503 microbalance, readable to 1 μg, was used for all weighings.

Bioassay procedures

Unfractionated or reconstituted extracts that had been solvent exchanged into DMSO as described above were evaluated for mutagenic activity in the Salmonella (Ames) mutagenicity assay using strain TA98. The standard plate-incorporation procedure using Aroclor 1254-induced male Sprague-Dawley rat liver S9 for metabolic activation was performed as described[5]. For the production of mutagenicity profiles (mutagrams)[22], a microsuspension assay was performed as described previously[29]. Briefly, 50 μl of a 5X overnight cell suspension were added along with 50 μl of either sodium phosphate buffer or S9 mix to a 2-dram vial containing the HPLC fraction that had been solvent exchanged into 2 μl of DMSO. This suspension was incubated at 37°C for 90 min, after which ~2.5 ml of molten top agar were added, and the contents poured onto minimal medium. Colonies (revertants) were counted after 2–3 days of incubation at 37°C. Mutagenic potencies were calculated based on the regression over the linear portion of the dose-response curves. All plate-incorporation assays were performed twice, each time in duplicate.

RESULTS AND DISCUSSION

Standards

Fractionation of the standards on the CN column using LC condition #1 is shown in the chromatogram in Figure 1. The results show that acceptable separation was achieved for these standards, which were selected based on their use with silica gel for the fractionation of environmental samples[6,17,18]. The elution times for some of these compounds on silica gel have been included for comparison in Table 1. These standards comprise a range of compounds that can be used to identify where representative aromatics, moderately polar neutrals, and highly polar species elute from the CN column.

Such a wide range is necessary to cover the range of chemical classes that may be present in a complex environmental sample. As illustrated later, characterization of the elution pattern of a set of standards is necessary for inferring the possible species responsible for the mutagenic activity produced within a certain region of a mutagram. This knowledge assists in the choice of ancillary analytical tests, such as

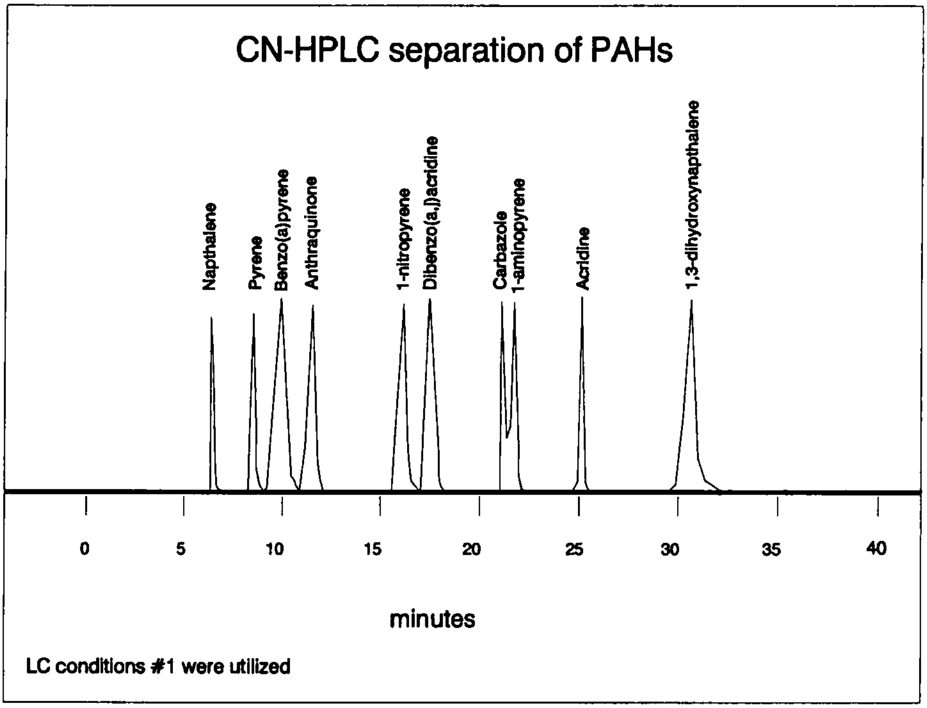

Figure 1 Chromatogram of standards fractionated on CN column under condition #1 (see Experimental).

Table 1 Absolute retention times of standards

Standard	*Retention time (min)*		*Polarity classification*
	CN[a]	*Silica*[b]	
1. Naphthalene	6.60	1.95	Aromatic
2. Pyrene	8.70	2.61	Aromatic
3. Benzo(a)pyrene	9.50	2.05	Aromatic
4. Anthraquinone	11.50		Moderately polar
5. 1-Nitropyrene	16.30	3.21	Moderately polar
6. Dibenzo(a, j)-acridine	17.30		Moderately polar
7. Carbazole	21.20		Moderately polar
8. 1-Aminopyrene	21.40		Moderately polar
9. Acridine	25.50	46.00	Highly polar
10. 1,3-Dihydroxynaphthalene	30.80		Highly polar

[a] LC condition #1; see Experimental.
[b] LC condition #2; see Experimental.

Table 2 Mass recovery of extracts from CN column

Extract[a]	*Injected mass* (μg)	*Percent recovery*
Incinerator A	203	81.2
Incinerator B	151	40.4
PE-After	216	100.8

[a] LC condition #1: see Experimental.

negative ion-mass spectroscopy if nitroaromatics are suspected of being present, or the selection of additional diagnostic bioassay tester strains.

Mass recovery

The CN column provided good mass recoveries for all samples except for Incinerator B, which had a recovery of only 40.4% (Table 2). This sample could have contained basic compounds that were susceptible to irreversible adsorption to residual silanols, which may occur with CN-bonded phases. The low mass recovery of the sample does not appear to be due to mass discrimination resulting from low mass loading because recoveries of 80–112% have been obtained for other samples, which were injected at comparable masses (150–500 μg). The CN column has given mass recoveries of 91–112% for two other incinerator emissions that we are currently evaluating for another study.

The loading range used was selected because of the requirement to fractionate mass/fraction sufficient to evaluate each fraction for mutagenic activity. However, this requirement was also restricted by the desire to not overload the analytical column, which would result in broader bands and poorer resolution. The mass recoveries for the extracts after CN-HPLC were as good or better than those obtained for other combustion emissions on silica gel, where mass recoveries of 85–95% have been obtained for diesel emissions, tobacco smoke, coke oven mains, and urban air particles[6,16,17,30].

Mutagenicity recovery

In addition to mass recovery, mutagenic recovery is also important when performing bioassay-directed fractionation. The fractionation procedure used to separate biologically active (mutagenic) mass from that which is not biologically active (nonmutagenic) should not cause degradation of the mutagens present in the sample or formation of mutagens. This important feature of an acceptable fractionation scheme can be characterized by comparing the mutagenic potency (rev μg^{-1}) of the unfractionated sample to that of a fractionated sample whose fractions have been pooled (a reconstituted sample). Similar mutagenic potencies between the two samples would indicate that the fractionation procedure did not alter the mutagenicity of the components of the mixture. However, chemical modifications could occur during fractionation that may not alter the net mutagenic potency of the extract.

Table 3 Mutagenic recovery from CN column

Sample	*Rev*$^{-1}$ *μg in TA98*[a]		*Recovery of mutagenic activity* (%)
	Before fractionation	*After fractionation*	
Incinerator A			
+S9	116	127	109
−S9	329	302	92
Incinerator B			
+S9	2	2	100
−S9	8	8	100

[a] Slope calculated from linear portion of dose-response curve.

Table 3 shows that such is the case for the complex mixtures fractionated on the CN column. All of the mutagenic recoveries were >90%. The high level of mutagenicity recovery for the extract from Incinerator B is especially interesting because only 40% of the mass of this sample was recovered from the CN column (Table 2). The high recovery of biological activity (mutagenicity) from the CN column suggests that this fractionation procedure did not retain or degrade the mutagens present in the extracts. Due to limited sample size, mutagenicity recovery was not performed with the PE-After extract. However, mutagenicity recovery (+S9) of a related extract (combustion of polyethylene without an afterburner) was 108% (unpublished data).

Mutagrams

The fractionation of the mutagenic activity of the complex combustion emissions on CN and silica columns is shown in Figures 2–4. Each figure shows a mutagram and its accompanying HPLC chromatogram. (Chromatograms were highly reproducible, and one representative chromatogram is shown with each mutagram.) Figure 2 shows that under the conditions used, the CN column separated the direct-acting (−S9) mutagenic activity of the DCM fraction of the organic emissions from Incinerator A into several fractions. As noted in Table 3, the mutagenic potency of the DCM fraction in the absence of S9 was approximately 3 times greater than in the presence of S9. The predominance of direct-acting mutagenic activity is confirmed by the mutagram in Figure 2. The relatively low level of indirect-acting (+S9) mutagenic activity is not due to inadequate recovery of this type of mutagenic activity from the CN column, which was 92% (Table 3). The mutagenic activity (−S9) elutes in the moderately polar region encompassed by standards 5–9. This includes compounds such as 1-nitropyrene, which is a direct-acting (−S9) mutagen in Salmonella TA98.

Because of limited sample size and the low mutagenic potency of the extract from Incinerator B in the presence of S9 (Table 3), the HPLC fractions from the DCM fraction of Incinerator B were bioassayed only in the absence of S9. Figure 3 shows that under HPLC condition #1 (see EXPERIMENTAL), the CN column did not

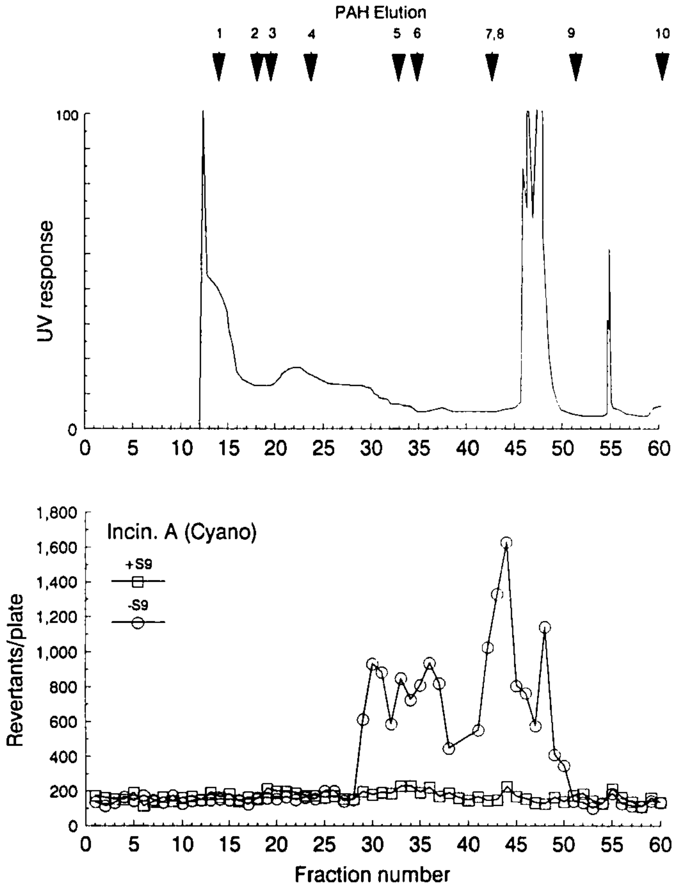

Figure 2 Chromatogram and mutagram of Incinerator A extract fractionated on CN column under condition # 1. Standards are identified by number in Table 1; see Experimental for details.

fractionate the mutagenic activity of this sample, i.e., much of the mutagenic activity eluted in one peak area, which was near the highly polar region. Consistent with the low mutagenic potency of this extract in the absence of S9 (8 rev μg^{-1}), the amount of mutagenic activity in the peak (maximum height of ~200 rev plate^{-1}) was also low. Thus, there was little mutagenic activity to fractionate, and that which was present may have been composed of species of similar chemical class/polarity.

A comparison of the chromatograms and mutagrams of the extracts from Incinerators A and B reveals some similarities, with major peaks in both the chromatograms and mutagrams between fractions 45–50 (Figures 2 and 3). Both also contain a small peak at fraction 55. One interpretation of the results wth these two incinerator samples is that they share some chemical similarity; however, the absolute amounts of mutagenic organics is considerably lower in the extract from Incinerator B, as evidenced by the much lower mutagenic potency of the extract from Incinerator

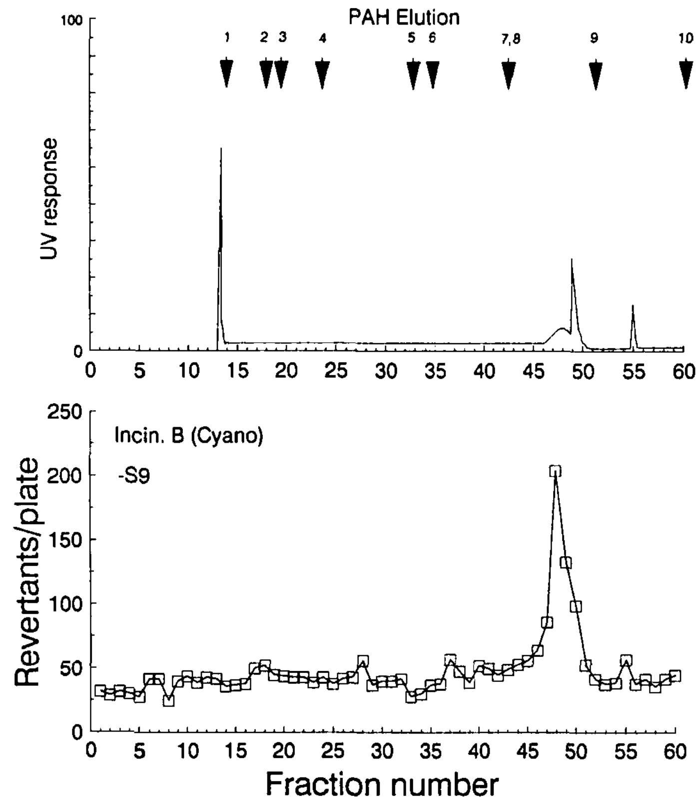

Figure 3 Chromatogram and mutagram of Incinerator B extract fractionated on CN column under condition #1. Standards are identified by number in Table 1; see Experimental for details.

B relative to that from Incinerator A (Table 3). Calculation of the mutagenic emission factor for these two incinerators has confirmed that incinerator B releases less mutagenic activity per hour than does Incinerator A[26].

Figure 4 shows the mutagrams produced by fractionation of the PE-After extract by the CN and silica columns. Both columns separated mutagenic activity; however, the mutagrams are different. As with the other incineration samples, direct-acting (−S9) mutagenic activity predominated. This is interesting because the mutagenic potency of the PE-After extract before fractionation was similar in the presence and absence of S9 (1.2 vs. 1.3 rev μg^{-1}, respectively). At least three distinct peaks of mutagenic activity were produced by the silica column; whereas two distinct peaks and a cluster of peaks were produced by the CN column (Figure 4). Fluorescence in the region of the clustered peaks revealed 10 resolved peaks (data not shown). These data suggest that both silica and CN-bonded silica may be useful for the purpose of bioassay-directed chemical analysis.

As with the other incinerator extracts, the PE-After contained mutagenic activity (both +S9 and −S9) that eluted in the moderately polar region of the CN column

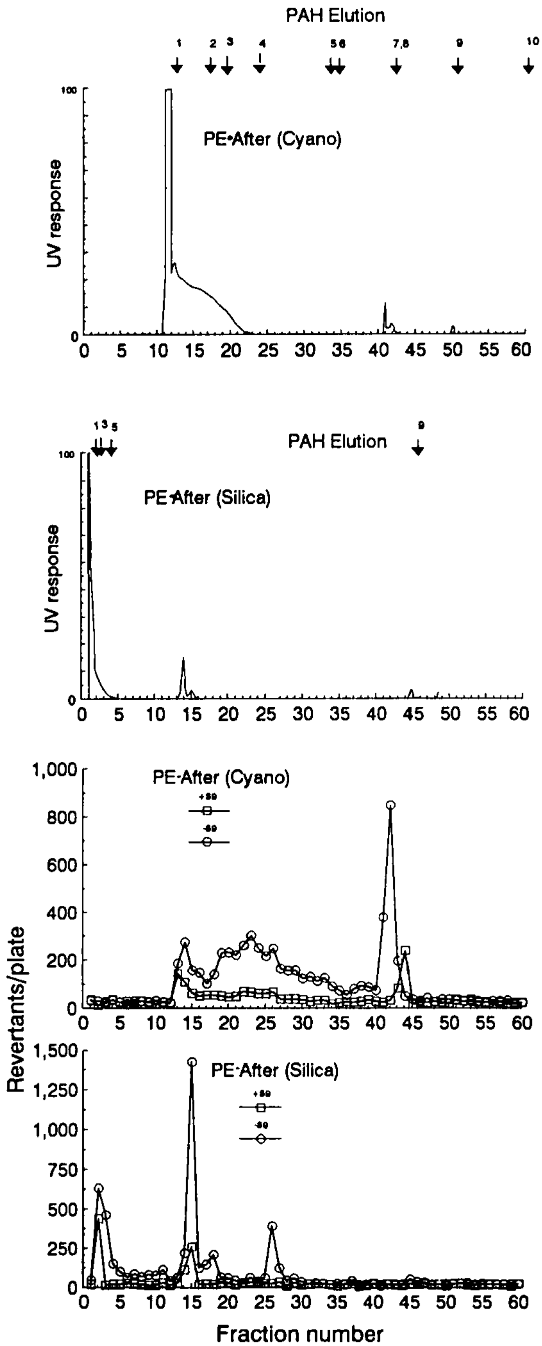

Figure 4 Chromatograms and mutagrams of PE-After extract fractionated on CN and silica columns under conditions #1 and #2, respectively. Standards are identified by number in Table 1; see Experimental for details.

(Figure 4). However, unlike the other incinerator extracts, the PE-After also contained mutagenic activity that eluted in the region in which the aromatic PAH standards (1–3) eluted from both columns (Figure 4). Although fractions from the PE-After extract have not yet been subjected to chemical analysis, examination by mass spectroscopy of selected fractions of PE combusted without an afterburner have revealed the presence of many PAHs[31].

CONCLUSIONS

The present study has shown that CN-bonded silica may be applicable for the fractionation of mass and mutagenic activity of some incinerator emissions. CN-bonded silica provided good mass and mutagenicity recoveries for most emission extracts, gave reproducible chromatograms, and provided useful separations of mutagenic activity. The results suggest that the emissions from municipal waste incinerators contain a high amount of direct-acting (−S9) mutagenic activity and that 1-nitropyrene, which is a direct-acting mutagen, elutes within the same region of the HPLC mutagram in which much of the direct-acting mutagenic activity elutes.

Recently, we have extended the present study by first generating a mutagram of a DCM extract of emissions from the combustion of PE without an afterburner. By performing further fractionation of selected mutagenic HPLC fractions and then analysing these mutagenic subfractions by gas chromatography/mass spectroscopy, we have been able to identify various mutagenic species within this complex incinerator emission[31]. These results and the present study demonstrate that the coupling of microsuspension bioassays to HPLC fractionation is a useful tool for characterizing the mutagenicity of incinerator emissions.

Acknowledgements

We thank Douglas Bell, Guy Lambert, and Randall Watts for their assistance; Paul Lemieux, Ray Grote, and Jeff Ryan for providing the incinerator emission samples; and Daniel Thompson for his helpful comments on the manuscript. This work was supported through EPA contract #68-02-4456. The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. This does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

1. P. Steinhart, *Audubon* **88**, 102–103 (1986).
2. E. Wingerter, *EPA Journal* **15**(2), 22 (1989).
3. International Agency for Research on Cancer (IARC), *Monograph Programme on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Polynuclear Aromatic Compounds, Part 4, Bitumens, Coal-tar and Derived Products, Shale Oils and Soots, Vol. 35*, IARC, Lyon, France, pp. 271 (1985).
4. J. Lewtas, *Fund. Appl. Toxicol.* **10**, 571–589 (1988).
5. D. Schuetzle and J. Lewtas, *Anal. Chem.* **58**, 1060A–1075A (1986).
6. D. B. Maron and B. N. Ames, *Mutat. Res.* **113**, 173–215 (1983).
7. J. Lewtas. In: *Toxicological Effects of Emissions from Diesel Engines* (J. Lewtas, ed) (Elsevier, New York, 1982), pp. 243–264.

8. K. Tokiwa, R. Nakagawa and K. Horikawa, *Mutat. Res.* **157**, 39–47 (1985).
9. J. Lewtas, J. Chuang, M. Nishioka and B. Petersen, *Intern. J. Environ. Anal. Chem.* **39**, 245–256 (1990).
10. D. A. Bell, H. Karam and R. M. Kamens, *Environ. Sci. Technol.* **24**, 1261–1264 (1990).
11. U. G. Ahlborg and K. Victorin, *Waste Manage. Res.* **5**, 203–224 (1987).
12. A. Kamiya and Y. Ose, *Sci. Total Environ.* **61**, 37–49 (1987).
13. R. Watts, H. Fitzgerald, H. Garabedian, R. Williams, S. Warren, L. Fradkin and J. Lewtas, *J. Air Pollut. Contl. Assoc.* **39**, 1436–1439 (1989).
14. W. P. Linak, J. V. Ryan, E. Perry, R. W. Williams and D. M. DeMarini, *J. Air Pollut. Contl. Assoc.* **39**, 836–846 (1989).
15. D. DeMarini, V. S. Houk, J. Lewtas, R. W. Williams, M. G. Nishioka, R. K. Srivastava, J. V. Ryan, J. A. McSorley and W. P. Linak, *Environ. Sci. Technol.* **25**, 910–913 (1991).
16. R. Williams, C. Sparacino, B. Petersen, J. Bumgarner, R. Jungers and J. Lewtas, *Intern. J. Anal. Chem.* **26**, 27–49 (1986).
17. M. Nishioka, C. Howard, D. Contos, L. Ball and J. Lewtas, *Environ. Sci. Technol.* **22**, 908–915 (1988).
18. D. J. Thompson, R. W. Williams, L. Brooks, D. A. Bell and M. G. Nishioka, *Proceedings of the 1990 EPA/AWMA Symposium on Measurement of Toxic and Related Air Pollutants*, EPA/600/9-90/026, 818–823 (1990).
19. C. Oestman and A. Colmjoe, *Chromatographia* **23**, 903–908 (1987).
20. W. Cooper and P. Smith, *J. Chromatography* **355**, 57–74 (1986).
21. P. Smith and W. Cooper, *Chromatographia* **25**, 55–60 (1988).
22. J. Lewtas, L. C. King, K. Williams, L. M. Ball and D. M. DeMarini, *Mutagenesis* **5**, 481–489 (1990).
23. R. Williams, T. Pasley, S. Warren, R. Zweidinger, R. Watts, A. Stead and L. Claxton, *Intern. J. Environ. Anal. Chem.* **34**, 137–154 (1988).
24. W. Linak, J. Kilgroe, J. McSorley, J. Wendt and J. Dunn, *J. Air Pollut. Contl. Assoc.* **37**, 54–65 (1987).
25. P. Lemieux, J. McSorley and W. Linak, In: *Remedial Action. Treatment and Disposal of Hazardous Wastes* (EPA/600/9-90/006, 1990), pp. 43–49.
26. R. R. Watts, P. M. Lemieux, R. A. Grote, R. W. Lowans, R. W. Williams, L. R. Brooks, S. H. Warren, D. M. DeMarini, D. A. Bell and J. Lewtas, *Environ. Health Perspect.* **98** (in press, 1992).
27. W. J. Steele, A. D. Williamson and J. D. McCain, U.S. Environ. Prot. Agency, Off. Res. Dev.; EPA 1988, EPA 600/8-88-069 (PB88-198551).
28. R. Williams, E. Perry and J. Lewtas, *Environ. Int.* **12**, 625–633 (1986).
29. D. M. DeMarini, M. M. Dallas and J. Lewtas, *Teratogen. Carcinogen. Mutagen.* **9**, 287–295 (1989).
30. D. Schuetzle, T. Jensen and J. Ball, *Environ. Int.* **11**, 169–182 (1985).
31. D. DeMarini, R. W. Williams, E. Perry, P. M. Lemieux and W. P. Linak, *Combust. Sci. Technol.* (in press, 1992).