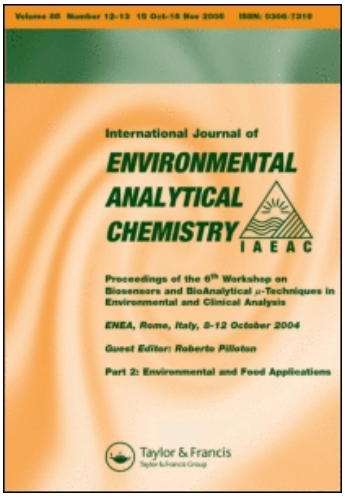

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Selection of a Suitable Extraction Method for Mutagenic Activity from Woodsmoke-Impacted Air Particles

Ron Williams[a]; Todd Pasley[a]; Sarah Warren[a]; Roy Zweidinger[b]; Randall Watts[b]; Andrew Stead[bc]; Larry Claxton[b]
[a] Environmental Health Research and Testing, Inc., Research Triangle Park, N.C., U.S.A. [b] Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, N.C., U.S.A. [c] Organon Teknika,

To cite this Article Williams, Ron , Pasley, Todd , Warren, Sarah , Zweidinger, Roy , Watts, Randall , Stead, Andrew and Claxton, Larry(1988) 'Selection of a Suitable Extraction Method for Mutagenic Activity from Woodsmoke-Impacted Air Particles', International Journal of Environmental Analytical Chemistry, 34: 2, 137 — 154
To link to this Article: DOI: 10.1080/03067318808027411
URL: http://dx.doi.org/10.1080/03067318808027411

Intern. J. Environ. Anal. Chem., Vol. 34, pp. 137–154
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in Great Britain

Selection of a Suitable Extraction Method for Mutagenic Activity from Woodsmoke-Impacted Air Particles

RON WILLIAMS, TODD PASLEY and SARAH WARREN

Environmental Health Research and Testing, Inc., P.O. Box 12199, Research Triangle Park, N.C., 27709, U.S.A.

and

ROY ZWEIDINGER, RANDALL WATTS, ANDREW STEAD* and LARRY CLAXTON

Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, N.C., 27711, U.S.A.

(*Received 13 October, 1987; in final form 23 March, 1988*)

Extraction methods were evaluated for recovery of mutagenic activity from woodsmoke-impacted air particles. Soxhlet and sonication techniques were utilized with a variety of solvents to ascertain the effect of solvent choice, extraction methods, or dissolved gases in extraction solvents on the recovery of mutagenicity. Sonication extraction gave slightly less mass recovery than the Soxhlet method. Methanol extracted more mass than the other solvents with dichloromethane recovering the least. Dissolved gases were not found to have any effect, while mutagenicity was shown to be dependent upon solvent and extraction method. Soxhlet extraction with acetone and toluene/ethanol yielded the highest recovery of mutagenic activity, however, results indicated a solvent/solute interaction which chemically altered one or

*Present address: Organon Teknika, Durham, N:C., 27713

more extract components. Extraction employing dichloromethane and sonication was selected as a suitable method since this treatment appeared not to alter extracted compounds, and good recovery of mutagenicity was obtained.

KEY WORDS: Woodsmoke particles, extraction techniques, mutagenicity, Integrated Air Cancer Project.

INTRODUCTION

One goal of the U.S. Environmental Protection Agency's ambient woodsmoke research was the selection of an appropriate extraction method for recovery of mutagens from ambient air particulate matter.[1] The selected method should recover organic compounds with minimal extraction of inorganics, provide rapid sample processing, be chemically inert to the analytes being recovered, and be compatible to further processing of extracted samples (gravimetric, gas and liquid chromatographic analysis, and bioassay).

It has been suggested from previous studies on ambient air particles that some solvents in combination with Soxhlet extraction techniques may be responsible for chemical alterations of extracted mutagenic species.[2,3] Some investigators hypothesized that other extraction factors such as solvent reflux temperatures may be responsible. This study examined the effects of various extraction conditions upon mass and mutagenicity recovery from woodsmoke-impacted ambient air particles. Methods were selected for extraction of woodsmoke particles to meet the above analysis requirements.

EXPERIMENTAL

Study design

Seventy identical ambient particle samples were prepared from 117 filters collected in a location with significant woodsmoke loadings. These samples were extracted in triplicate and evaluated for the effects of various solvents and extraction methods upon the recovery of mass and mutagenicity. Methanol, acetone, dichloromethane and 3:1(v/v) toluene/ethanol were used as solvents and each was tested using sonication and Soxhlet extraction. The effect of dissolved gases

upon extraction solvents was evaluated by degassing one set of samples prior to sonication and also by using a continuous nitrogen purge during a second set of Soxhlet extracted samples. Sample extracts from all trials were filtered to remove suspended particles and concentrated by rotary evaporation to facilitate determination of extractable mass via gravimetric analysis. Extracts were solvent exchanged into dimethyl sulfoxide (DMSO) in preparation for the Salmonella bioassay. The presence of residual extraction solvent in each sample (a potential source of Salmonella toxicity) was determined by gas chromatography. High performance liquid chromatography (HPLC) with fluorescence detection was performed upon selected samples to compare the result when different solvents and extraction methods were utilized. An outline of this study design is presented in Figure 1.

Those solvents for which the Soxhlet method appeared to extract more mutagens than sonication were tested to determine if the increased mutagenicity resulted from enhanced extraction efficiency

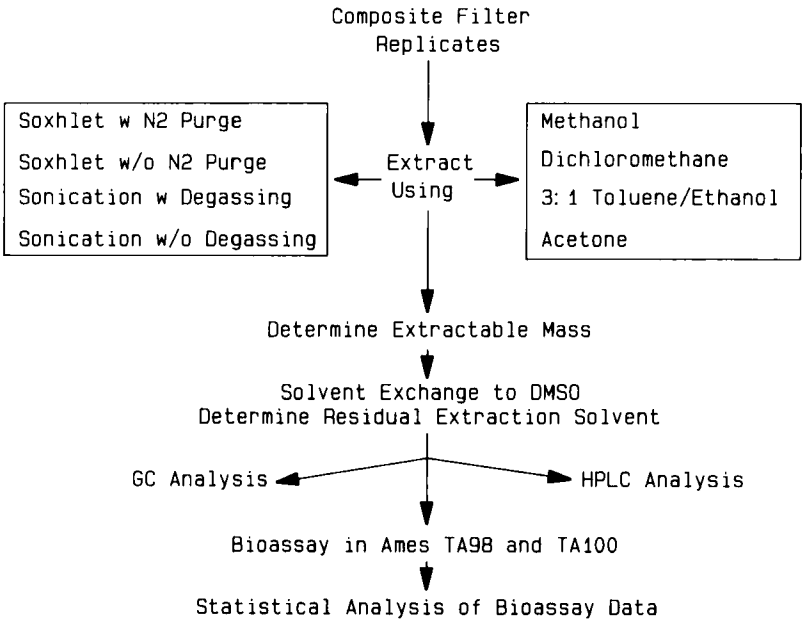

Figure 1 Study design used in comparison of extraction solvents and methods for woodsmoke impacted air particles.

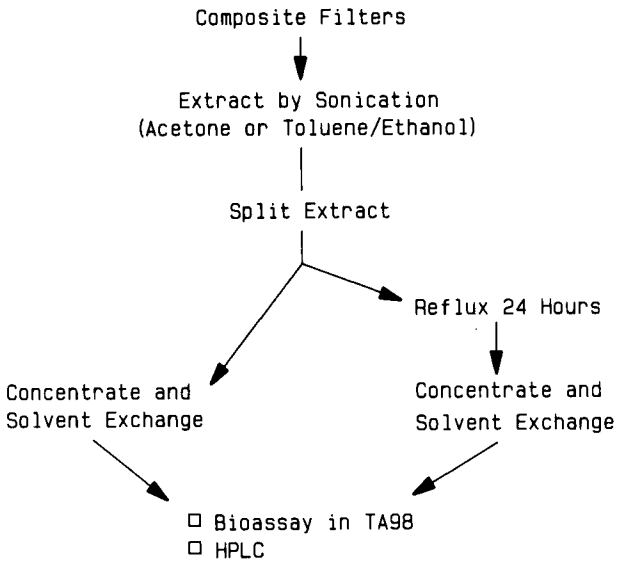

Figure 2 Procedures utilized to compare refluxed woodsmoke extracts to non-refluxed samples.

or from an interaction of the solvent with the extracted components. A split sample analysis (Figure 2) was used. Five particle samples were extracted by sonication with acetone and 5 with 3:1 toluene/ethanol. Half of each was solvent exchanged into DMSO for bioassay. The remainders were refluxed for 24 hours to simulate the conditions found in a Soxhlet extraction of woodsmoke organics. All samples were bioassayed and HPLC chromatograms obtained.

Materials and methods

Particulate matter was collected from a residential site in Raleigh, NC between January and March 1985 as part of the Integrated Air Cancer Project using Hi-Vol PM-10 and Hi-Vol PM-2.5 samplers.[4] Teflon-coated ($8'' \times 10''$) glass fiber filters (Pallflex, No. T6820) were employed for particle capture. A total particle loading of approximately 3 grams was collected from 42,002 m^3 of air of which there were 67 PM-10 filters (particles ≤ 10 microns) and 50 PM-2.5 filters (particles ≤ 2.5 microns). Filters were stored in darkness at $-80°C$ prior to extraction to prevent sample degradation and loss of semi-volatile materials.

One hundred seventeen filters were composited into 70 replicate samples to compensate for any variability in mass loading between filters and to provide consistent mass quantities for each extraction. A previously designed cutting jig was used to cut the loaded area of each filter into 70 sections of equal area.[5] These sections were distributed into 70 samples, each containing 117 sections (one from each filter). Randomness of distribution was assured by the use of random number tables in assigning filter sections to composite samples. Average particulate mass per composite sample was calculated to be 41.6 mg.

Glassware to be used in the study was acid cleaned followed by distilled/deionized water and solvent rinsed. Glassware was then baked out overnight. Extraction solvents were all Burdick and Jackson (B&J™) with the exception of the ethanol (Aaper Chemical, Lexington, KY, USA). Only one lot of each solvent was used for consistency.

Soxhlet extractions

Soxhlet extractions involved size 23 Soxhlets in conjunction with Allihn condensers and 500 mL round bottom flasks (Kontes K-585000). Borosilicate glass extraction thimbles with extra coarse filter frits (size 222, Kontes K-586500) were used to contain the filter pieces within the extraction tubes. Heat was supplied to the flasks by hemispherical heating mantles powered and controlled by variable autotransformers.

Soxhlet extraction with a nitrogen purge required a nitrogen blanketing system to be utilized. This system replaced normal atmospheric gases in the reflux pot with a closed system of nitrogen, thus reducing chances of extracted analyte interactions with dissolved solvent gases. A nitrogen atmosphere was maintained within each Soxhlet prior to and during extraction by passing nitrogen into the units at approximately 150 mL/min. Bubblers were attached to the purge system to ensure it was working.

Composite samples (1/Soxhlet) were placed in thimbles crowned with a plug of extracted silanized glass wool and were extracted with 300 mL of the test solvent. Only one lot of each solvent (B&J™) was used for consistency. Teflon™ boiling chips were utilized in the solvent flasks to aid ebullition. Heat was applied to achieve a Soxhlet cycle rate of 2 cycles/hr for 24 hours.

Sonication extraction

A Branson model B-32 100 watt ultrasonic cleaner was used for the sonication extractions. A Gast (model 4143) vacuum pump was used for samples requiring degassing. Samples were contained in 250 mL glass bottles with Teflon™ lined caps. Composite samples were placed in the vessels and ultrasonically extracted 2 times, each time using 150 mL of fresh solvent for 10 minutes. Earlier work had established that no greater extraction efficiency could be gained with more than 2 extraction periods. Vacuum for samples to be degassed was applied via a Teflon™ coated one-hole stopper connected to the hose of the vacuum pump for 15 seconds (60 torr) at the start of each extraction period. Caps were immediately replaced on extraction vessels after degassing and the extraction continued for a total of 10 minutes. The two 150 mL extracts from each composite were combined to form one sample.

Extraction concentration

Extracts were filtered through individual 0.5 micron Teflon™ filters using a borosilicate glass filter apparatus and quantitatively transferred to 500 mL round bottom flasks, where they were reduced in volume to less than 10 mL using rotary evaporation at 35°C. Concentrates were transferred to volumetric flasks and diluted to exactly 10 mL with the appropriate extraction solvent. Duplicate mass determinations (0.5 mL) were placed into individually tared aluminum weighing pans. Solvent was allowed to evaporate passively from the pans followed by placement in a desiccator for 24 hours prior to weighing. Means of the mass residue were used to determine the average mass concentration in extracts. A balance readable to 0.01 mg was used to determine mass residues. Residues were in the range of 1–3 mg per 0.5 mL aliquot.

Eight mL of the 10 mL extracts were transferred to 10 mL Kunderna-Danish concentrator tubes equipped with ball-less micro Snyder columns (Kontes K-570050, K-569251). A Meyer N-Evap™ nitrogen evaporator at 35°C was used to complete the sample concentration and solvent exchange. Nitrogen was bubbled through the extracts via Teflon™ tubing attached to stainless steel needles. When the solvent volume reached 0.5 mL, 0.5 mL of DMSO was added. Nitrogen ebullition was continued until volume again reached

0.5 mL. An additional 0.5 mL of DMSO was added followed by nitrogen ebullition for an additional 10–15 minutes to remove any traces of remaining extraction solvent. Samples were then transferred to volumetric flasks and brought up to exactly 5.0 mL with DMSO for bioassay.

Aliquots (0.1 mL) of all bioassay samples were placed in autosampler vials containing 1.4 mL of DMSO. Samples were analyzed by packed column gas chromatography with flame ionization detection to ensure that solvent exchanged extracts contained less than 1%(v/v) residual extraction solvent. Samples were found to actually contain on the order of 0.1% artifact solvent. Methods utilized for this determination have previously been reported.[6]

High performance liquid chromatography

Samples of each extract were chromatographed on a Hewlett Packard 1090 liquid chromatograph equipped with a Perkin Elmer 650 fluorescence detector operating at nm wavelengths of 340ex/400em. A 25 cm × 4.6 mm C18 reverse phase column (Dupont Zorbax, 5 micron) was employed at a flowrate of 1 mL/minute with a 40 minute linear gradient of 45% methanol-water to 100% methanol and 35 minutes at 100%. Data was acquired with a Nelson Analytical laboratory data system.

Bioassay

The bioassays were performed using a *Salmonella typhimurium* reversion assay with modifications.[7] Duplicate doses of 10, 30, 50, 100, and 200 ul from each 5.0 ml sample were tested. Strains TA98 and TA100 were kindly supplied by B. Ames (Univ. of California, Berkeley, CA) and used with and without exogenous metabolic activation (±CD male rat liver S9) for initial comparisons. Only strain TA98 was used to investigate the effects of the different solvents during reflux experiments. Positive control 2-anthramine (0.5 ug/plate) was used for strains TA98 and TA100 with S9 activation, while 2-nitrofluorene (3.0 ug/plate) and sodium azide (3.0 ug/plate) were used as controls without S9 activation for strains TA98 and TA100 respectively. Single technicians were responsible for each Salmonella strain.

Statistical methods

Mutagenic activity in each sample was estimated by finding the initial linear slope using the method of Bernstein *et al.*[8] Effects on these slopes arising from either solvent or extraction method were examined by fitting a two-way analysis of variance (ANOVA) model. An interaction term was included to test for non-additivity between the 2 factors. A separate ANOVA was performed for each combination of metabolic activation and bacterial strain and choice of slope units.

If there was evidence of an interaction between solvent and method, the data was reanalyzed within a 1-way ANOVA framework with 16 treatment combinations. The sixteen treatments are described in Table 1. A multiple comparison procedure[9] was then invoked to determine which treatment means were significantly different. If no interaction was detectable, then specific contrasts were of interest. Were the Soxhlet methods with and without nitrogen purging different? Was sonication with and without degassing different? Finally, was the Soxhlet method different from the sonication method?

Ratios such as slopes often have non-Gaussian properties, the analyses described above were performed with and without using logarithmic transformation. However, residual variation from log slope ANOVA models departed more from normality than did the untransformed data.

Bioassay results from the extract-reflux interaction studies were evaluated by estimating initial linear slopes, again using the method of Bernstein *et al.*,[8] and subsequently fitting these slopes to a one-way ANOVA model. All analyses were conducted using the general linear models (GLM) procedure within the basic SAS software[10] package.

Table 1 Relative amounts[a] of mass recovered using various extractions

Solvent	*Soxhlet with N_2 purge*	*Soxhlet without N_2 purge*	*Sonication with degassing*	*Sonication without degassing*
Methanol	1.00	0.91	0.96	0.90
3:1 v/v Toluene/ethanol	0.51	0.64	0.47	0.51
Dichloromethane	0.55	0.55	0.46	0.42
Acetone	0.76	0.77	0.68	0.74

[a]% Mass recovery of treatment effects normalized to the recovery obtained by methanol Soxhlet with nitrogen purge. Values represent means from triplicate identical treatments.

RESULTS

Mean mass recovery for each extraction effect is presented in Table 1. Values have been normalized to those of the methanol Soxhlet extraction with nitrogen purge which averaged 46.1 mg per replicate treatment. The greatest recovery of mass was by methanol extraction followed by acetone, 3:1 toluene/ethanol and then dichloromethane.

Mutagenicity data was calculated in both revertants/ul and revertants/ug of extract for solvent-method comparisons (Figures 3 and 4). The choice of these units for mutagenic potency estimation

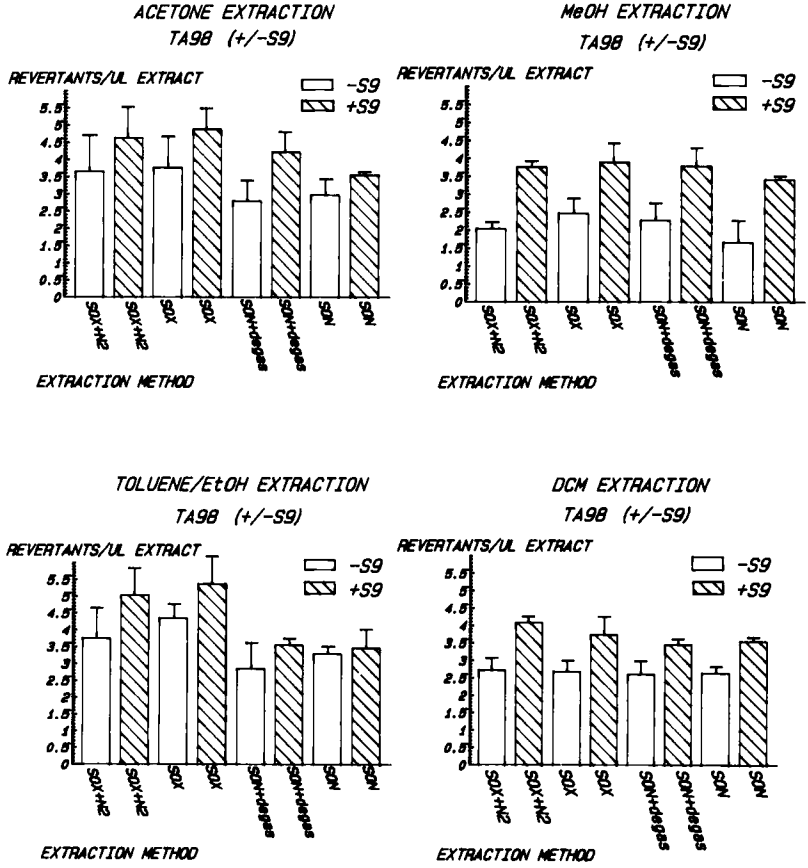

Figure 3 Mean bioassay dose response of woodsmoke extracts based upon revertants/ul extract. Means of mutagenic activity from triplicate identical treatments. Sox = Soxhlet, Son = Sonication, N2 = Nitrogen purge, Degas = Vacuum degassing.

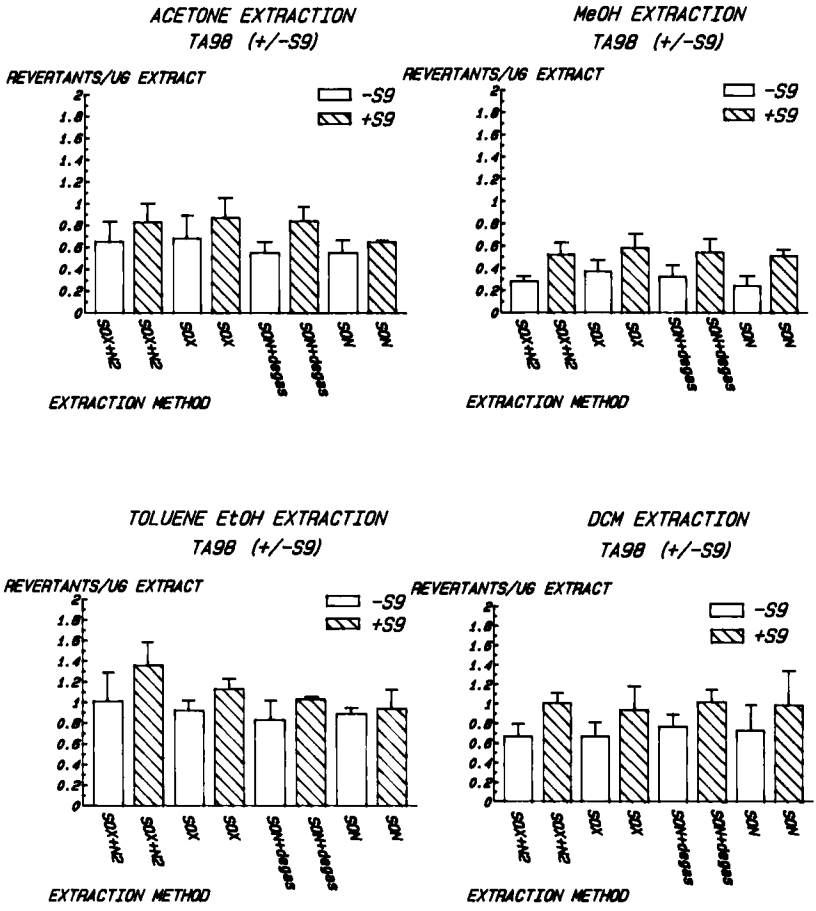

Figure 4 Mean bioassay dose response of woodsmoke extracts based upon revertants/ug extract. Means of mutagenic activity from triplicate identical treatments. Sox = Soxhlet, Son = Sonication, N2 = Nitrogen purge, Degas = Vacuum degassing.

affected analysis of variance results which are presented in Table 2. The interaction between solvent and method for TA98 + S9 arises due to a large potency difference between sonication and Soxhlet prepared toluene/ethanol and acetone samples as compared to the other solvent systems. No other interactions were evident. Solvent effect differences were noted in all systems except TA100 + S9 (revertants/ul). Extraction method slope differences were noted in TA98 except for the −S9 revertants/ul set. Soxhlet extraction

Table 2 Significance probabilities associated with *F*-tests from 2-way ANOVA for each strain and activation system

Effect	*Strain dose unit activation*	*TA100 ug +S9*	*TA100 ug −S9*	*TA100 ul +S9*	*TA100 ul −S9*	*TA98 ug +S9*	*TA98 ug −S9*	*TA98 ul +S9*	*TA98 ul −S9*
Solvent effect	—	0.0005	0.0001	0.7855	0.0030	0.0001	0.0001	NA[a]	0.0001
Method effect	—	0.4834	0.9750	0.7451	0.5943	0.0695	0.7865	NA[a]	0.0376
Solvent-method interaction	—	0.1855	0.8840	0.2906	0.8286	0.2100	0.8641	0.0172	0.6246
Degassing vs. no degassing	—	0.8699	0.7917	0.6297	0.6760	0.1476	0.8052	NA[a]	0.9689
Nitrogen vs. no nitrogen purge	—	0.2222	0.7253	0.3533	0.9359	0.3918	0.9673	NA[a]	0.3039
Sonication vs Soxhlet	—	0.3413	0.3526	0.7406	0.2025	0.0351	0.3268	NA[a]	0.0067

[a]Significant non-additivity of main effects precludes examining this effort. Values $\leqq 0.05$ constitute a significant probability.

methods were generally found to yield higher mutagenic activity in strain TA98 than sonication. No statistical differences in slope could be detected due to degassing or nitrogen purge.

Expressing mutagenic response in units of revertants/ug yielded more significant differences due to the extraction solvent than using units of revertants/ul. However, the latter units showed differences due to the extraction method more readily. Although we were interested in both types of effects, revertants/ul was proportional to revertants/sample and seemed the most appropriate units to use in estimating extraction efficiency and looking for interactions. Final method comparisons were made based upon the TA98+S9 revertants/ul data (Table 3). The TA98+S9 data was chosen over the other mutagenicity data sets due to its consistently higher yield in treatment dose response.

Comparisons between the various treatments can be made using Table 3. Greatest mutagenic yield is seen with the toluene/ethanol and acetone extracts. This table reveals that there is a general disparity between Soxhlet and sonication recoveries, particularly in the toluene/ethanol and acetone extracts. Mutagenicity differences between the two extraction techniques could have resulted from the Soxhlet method being more efficient or a result of solvent/analyte interaction with one or both extraction methods.

HPLC chromatograms of representative sonication extracts were found to be strikingly similar to each other while Soxhlet extract compositions were found to vary slightly by solvent as a group (Figure 5 and 6 respectively). This was believed to be due to a complex interaction between solvent, analytes and temperature of the refluxing solvent which is not fully understood. Sonication samples

Table 3 Comparison of mutagenic potency between solvent and extraction method treatments[a]

Solvent	*Soxhlet with N_2 purge*	*Soxhlet without N_2 purge*	*Sonication with degassing*	*Sonication without degassing*
Methanol	3.79±0.19	3.92±0.68	3.82±0.54	3.45±0.11
3:1 v/v				
Toluene/ethanol	5.07±0.73	5.40±0.81	3.59±0.17	3.50±0.54
Dichloromethane	4.12±0.16	3.77±0.56	3.49±0.16	3.58±0.15
Acetone	4.66±0.84	4.91±0.59	4.25±0.56	3.58±0.16

[a]Means of Bernstein[8] modeled dose responses for the TA98+S9 revertants/ul data. Values represent means from triplicate identical treatments with their respective standard deviations.

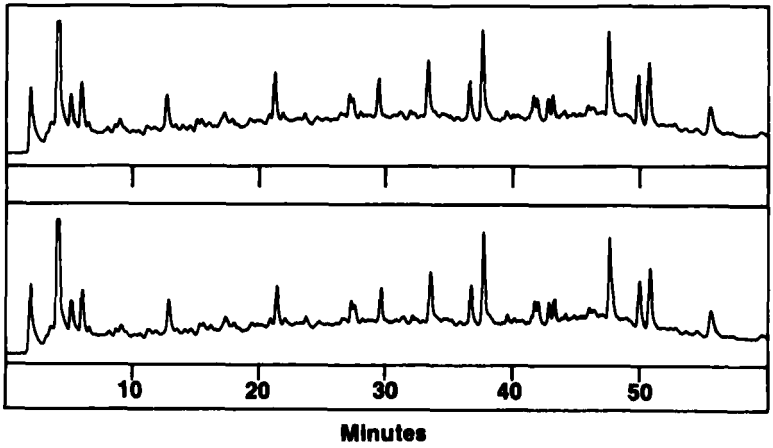

Figure 5 Fluorescence HPLC chromatograms of DCM Sonication extracts prepared with or without nitrogen purge. Upper trace with purge, lower trace prepared without nitrogen purge. Other treatments gave similar chromatograms.

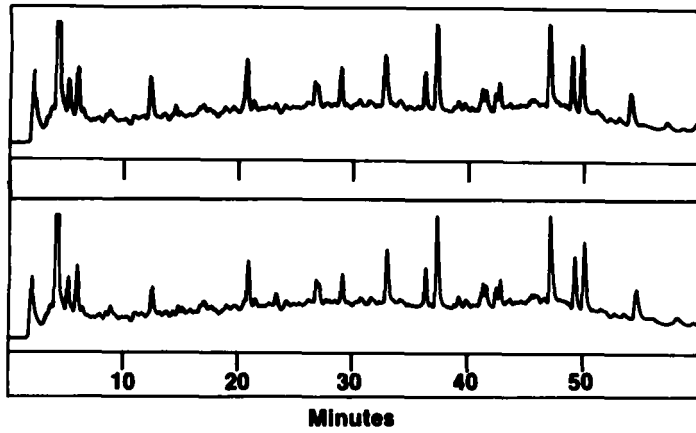

Figure 6 Fluorescence HPLC chromatograms of DCM Soxhlet extracts prepared with or without solvent degassing. Upper trace with solvent degassing, lower trace without. Other treatments gave similar chromatograms with some individual variations.

had peaks of the same retention times and areas while variations between Soxhlet samples were indicated by changing peak areas at various retention times. None of the Soxhlet samples yielded identical HPLC spectra by solvent comparison. When extracts were first prepared by sonication in either toluene/ethanol or acetone and

subsequently refluxed for 24 hours (simulating Soxhlet extraction), compositional changes were noted in fluorescence spectra (Figure 7–8). These changes were seen at retention times of approximately 12 and 30 minutes in the acetone extracts and between 12–17 minutes in the toluene/ethanol samples. The identities of these components have not been established. Reflux studies were not performed using dichloromethane or methanol because ANOVA analysis failed to detect significant differences between Soxhlet or sonication extracts with these solvents.

Sonicated portions of the toluene/ethanol extracts exhibited statistically greater direct acting mutagenicity in TA98 as compared to

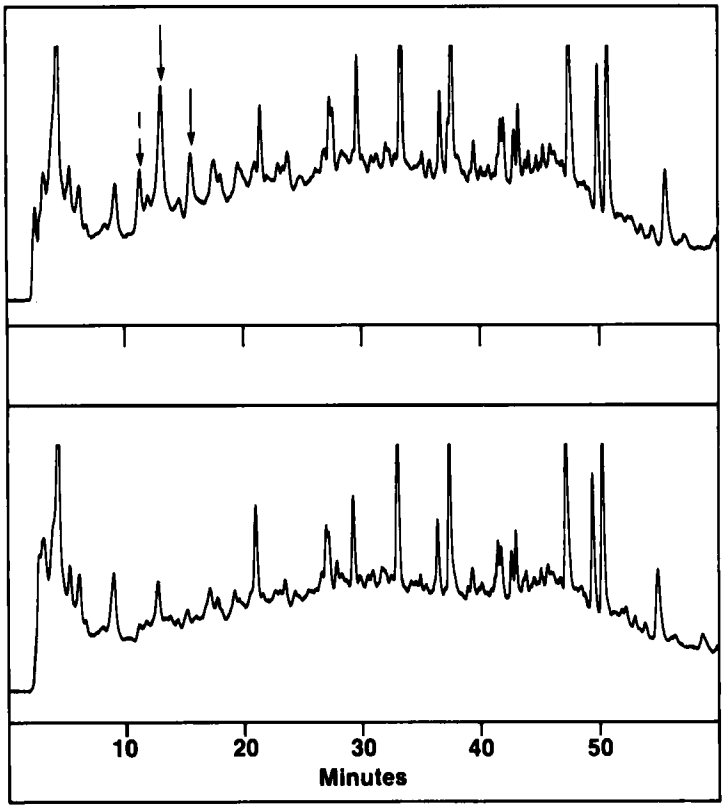

Figure 7 Fluorescence HPLC chromatograms of refluxed (upper) versus non-refluxed (lower) toluene/ethanol woodsmoke extracts. Note compositional changes between the two by positions of arrows on the upper trace.

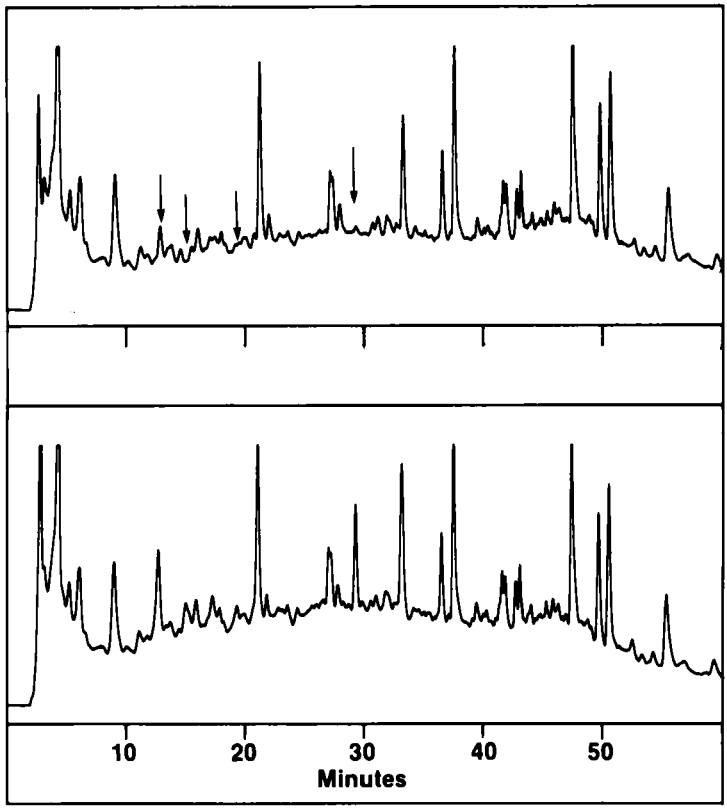

Figure 8 Fluorescence HPLC chromatograms of refluxed (upper) versus non-refluxed (lower) acetone woodsmoke extracts. Note compositional changes between the two by positions of arrows on the upper trace.

refluxed portions ($p = 0.004$). Conversely, refluxed acetone extracts displayed greater direct acting mutagenic activity than sonicated portions ($p = 0.002$). Statistical differences were not observed when metabolic activation was utilized.

DISCUSSION

In the selection of an extraction method for woodsmoke-impacted air particles, clear choices were available. Acetone and toluene/

ethanol were not selected as possible extraction solvents because synergistic or degradation effects were seen when these extracts were refluxed. Compositional and mutagenicity changes were noted for these Soxhlet-produced samples when compared to their sonicated counterparts. It must be noted that compositional changes may have been present in the other extracts and not detected. The fluorescence HPLC technique was capable of detecting fluorescing components at only the specified wavelengths and not necessarily all of the mutagens present. This study failed to detect differences in mean mutagenic potencies of extracts prepared with solvent systems other than the toluene/ethanol and acetone ones in the TA98 + S9 data.

As stated earlier, neither nitrogen purging nor solvent degassing was shown to influence mutagenic recovery. Thus the selection of an appropriate method and solvent could be based upon other considerations. Methanol as an extraction solvent gave results similar to dichloromethane. Use of this solvent requires extensive solvent concentration and solvent exchange steps due to its low volatility. Dichloromethane was by far the most desirable extraction solvent. It was easily concentrated, amenable to gravimetric and chromatographic techniques and based upon HPLC chromatograms its extracts were compositionally similar to the other sonication extracts. Sonication procedures were found to be preferable to Soxhlet extracts due to reduced extraction times, minimal equipment requirements and ease of technical procedures. Other thermal degradation effects not amenable to fluorescence HPLC analysis would also be minimized.

Earlier studies have reported comparisons between extraction techniques and solvents in the recovery of urban ambient air particulate mutagens.[2,3,11–14] None of the above used sources of air particles where woodsmoke impaction was a major known contributor. Therefore, only generalized comparisons could be made. Acetone,[14] mixtures of benzene/acetone,[12] acetone followed by dichloromethane[2] and benzene/ethanol[3] have been found to extract greater quanitites of air particulate matter mutagens as compared to a variety of other solvent systems. Use of polar solvents, such as methanol, have been shown to increase extraction recovery of unwanted inorganics as compared to mildly or non-polar solvents (such as dichloromethane).[15] Mass recovery of extracts in this study was also shown to correlate with solvent polarity.

This study determined that greater quanitities of mutagens could be recovered with acetone and aromatic mixtures but that unknown solvent/analyte interactions affected mutagenic yields with the Soxhlet method. Results indicate that dichloromethane sonication offers acceptable mutagen recovery based upon consideration of all factors. Questions remain over what effect Soxhlet reflux had upon woodsmoke extracts. The mechanism(s) by which acetone and toluene/ethanol interact, warrants in-depth research greater than the scope presented here. Widespread use of acetone and Soxhlet extraction in the recovery of other environmental mutagens should be investigated to ascertain what effect these parameters have upon reported levels of activity.

Acknowledgements

The authors wish to thank Joyce Chappell for her assitance with the bioassay and John Fitzgerald for graphics assistance. EHRT was supported through EPA Contract No.68-02-4456. The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval 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. J. Lewtas, The Integrated Air Cancer Project, presented at the Environmental Protection Agency/Air Pollution Control Association Symposium, May 6, 1987, Research Triangle Park, N.C.
2. G. Krishna, T. Ong, W. Whong and J. Nath, *Mutation Res.* **124,** 113 (1983).
3. S. Goto, A. Kawai, T. Yonekawa, Y. Hisamatsu and H. Matsushita, *J. Japan Soc. Air Pollut.* **17,** 18 (1981).
4. R. Highsmith, R. Baumgardner, B. Harris, T. Lumpkin, R. Drago, R. Zweidinger and R. Merrill, The Collection of Wood Combustion Samples in Raleigh, N.C. and Albuquerque, N.M., Presented at the Environmental Protection Agency/Air Pollution Control Association Symposium, May 6, 1987, Research Triangle Park, N.C.
5. R. Williams, L. Forehand and P. Killough, *Atmos. Environment* **20,** 1505 (1986).
6. R. Williams, E. Perry and J. Lewtas, *Environ. Intern.* **12,** 625 (1986).
7. L. Claxton, *Environ. Intern.* **5,** 389 (1981).
8. L. Bernstein, J. Kaldor, J. McCann and M. C. Pike, *Mutation Res.* **97,** 267 (1982).
9. T. A. Ryan, *Psychological Bulletin* **57,** 318 (1960).
10. *Statistical Analysis System User's Guide:* Statistics, Version 5, SAS Institute Inc., Cary, N.C., U.S.A. (1985).

11. G. Krishna, J. Nath, W. Whong and T. Ong, *Mutation Res.* **124,** 121 (1983).
12. C. Viau, S. Sherman and P. Sabharwal, *Mutation Res.* **105,** 133 (1982).
13. S. Goto, A. Kawai, T. Yonekawa and H. Matsushita, *J. Japan Soc. Air Pollut.* **17,** 53 (1982).
14. R. Talcott and E. Wei, *J. Natl Cancer Inst.* **58,** 449 (1977).
15. R. Jungers, R. Burton, L. Claxton and J. Lewtas Huisingh. In: *Short-Term Bioassays in the Analysis of Complex Environmental Mixtures II*, Plenum Publishing, New York, **45** (1981).