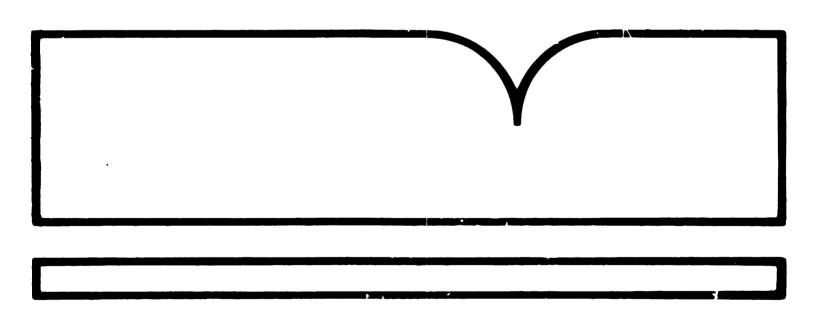
PAH (Polycyclic Aromatic Hydrocarbons) Uptake by Plants: Methodology and Initial Investigations

Clemson Univ., SC

Prepared for

Environmental Research Lab., Athens, GA

Feb 85



PAH UPTAKE BY PLANTS

Methodology and Initial Investigations

by

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAKs) are formed during the pyrolysis of naturally occurring organic materials. Pyrolytic reactions may be induced anthropogenically or by natural events (e.g., forest and grassland fires or volcanic activity). Background levels and the ubiguitous occurrence of PAHs in the environment may result from synthesis in terrestrial vegetation, microbial systhesis and volcanic activity (Andelman and Suess, 1970; Suess, 1976; Shabad, 1980). The greatest amounts of PAHs released into the environment, however, come from anthropogenic sources (Andelman and Suess, 1970). Fossil fuel burning for power production appears to be the most significant source, with motor vehicle fuel combustion and anthropogenic forest and agricultural fires contributing an additional 1% and 8% of the total, respectively (Suess, 1976). Recent reviews by Howard and Fazio (1980) and Edwards (1983) provide summaries addressing the sources, occurrence, plant uptake, degradation and analytical methodologies for PAHs in the environment.

Although a large data base has accumulated on the presence of PAHs in food products and plants, routine measurement of the uptake and accumulation of PAHs in vegetation has been hampered by analytical techniques. Sensitivity is often a problem with these techniques and the majority of the procedures cited in the literature are tedious, lengthy and expensive to perform and therefore are not amenable to routine analyses. For example, Winkler et al. (1977) described a method for the determination of PAHs with three to seven rings in wet and dry maize. Samples were extracted using a Soxhlet apparatus; extracts were rotary evaporated, saponified, filtered over silica gel, evaporated for a second time and partitioned with Sephadex LH 20. Howard et al. (1968) described a method of similar complexity for root vegetables using saponification, multiple solvent partitioning, solvent replacement, and adsorption chromatography on Florisil. Recently, Coates et al. (1984) have reported a simpler method suitable for routine analysis of PAHs in plant tissues with sufficient sensitivity for screening PAH uptake by plants growing under conditions of low to moderate exposure to PAH contamination,

such as might occur next to a roadway, upon irrigation with treated wastewater, or during overland flow treatment of wastewater. As reviewed brlow, the sonification extraction procedure coupled to a silicic acid clean-up scheme was found to be reliable and convenient.

The new sonification method of Coates et al. (1984) was applied in a field study of plants irrigated with PAH-spiked domestic wastewater or grown on contaminated soil. The method was found appropriate to measure concentrations in hybrid grain sorghum and fescue down to the 25 $\mu g/kg$ level. Apparent uptake of some PAHs from contaminated soil and/or irrigation water was demonstrated using this technique.

MATERIALS AND METHODS

Chemicals

- (a) Acetonitrile and pentane (pesticide grade)
- (b) Sodium sulfate (anhydrous granular, ACS quality)
- (c) Sodium chloride (granular, ACS quality)
- (d) Mercuric chloride (granular, ACS quality)
- (e) Silicic acid (UnisilR activated silicic acid, 100-200 mesh, Clarkson Chemical Co., Inc.)
- (f) PAHs (Aldrich Chemical Co.)
 Acenaphthylene, 95%
 Flouranthene, 98%
 Pyrene, 99+%
 Benzo[a]Pyrene, 98%
 Benzo[q.h.i]Perylene, M.P. 277-279°C
- (g) PAH standards (National Bureau of Standards SRM 1647, 16 PAHs in acetonitrile)
- (h) Laboratory blank water (twice distilled in glass)

<u>Apparatus</u>

- (a) Sample preparation (Hobart^R food processor Mode! 8181 D)
- (b) Sonification extraction (Brinkman Polytron^R with PT-20 ST probe generator)
- (c) Centrifuge (Sorvall Superspeed RC-28 automatic, refrigerated, with GSA heac)
- (d) Sample concentration (Kuderna-Danish concentrator, 500 ml)
- (e) Sample clean-up columns (Pasteur pipets)
- (f) Gas-liquid chromatography (Hewlett-Packard Model 5880 Capillary GC equipped with an FID detector and

a 30M x 0.21mm ID SE-54 capillary column having a 0.25-µm film thickness). Instrument conditions: linear velocity, 33 cm/sec; injector temperature, 250°C; detector temperature, 350°C; temperature program, 70°C for 2 minutes then 20°C/min to 125°C then 4°C/min to 290°C, held for 15 minutes.

Plant Extraction Cleanup Technique

As reported by Coates et al. (1984), acetonitrile (75 mL) was added to the centrifuge tube containing the plant mulch. The sonification probe generator was immersed into the suspension tube and the mixture sonicated for 2 minutes at maximum speed. Contents were then centrifuged at 5500 g for 20 minutes and the centrifugate quantitatively transferred to a 500 mL separatory funnel. The solid was washed with 25 mL of acetonitrile and centrifuged; the centrifugate was combined with the first extract. Pent (50 mL) was added to the separatory funnel containing t retonitrile and shaken vigorously for 2 minutes. Water siturated with sodium chloride (10 mL) was then added to the separatory funnel, followed by 350 mL of laboratory blank water, and again shaken for 30-40 seconds. The immiscible phases were separated, and the pentane layer was dried by passing through a sodium sulfate column and drained into a 500-mL K-D apparatus.

The pentane extract was concentrated to a volume of 3 to 4 mL in a water bath at 65°C, removed, and allowed to cool for 10 minutes. The K-D flask was rinsed with 3 mL of dry pentane and the 10-mL concentrator tube was removed from the flask. All ground glass joints were rinsed with 2 mL of pentage, which also was collected in the concentrator tube. The extract was then blown down to 0.5 mL with dry nitrogen at room temperature and quantitatively transferred to a prewashed micro clean-up column containing 1.5 gm of Unisil silicic acid; the column was then eluted with 5 mL of dry pentane to remove primarily aliphatic hydrocarbons from the extract. The second and third 5-mL fractions were eluted with a 85:15 solvent mixture of pentane and methylene chloride (DCM). All fractions were again concentrated to 0.5-mL with dry nitrogen and analyzed by GC-FID. All PAHs appeared in the second 5-mL fraction collected. No PAHs were ever found in the first (pentane) fraction nor the third (pentane:DCM) fraction. The clean-up procedure was sufficient for extracting up to 15 gm of air dried plant material; additional silicic acid was required to remove interfering polar constituents when extracting more than 15 gms of plant

material. The method was scaled up when necessary on the basis of silicic acid pore volume to plant mass ratios.

Sample Collection and Spiking for Recovery Studies

Immature hybrid grain sorghum was harvested from the Clemson University Agricultural Experiment Station - Simpson Farm during the early fall of 1982 and stored at -10°C until recovery studies were initiated. All plants were removed from storage and the aerial plant parts homogenized in a food processor prior to spiking for recovery studies.

A weighed portion (2-10 gm) of plant mulc' was placed in a 150-mL glass centrifuge tube with 1 mL of a PAH mixed standard (NBS SRM 1647). Laboratory blank water (150 mL) and 1 mL of 185 immolar mercuric chloride as a bacteriocide were added to the tube. The tube was capped and placed on a slide shaker in a dark room for 24 hours. After shaking, the contents were centrifuged at 5500 g for 20 minutes. The aqueous phase was transferred to a separatory funnel and extracted 3 times with 30-mL portions of pentane. Extracts were combined and concentrated using a K-D apparatus to approximately 3 to 4 mL, then by dry nitrogen to 0.5 mL. The centrifuge tube wall was extracted 3 times with 10-mL portions of pentane and concentrated to 0.5 mL is above. The plant residue was extracted by sonificatio.

Experimental Design for Field Study.

A field study was originally initiated (Coates et al., 1984) to demonstrate that the sonification technique was suitable to extract plant tissue that had accumulated PAHs during growth as well as with spiked samples. The additional data reported here were obtained to make an initial assessment or uptake of selected PAHs and to investigate whether uptake could occur from contaminated soil as well as from irrigation water. In the irrigation water study, hybrid grain sorghum and fescue were grown from seed in 20-L containers and irrigated daily with PAH-spiked municipal sewage effluent for an 8-week period during the summer of 1983. Treated activated sludge effluent wastewater was obtained from the Coneross Waste Treatment Plant, Oconee County, South Carolina, and stored with aeration at 4°C during the study period. The experimental design was a completely random design with triplicate controls and triplicate PAH treatment representing two levels of PAH contamination for both sorghum and fescue. Polycyclic aromatic

hydrocarbons used in this 'nvestigation were acenaphthylene, fluoranthene, pyrene, benzo[a]pyrene, and benzo[g,h,i]perylene. The concentration of the five PAHs in the spiked wastewater and the total mass of PAHs introduced to the growing plants during the study period are listed in Table 1.

TABLE 1

CONCENTRATIONS AND MASSES OF PAHS USED IN THE IRRIGATION STUDY

| PAH | | tration Wastewater | in Spike water | ss Applied ed Waste- to Each tainer |
|--|-----------------------------------|----------------------------------|-------------------------------|--|
| | 1 | atment 2 ug/L | Trea 1 | atment 2 µ9 |
| Acenaphthylene Fluoranthene Pyrene Benzo[a]pyrene Benzo[g,h,i]perylene | 2.0 2.0 1.0 0.05 0.10 | 1000 200 100 1.0 0.5 | 640 640 320 16 32 | 320,000 64,000 32,000 320 160 |

Plants were irrigated using hand-pump pressurized spray cans. Containers were moved frequently to eliminate position in the growing area as a variable. The aerial part of each plant was harvested at the end of the growing period and frozen until extracted. The seeds were planted in July, irrigation with spiked wastewater was begun in September, and the plants were harvested in November.

To obtain some indication of the rotantial for root uptake of PAHs, the soil in two 20-L con liners was contaminated with three PAHs (acenaphthylene, fooranthene and pyrene). One gram of each PAH was added to the top two inches of soil and thoroughly mixed. The soil density was estimated to be 1.25 gm/cm³, which translated into a calculated 217 ppm PAH concentration in soil (not verified by extraction).

Contaminated soil was incubated for 1 month prior to seeding. Fescue was planted in one container and hybrid grain sorghum was planted in the second container. Two controls were planted at the same time and all four containers were irrigated with tapwater during the 8-week growing period. During this time the soil surface was not allowed to dry, thus reducing the effect of "dusting," which could contaminate aerial plant parts.

RESULTS AND DISCUSSION

Evaluation of Analytical Technique

As reported by Coates et ai. (1984), the elution in and corresponding relative retention times for the 16 is present in the NBS SRM 1647 standard are listed in Table 2. The chromatographic conditions used in this investigation are listed in the Materials and Methods section. Retention orders for PAHs having different molecular weights were verified using gas chromatography on an SE-54 capillary column and selected ion monitoring mass spectrometry (GC-MS). The retention order for the remaining PAH molecular weight pairs (phenanthrene-anthracene, fluoranthene-pyrene, chrysene-B[a]A, and B[b]F-B[k]F) were determined by spiking the NBS standard with an EPA quality control sample containing one of the PAHs of the paired combinations and analyzing by the GC-MS technique.

RETENTION TIMES FOR 16 PAHS PRESENT IN THE NBS SRM 1647
STANDARD*

| PAH | | Mol. Wt. | Relative Retention (referenced to Pyrene) | | |
|-----|----------------|----------|---|--|--|
| 1. | Naphthalene | 128.2 | 0.272 | | |
| 2. | Acenaphthylene | 152.2 | 0.457 | | |
| 3. | Acenaphthene | 154.2 | 0.485 | | |
| 4. | Fluorene | 166.2 | 0.566 | | |
| 5. | Phenanthrene | 178.2 | 0.733 | | |
| 6. | Anthracene | 178.2 | 0.741 | | |

TABLE 2 (Continued)

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| | РАН | Mol. Wt. | Relative Retention (referenced to Pyrene) |
|-----|-----------------------------------|----------|---|
| 7. | Fluoranthene | 202.3 | 0.959 |
| 8. | Pyrene | 202.3 | 1.000 |
| 9. | Benzo[a]anthracene | 228.3 | - · 1.238 |
| 10. | Chrysene | 228.3 | 1.246 |
| 11. | Benzo[b]fluoranthene | 252.3 | 1,439 |
| 12. | Benzo[k]fluoranthene | 252.3 | 1.443 |
| 13. | Benzo[a]pyrene | 252.0 | 1.494 |
| 14. | <pre>Indeno[1,2,3-cd]pyrene</pre> | 276.3 | 1.739 |
| 15. | D[a,h]anthracene | 278.4 | 1.749 |
| 16. | Benzo[g,h,i]perylene | 276.3 | 1.805 |

^{*}After Coates et al., 1984

Plant extracts when concentrated typically contain high percentages of polar organic compounds that co-elute and significantly interfere with many chromatographic analyses. Many of the available clean-up schemes are complex and tedious to implement for routine analyses. The single sorbent clean-up scheme used by Coates et al. (1984) coupled with solvent partitioning was sufficient to allow quantification of all PAHs listed in Table 2 at the 25 µg/kg level. PAHs extracted by acctonitrile were partitioned into pentane, dried on sodium sulfate, concentrated to 0.5 mL and cleaned up with a micro-silicic acid column, as noted in the Materials and Methods Section. Two 5-mL fractions were eluted with a pentane:DCM (85:15) solvent mixture. Optimization of the solvent ratio was necessary to maintain the usefulness of the single adsorbent clean-up step (Coates et al., 1984).

Figure 1a is a chromatogram of the PAH standard and figure 1b is a chromatogram of the first 5-ml eluant fraction of a plant extract using pentane:DCM (85:15) as eluant. Coates et al. (1984) showed quantitative recovery for most of the PAHs from hybrid grain sorghum extracts for the concentration and cleanup steps with the exception of indeno[1,2,3-cd] pyrene and benzo[g,h,i]pervlene, which showed losses of 12 and 17 percent, respectively.

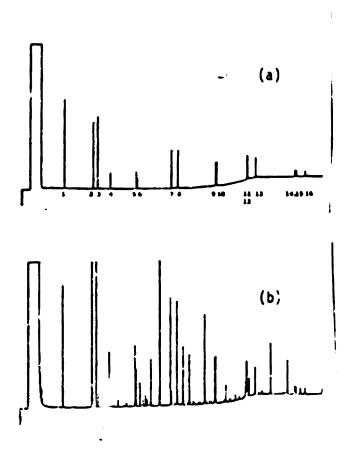


FIGURE 1. Chromatogram of (a) NES 1647 standard (numbered peaks appear in order as in Table II) and (b) sample eluted with 85:15 pentane:DCM.

Overall method calibration and recovery also was evaluated (Coates et al., 1984). Initial calibration studies for the 16 PAHs used in this investigation indicated detector linearity over the analytical working range so a single point calibration curve was used to quantify PAH concentration and calculate recovery efficiencies. Standards (NBS SRM 1647) were injected in the gas chromatograph every 4 to 5 samples to monitor system drift. Results of overall method recovery studies are listed in Table 3. These data were corrected for residual naphthalene, acenaphthylene and acenaphthene concentrations remaining in the aqueous phase after spiking and incubation with the plant mulch. Aqueous phase concentrations of the remaining PAHs were below detectable limits (C.05-1

PAHS IN PLANTS

ng/ml). See Materials and Methods for spiking details. Concentrations of PAHs spiked into the plant tissue homogenates were between 600 and 4500 μ g/kg.

TABLE 3

OVERALL METHOD RECOVERY EFFICIENCIES (PAH PLANT CONCENTRATION 600-4500 µg/kg)

| | PAH | Mass in Spike µ9 | Percent Recovered* |
|-----|------------------|------------------------|-----------------------|
| 1. | Naphthalene | 22.5 | 47+(1) |
| 2. | Acenaphthylene | 13.1 | 81 <u>∓</u> (8) |
| 3. | Acenaphthene | 21.0 | 80 - (8) |
| 4. | | 4.92 | 82 <u>∓</u> (8) |
| | Phenanthrene | 5.06 | 90 <u>+</u> (9) |
| | Anthracene | 3.29 | 82 <u>∓</u> (8) |
| 7. | Fluoranthene | 10.1 | 83 + (12) |
| 8. | Pyrene | 9.84 | 83 <u>∓</u> (13) |
| 9. | B[a]A | 5.03 | 72 + (6) |
| 10. | Chrysene | 4.68 | 71 , ∓(9) |
| 11. | B[b]r | 5.11 | 62+(16) |
| 12. | B[k]F | 5.02 | 61 <u>∓</u> (15) |
| 13. | | 5.30 | 5 7+(5) |
| 14. | | 4.06 | 49+(17) |
| 15. | D[a,h]A | 3.68 | 45+(17) |
| 16. | B[g,h,i]Perylene | 4.01 | 61+(14) |

^{*}Average of four replicates with standard deviations in parentheses.

Data in Table 3 suggest that the sonification procedure was generally efficient in removing most of the PAHs from the plant matrix. Recovery efficiencies, however, for the more volatile naphthalene and for a few of the more hydrophobic PAHs were lower (45-62%). The practical detection limit was approximately $0.025~\mu g/g$.

Field Application Studies

Irrigation Study. At the end of the 8-week growing period, the hybrid grain sorghum and fescue that were irrigated with PAH-spiked domestic wastewater were harvested and stored frozen until extracted. All plants were extracted using the sonification technique with the clean-up scheme outlined above, and extracts were analyzed by GC-FID.

Results are listed in Tables 4 and 5 for hybrid grain sorghum and fescue, respectively. Concentrations for each plant type are in units of microgram PAH per gram of air dried plant extracted. Error estimates for the data presented in Table 4 were not available because replicates for both control and treatments 1 and 2 had to be combined to have enough plant material to extract. Error estimates for the control and the two treatment levels for the fescue uptake study were calculated and were used in significance analysis.

PAH CONCENTRATIONS IN HYBRID GRAIN SORGHUM IRRIGATED WITH PAH-SPIKED DOMESTIC WASTEWATER

| PAH | Control | Treatment 1 | Treatment 2 |
|--|--------------|----------------|----------------|
| | µg /g | µg/g | µg/g |
| Acenaphthyl ene | ND+ | ND | ND |
| luoranthene | 0.05 | 0.82 | 1.32 |
| Pyrene | 0.02 ND | 0.49 ND | 0.63 ND |
| Benzo(a)pyrene Benzo(g,h,i)perylene | ND ND | ND | ND |

^{*}ND means not detected (less than approximately 0.02 µg/g).

PAH CONCENTRATIONS IN FESCUE IRRIGATED WITH PAH-SPIKED DOMESTIC WASTEWATER

| PAH | Control (avg. : | Treatment 1 + standard de | Treatment 2 viation) |
|--------------------------|------------------------------|---------------------------|----------------------------|
| Acenaphthylene | ND* | ND | 0.58+0.16 |
| Fluoranthene | 0.12+0.03 | 0.20 <u>+</u> 0.07 | 50+13 24+6 |
| Pyrene Benzo(a)pyrene | 0.07 - 0.03 ND | 0.10∓0.04 ND | 24+0 ND |
| Benzo(g,h,i)perylene | ND | ND | 0.13+0.03 |

^{*}ND means not detected (less than approximately 0.025 µg/g).

Fluoranthene and pyrene were found in the sorghum at concentrations significantly greater than the control. Four of the five PAHs were present in the fescue irrigated with spiked wastewater at concentrations greater than in the controls. Concentration differences between treatment 1 and treatment 2 and the controls were significant at the <0.01 level as determined by the Student's T test.

Table 1 gives the total mass of each PAH applied to the plants for each treatment through the spiked irrigation water. No attempt was made to account for losses of PAHs due to sorption, biodegradation, photolysis, volatilization from soil surface or transpiration, but only to determine whether measureable quantities of PAHs could be found associated with growing plants irrigated with PAH contaminated wastewater. Similarly, although the experiment indicated uptake by the plants, it does not distinguish between foliar or root uptake or a combination of the two. As expected, given different physical and chemical characteristics, some PAHs accumulated more than others. Further work is required to elucidate mechanisms of uptake and accumulation, however.

Contaminated Soil Study

Results are listed in Tables 6_and 7 for hybrid grain sorghum and fescue and indicate the magnitude of plant uptake for the PAHs used in this study. It is important to note that treatments were not replicated and the numbers should only be viewed as indications of potential accumulation. No attempt was made to correlate uptake or accumulation as a function of plant growth. The data strongly suggest, however, that some PAHs may be taken up by plant roots and translocated to the aerial plant. Two of the three PAHs studied, acenaphthylene and fluoranthene, exhibit greater aqueous solubilities and lower hydrophobic character than pyrene and were found at higher levels in the plant tissue than pyrene in the contaminated soil study. In addition, acenaphthylene was the most volatile of the PAHs studied and may have been lost from the system. If the results presented are order of magnitude indicators of plant accumulation tendencies, they are consistent with the above characteristics of PAHs and indicate lower uptake of PAHs with lower aqueous phase concentrations.

TABLE 6

PAH CONCENTRATIONS IN SORGHUM GROWN ON CONTAMINATED SOIL

| PAH . | Control | Contaminated Soil |
|----------------|---------|----------------------|
| | µg/g | μg/g |
| Acenaphthylene | ND* | ND |
| Fluoranthene | 5.23 | 73.49 |
| Pyrene | · ND | 2.38 |

^{*}ND means not detected (approximately less than 0.02 µg/g)

TABLE 7

PAH CONCENTRATION IN FESCUE GROWN ON CONTAMINATED SOIL

| PAH | Control µg/g | Contaminated Soil ug/g |
|------------------------------------|---------------------|------------------------------|
| Acenaphthylene Fluoranthene Pyrene | ND* 0.13 0.06 | 3.09 -10.32 3.29 |

^{*}ND means not detected (approximately less than 0.02 µg/g)

Further work is required to elucidate and verify uptake mechanisms and to determine the impact of application rates and methods, volatilization, photodegradation, biodegradation, plant metabolism and translocation as possible explanations for the ranges of PAH concentrations observed in aerial plant parts.

<u>ACKNOWLEDGEMENTS</u>

This work was supported primarily by a cooperative agreement to Environmental Systems Engineering, Clemson University from the U.S. Environmental Protection Agency through the Athens Environmental Research Laboratory, for which appreciation is expressed. Partial support through NSF grant No. ISP-8011451, "Environmental Engineering Chemistry" is also gratefully acknowledged. Finally, our thanks to Elaine McGarity and Sherry Jarrard for their excellent work on the manuscript.

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| 4. TITLE AND SUBTITLE | 5. REPORT DATE | o.e | | |
| PAH UPTAKE BY PLANTS: Methodology and Init Investigations | ial February 198 | | | |
| 7 AUTHORIS) | 8. PERFORMING OR | GANIZATION REPORT NO | | |
| John T. Coates, Alan W. Elzerman and A. Wa | yne Garrison | | | |
| Prenforming organization name and address Environmental Systems Engineering, Clemson Clemson SC 29631 | University, CCULIA | | | |
| *Environmental Research Laboratory, U.S. El Protection Agency, Athens GA 70613 | | | | |
| 12 SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection AgencyAthe | ns GA Published p | T AND PERIOD COVERED | | |
| Office of Research and Development | 14 SPONSORING AC | | | |
| Environmental Research Laboraton, Athens GA 30613 | EPA/600/01 | | | |
| 15. SUPPLEMENTARY NOTES | | | | |
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| 17. KEY WORDS AND DO DESCRIPTORS | CUMENT ANALYSIS | c. COSATI Leid Group | | |
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