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A Routine Method for the Analysis of Mononitro-*PAH* in Immission and Emission Samples

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Summary. A routine method for the identification and quantification of mononitrated polycyclic aromatic hydrocarbons in environmental samples has been developed. Micro-scale liquid-liquid partition between dimethylformamide and cyclohexane and HPLC on normal-phase silica gel columns are used for clean-up of samples. This preconcentration scheme is time-saving and uses a minimal amount of solvents. Identification and quantification are performed by GC/ECD and GC/MS in the "selected ion monitoring" mode. The analytical scheme has been optimized under use of standard substances by variation of several parameters in different steps of the analysis. The method was applied to the NBS Reference Material SRM 1649 "Urban Dust", to urban air samples from the city of Vienna as well as to combustion emissions.

Keywords. Nitro-*PAH*; GC/MS; Urban air; Wood combustion.

Eine Routinemethode zur Immissions- und Emissionsmessung von Mononitro-*PAH*

Zusammenfassung. Es wurde eine Routinemethode zur Identifizierung und Quantifizierung von mononitrierten polycyclischen aromatischen Kohlenwasserstoffen in Umweltproben entwickelt. Zur Vortrennung der Proben wurde eine Flüssig-flüssig-Verteilung im Mikromaßstab zwischen Dimethylformamid und Cyclohexan und eine HPLC-Auftrennung an Silicagel-Säulen angewandt. Diese Aufarbeitungsmethode erwies sich gleichzeitig als zeit- und lösungsmittelsparend. Die Identifizierung und Quantifizierung der Nitro-*PAHs* erfolgt mittels GC/ECD und GC/MS im „selected ion monitoring"-Modus. Der Analysenvorgang wurde unter Verwendung von Standardsubstanzen durch Änderung der verschiedensten Parameter in den einzelnen Analysenschritten optimiert. Praktisch angewandt wurde die vorliegende Methode schließlich zur Untersuchung von Proben des Standardstaubs NBS Standard Reference Material SRM 1649 „Urban Dust", von Stadtluft an einer verkehrsreichen Kreuzung in Wien und von Emissionen verschiedener Holzverbrennungsanlagen.

Introduction

Nitrated polycyclic aromatic hydrocarbons (nitro-*PAH*) have been determined in samples of very different origin such as combustion emissions, automobile exhaust, rural, urban and indoor air, cigarette smoke condensates, xerographic toners, motor oil and food [1–14]. The toxicological significance of these substances lies in their high direct-acting mutagenicity in bacteria and their suspected carcinogenicity for man. Biological effects, experiments and their relevance have been reviewed recently [15–17]. Nitro-*PAH* are present in environmental samples in much lower amounts than the unsubstituted *PAH* (for urban particulate matter nitro-*PAH* concentrations are about two orders of magnitude lower than *PAH* concentrations). Their identification can be complicated by the presence of the parent *PAH* as well as by interference of heteroaromatic polycyclic compounds. The reducibility of nitro-*PAH* is a serious inconvenience in their quantification [18, 19]. Artifact formation by nitration of parent *PAH* has also been observed [20, 21]. Aspects of nitro-*PAH* analysis and toxicology have been summarized in a recently published monography [22].

A reproducible routine method for the determination of mononitro-*PAH* in environmental samples minimizing the disturbances discussed above is described here. Its applicability has been investigated with urban air and combustion emissions.

Experimental

Nitro-*PAH* standards were synthesized and provided by Dr. W. Posch, Karl-Franzens-Universität Graz [23, 24]. Standard solutions were prepared with chloroform and further diluted with cyclohexane.

Solvents: Distilled cyclohexane p.a. (Merck) or cyclohexane "zur Rückstandsanalyse" (pesticide grade, Promochem); chloroform and methylene chloride in p.a. grade (Merck); distilled dimethylformamide (*DMF*) p.a. (Merck), stored at 4°C in the dark; bidest. water.

HPLC system: Pump: LDC Constametric III. UV detector: LDC Spectromonitor 1204A, operated at 254 nm. Precolumn: 120 × 4.6 mm, Merck Kieselgel 60, 70–230 mesh. Analytical column: 250 × 4 mm, Merck Lichrosorb Si 100 (10 μm) or Si 60 (10 μm). Mobile phase: 1 ml/min cyclohexane/chloroform 99 : 1 or 1.5 ml/min cyclohexane/methylene chloride 90 : 10.

GC analyses were carried out on a Carlo Erba Fractovap 4160 equipped with split/splitless and on-column injectors and an ^{63}Ni-ECD. Analyses of samples were performed on a 30 m, 0.32 mm i.d. J & W DB-17-column with a film thickness of 0.15 μm. Conditions of operation were as following: carrier gas: helium, 1.5 kg/cm^2; on-column injection at 65°C, followed by temperature programming from 125°C to 275°C at 4°C/min, the temperature being held at 80°C for 1 min for solvent evaporation; ECD: make-up gas: N_2, 1.5 kg/cm^2; temperature: 300°C, constant current mode. GC/MS analyses were carried out on the same GC instrument coupled by an open split interface to a Finnigan MAT 8230 mass spectrometer, under EI conditions at 70 eV. Ion source and interface temperatures were 300°C and 280°C respectively.

Method

Sampling

Urban air sampling was performed on a low-volume air sampler as described in ref. 25. The apparatus consisted of a Gelman filter holder 1235 and a glass tube carrying a Whatman GF/A 47 mm dia. glass fibre filter for particles followed by three polyurethane foam plugs for the volatile portion. In order to assess the importance of artifact formation and losses of volatile compounds, simultaneous sampling was conducted in two parallel devices in two instances. A sample was collected without interruption on an apparatus for 4 days and compared with a composite of four separate 24 h-samples taken in the same time interval. As can be seen in Table 1, losses of nitronaphthalenes can be observed during prolonged sampling. Therefore, the concentrations measured for these substances are to be regarded as the lower limit. Artifact formation by nitration of *PAH* is estimated to be negligible.

Table 1. Comparison of samples differing in sampling time: a: 4-day-sample; b: composite of four 24 h-samples

Compound	Concentration [ng/Nm^3] a	b
1-nitronaphthalene	0.11	0.19
2-nitronaphthalene	0.11	0.23
4-nitrodiphenyl	0.02	0.01
2-nitrofluorene	0.01	0.01
9-nitroanthracene	0.26	0.24
2-nitrofluoranthene	0.42	0.33
3-nitrofluoranthene	0.01	0.01
1-nitropyrene	0.08	0.08
2-nitropyrene	0.08	0.06
6-nitrochrysene	0.01	0.01

Emission samples were taken from the stack of a medium-sized burner by means of a heated glass probe. Quartz wool was used as a prefilter. After the collection of condensate (at 4°C), the stack gases were lead through a glass fibre filter and polyurethane foams. The condensate was filtered through a glass fibre filter and extracted with cyclohexane, this extract being added subsequently to the filter and absorption media extract.

Sample Preparation

Glass fibre filters and polyurethane foams were subjected to a soxhlet extraction with cyclohexane for 24 h in the dark after addition of 2,2′-binaphthyl and 2-methyl-1-nitronaphthalene as internal standards for the quantification of *PAH* and nitro-*PAH* respectively. 2-methyl-1-nitronaphthalene could not be found in notable amounts in unspiked samples of different origin and therefore seemed suitable as an internal standard.

Further treatment was performed by micro-scale liquid-liquid partition between dimethylformamide (*DMF*) and cyclohexane as described by Jaklin [26]. Due to the interaction of aromatic π-electron systems with *DMF* (formation of charge-transfer complexes), aromatic species can be transferred to *DMF*, back-extracted with cyclohexane after addition of water and thereby separated from aliphatic compounds, which remain in the raw extract. Losses of nitro-*PAH* occurring in the partition step were investigated in three parallel experiments with standard substances. All investigated mononitro-*PAH* could be preconcentrated with a recovery of 90–100%, whereas dinitro-*PAH* could not be transferred reproducibly, because they partly remain in the water phase due to their higher polarity.

PAH and mononitro-*PAH* were further separated by HPLC on normal-phase silica gel columns. The HPLC prefractionation on silica gel, which is widespread in the analysis of nitro-*PAH*, often in conjunction with gradient elution, seems poorly reproducible due to the varying activity of the stationary phase and to adsorption phenomena, a fact which is not explicitely mentioned in the current literature on the subject. Small amounts of solvent indispensable to purge the analytical column after each sample run (despite the use of a precolumn) can remain adsorbed on the particles, altering the next separation. Chloroform, first in use for these means in the present investigation, was therefore replaced by methylene chloride. The following fractionation scheme was applied: Before each run, the column was activated with 2,2-dimethoxypropane/glacial acetic acid/cyclohexane 2.5 : 10 : 90 as described by Bredeweg et al. [27] and equilibrated with solvent. Then the retention interval of the mononitro-*PAH*-fraction and its separation from the unsubstituted *PAH* was verified by injection of a standard solution. After frac-

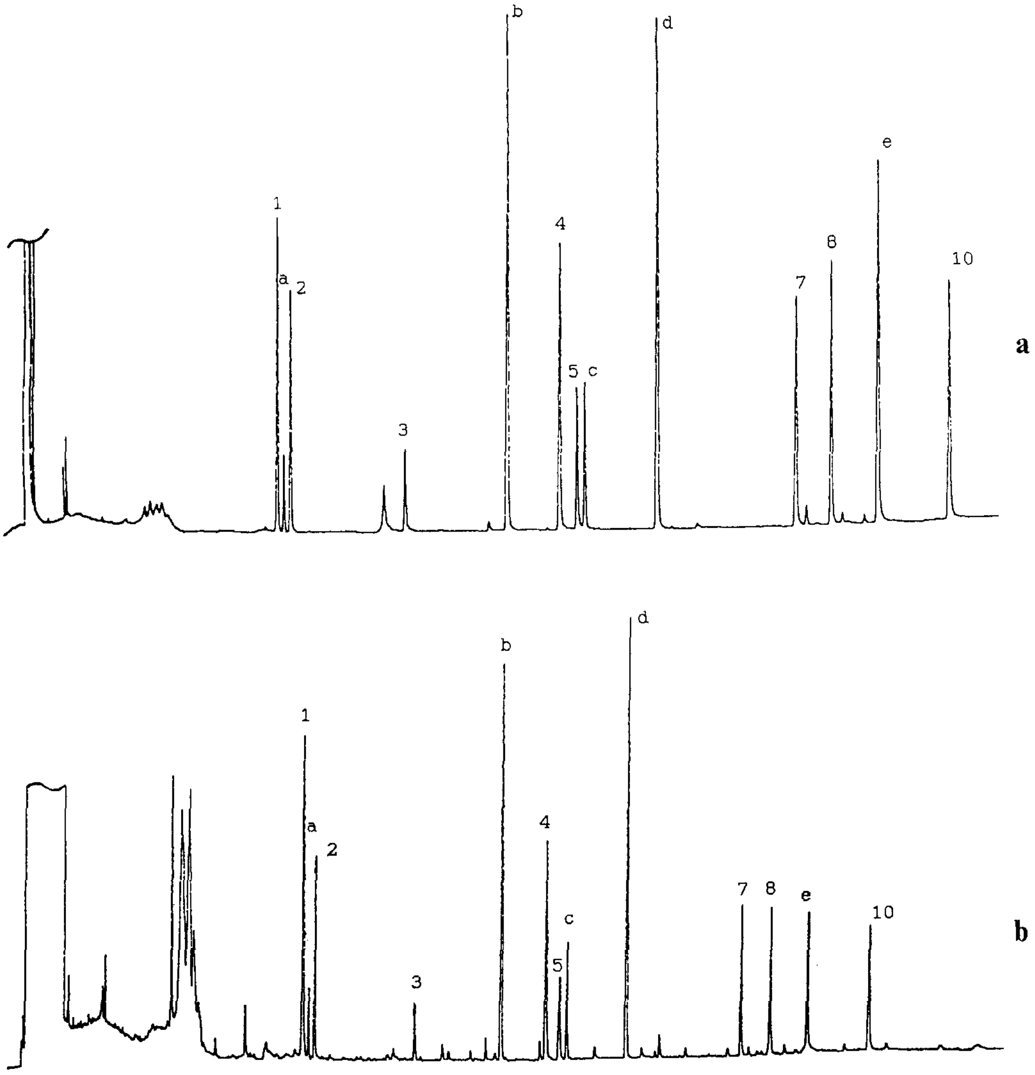

Fig. 1. Comparison of on-column (**a**) and splitless (**b**) injection with respect to discrimination of higher-boiling compounds. *a* 2-methyl-1-nitronaphthalene, *b* 3-nitro-9-fluorenone, *c* 1,5-dinitronaphthalene, *d* 1,8-dinitronaphthalene, *e* 2,7-dinitrofluorene (compound names corresponding to the numbers are given in Table 3)

tionation of each sample, the columns were purged separately with methylene chloride.

GC and GC/MS

The identification and quantification of mononitro-*PAH* was performed by GC/ECD and GC/MS with selected ion monitoring (SIM). On-column and splitless injection were compared. As can be seen in Fig. 1, on-column injection prevents discrimination of higher boiling compounds, which occurs in the splitless mode of injection.

Columns with different lengths, stationary phases and layer thicknesses were also compared. The DB-17-column was found to be more effective for the separation of the nitro-*PAH* in question than the DB-5-column most commonly used by other authors.

The detection of nitro-*PAH* by ECD and GC/MS (SIM) were compared as well. For standards, the ECD is slightly more sensitive than the GC/MS method. Its detection limits are in the sub-pg-range. The identification of nitro-*PAH* in complex samples is supported by the calculation of retention indices (RI) newly defined in the present publication, 2-methyl-1-nitronaphthalene, 1,5-dinitronaphthalene and 2,7-dinitro-fluorene being used as reference compounds:

$$RI = 100 \cdot \frac{Rt_c - Rt_n}{Rt_{n+1} - Rt_n} + \mathrm{n} \cdot 100$$

Rt_c ... retention time of compound
Rt_n,
Rt_{n+1} ... retention times of flanking reference compounds
$n = 1$: 2-methyl-1-nitro-naphthalene
$n = 2$: 1,5-dinitronaphthalene
$n = 3$: 2,7-dinitrofluorene

Table 2. Retention indices (RI) of nitro-*PAH* (reference compounds are marked with *)

Compound		Retention index
1-nitronaphthalene		97.0 ± 0.1
2-methyl-1-nitronaphthalene	*	100
2-nitronaphthalene		102.9 ± 0.1
4-nitrodiphenyl		154.6 ± 0.2
1,5-dinitronaphthalene	*	200
2-nitrofluorene		214.1 ± 0.1
9-nitroanthracene		220.7 ± 0.2
2-nitrofluoranthene		273.2 ± 0.1
3-nitrofluoranthene		277.7 ± 0.2
1-nitropyrene		287.0 ± 0.2
2-nitropyrene		288.4 ± 0.2
2,7-dinitrofluorene	*	300
6-nitrochrysene		319.3 ± 0.2

The indices defined by Lee and White [28] relative to four *PAH* cannot be used in conjunction with the ECD, as the reference compounds are not detected. Thew new retention indices, shown in Table 2, have proven to be reliable and reproducible over a range of several months.

The GC/MS (SIM)-identification was supported by registration of the three or four most abundant fragment ions of every substance in addition to the molecular peak. Detection limits are in the pg-range.

Application

The method for the determination of mononitro-*PAH* described above was applied to reference material "Urban Dust" from the National Bureau of Standards, to stack gases from wood-burning plants and to urban air sampled near an arterial road (Table 3). The identification of nitro-*PAH* in the very complex ECD chromatograms (Fig. 2) is considerably facilitated by use of the retention indices described above. Nevertheless, identification and quantification by GC/MS are more reliable. Although the detection limits of nitro-*PAH* in the samples are in the range of a few pg for both methods, the GC/MS (SIM) method shows more selectivity.

The reference material NBS SRM 1649 represents a time-integrated sample collected over a range of several months. In Table 3, concentrations as determined from three 1 g-samples are given. Ramdahl et al. [29] found approximate amounts of 600, 200 and 50 ng/g for 2-nitrofluoranthene. 1-nitro-pyrene and 2-nitropyrene in the same NBS reference material.

Emission samples were collected from the stack of a medium-capacity burner fired with bark and wood chips (Table 3, column A) and from a industrial plant fed with wood chips and flue gases from a coal-tar-consuming process (Table 3, column B). Very little is known of nitro-*PAH*-concentrations in this type of combustion emissions, especially with wood used as a combustible. Gibson [30] found 1-nitropyrene and 6-nitrobenzo[a]pyrene in low amounts in emissions from a wood-

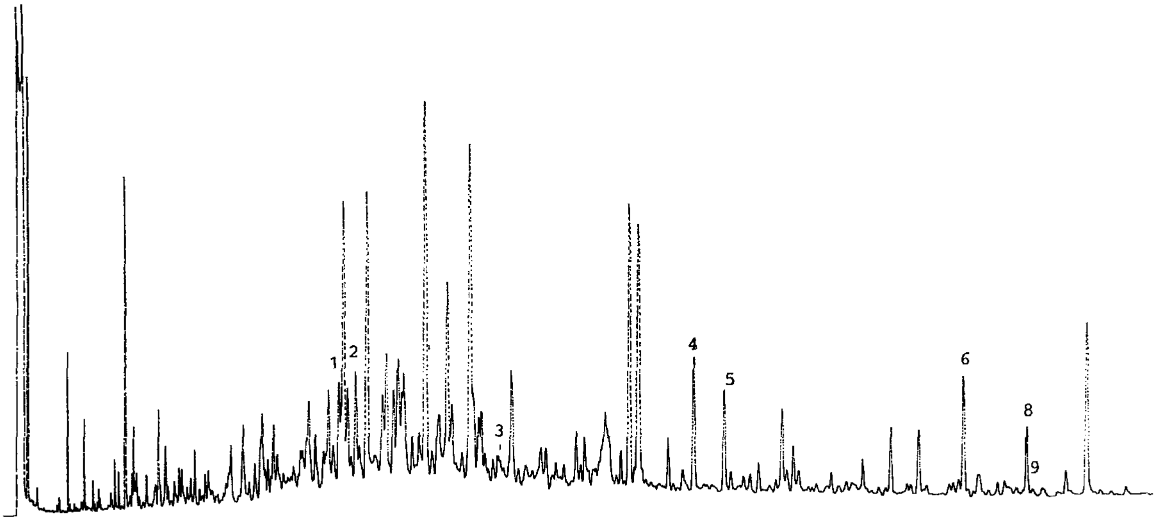

Fig. 2. ECD-chromatogram of an urban air sample (compound names corresponding to the numbers are given in Table 3)

Table 3. Concentration of nitro-*PAH* in environmental samples as determined by GC/MS (SIM). A: Stack gas from a medium-capacity burner (fired with bark and wood chips); B: Stack gas from an industrial plant (fed with wood chips and flue gases from a coal-tar-consuming process); I: Urban air, sampled near an arterial road: cold period (mean of 4 sampling events); II: Urban air, sampled at the same location: mild period (mean of 4 sampling events)

No.	Compound	Concentration					Relative standard deviation[b] [%]
		Emission samples[a] [$\mathrm{ng/Nm^3}$]		Immission samples [$\mathrm{ng/Nm^3}$]		Reference material NBS SRM 1649 [ng/g]	
		A	B	I	II		
1	1-nitronaphthalene	95	465	0.4 ± 0.2	0.2 ± 0.1	3	20
2	2-nitronaphthalene	140	900	0.5 ± 0.2	0.2 ± 0.1	5	20
3	4-nitrodiphenyl	120	75	0.04 ± 0.01	0.01 ± 0.01	5	20
4	2-nitrofluorene	n.d.	1	0.07 ± 0.03	0.01 ± 0.01	3	100
5	9-nitroanthracene	115	50	1.6 ±0.4	0.3 ± 0.1	30	30
6	2-nitrofluoranthene	35	n.d.	1.8 ± 0.9	0.3 ± 0.2	420	10
7	3-nitrofluoranthene	105	n.d.	0.06 ± 0.03	0.01 ± 0.01	10	80
8	1-nitropyrene	25	n.d.	0.6 ± 0.4	0.09 ± 0.03	65	20
9	2-nitropyrene	20	n.d.	0.6 ± 0.3	0.06 ± 0.04	30	30
10	6-nitrochrysene	3	1	0.1 ± 0.01	0.01 ± 0.01	30	60

[a] *n.d.* not detectable

[b] Reflects the reproducibility of the whole analysis scheme, including extraction and preconcentration steps. The relative standard deviations were determined with immission samples and therefore show high values for compounds with concentrations near the detection limit

burning fireplace. Kamens et al. [31] identified 1-nitropyrene in wood combustion effluents reacted with O_3 and NO_2. In the experiments presented here, 1- and 2-nitronaphthalene, 4-nitrodiphenyl and 9-nitroanthracene were identified in both samples, whereas the isomeric nitrofluoranthenes and nitropyrenes were detected only in sample A. 2-nitrofluorene, 6-nitrochrysene and undefined isomers of these compounds could be determined only in minor amounts.

All of the 10-mononitro-*PAH* mentioned were detected in the urban air samples collected on a heavily frequented road crossing in the city of Vienna. There was substantial evidence for the presence of 2-nitrofluoranthene and 2-nitropyrene which have been discovered in urban air samples only recently and which are considered to be ubiquitarily present in the atmosphere. 2- and 3-nitrofluoranthene have been subject to confusion in the past because of their very similar retention characteristics on DB-5-columns [7, 8, 29, 32]. As shown in Fig. 3, the DB-17-column in use in the present analyses allows for an unequivocal distinction of these two isomers, as well as of the 1- and 2-isomers of nitropyrene. The identification of 2-nitrofluoranthene and 2-nitropyrene is further supported by the presence of the corresponding fragment ions in addition to the molecular peak.

Looked at more closely, the urban air samples formed two groups, which could be related to seasonal variations in meteorological conditions. Column 1 in Table

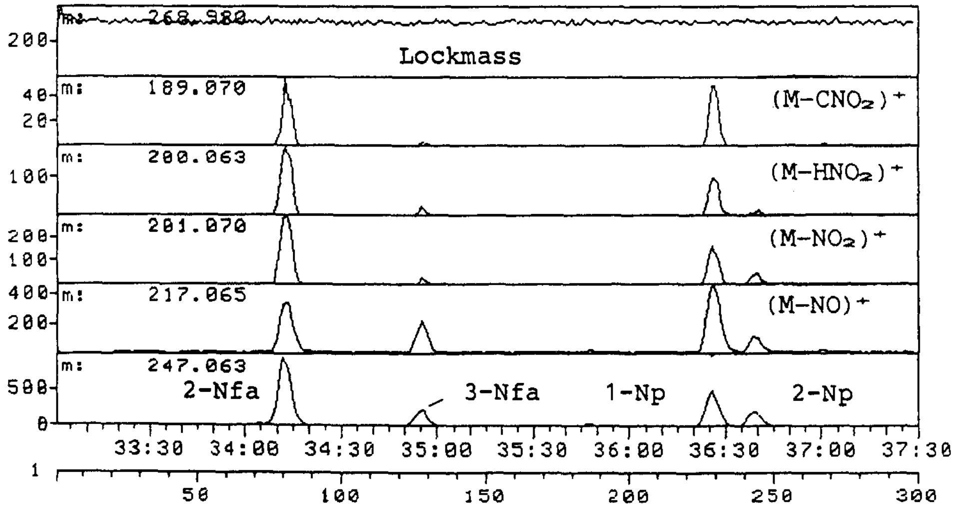

Fig. 3. Nitropyrene (*Np*) and nitrofluoranthene (*Nfa*) isomers in an urban air sample as determined by GC-MS (SIM)

3 shows the average of 4 samples collected in a cold winter between November 1985 and March 1986, whereas column II represents the mean of 4 samples collected in a very mild period between March and June of 1988. The effect of domestic heating on the pollution situation has apparently varied considerably in these two sampling periods. The winter samples show higher concentrations in nitro-*PAH* as well as in *PAH* than the spring samples.

It was also investigated whether *PAH* and nitro-*PAH* amounts determined in urban air samples could be related to each other. A linear correlation could be found for the amounts of pyrene and nitropyrene isomers (correlation coefficient $r = 0.96$, with a statistical significance [33] of 99%) as well as for the amounts of fluoranthene and nitrofluoranthenes ($r = 0.89$, statistical significance 99%).

The total amounts of *PAH* (sum of concentrations of 25 *PAH*) and nitro-*PAH* (sum of concentrations of 10 mononitro-*PAH*) were also linearly correlated with a correlation coefficient of 0.87 and a statistical significance of 99%.

Conclusion

By application of the analysis scheme described 10 mononitro-*PAH* were determined in urban air samples. Most of these compounds were also detected in flue gases of wood combustion plants for the first time. Concentrations of pyrene and nitropyrenes, fluoranthene and nitrofluoranthenes as well as the total amounts of 25 *PAH* and 10 nitro-*PAH* in urban air samples showed linear correlation.

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References

[1] Tokiwa H., Nakagawa R., Horikawa K. (1985) Mut. Res. **157**: 39–47
[2] Harris W. R., Chess E. K., Okamoto D., Remsen J. F. (1984) Environ. Mutagen. **6**: 131–144
[3] Liberti A., Ciccioli P., Cecinato A., Brancaleoni E., Di Palo C. (1986) J. High Res. Chrom. Chem. Commun. **7**: 389–397
[4] Draper W. M. (1986) Chemosphere **15**: 437–447
[5] Paputa-Peck M. C., Marano R. S., Schuetzle D., Riley T. L., Hampton C. V., Prater T. J., Skewes L. M., Jensen T. E. (1983) Anal. Chem. **55**: 1946–1954
[6] Hartung A., Kraft J., Schulze J., Kiesz H., Lies K.-H. (1985) Chromatographia **19**: 269–273
[7] Gibson T. L., Korsog D. E., Wolff G. T. (1986) Atmos. Environ. **20**: 1575–1578
[8] Arey J., Zielinska B., Atkinson R., Winer A. M. (1987) Atmos. Environ. **21**: 1437–1444
[9] Chuang C. C., Mack G. A., Petersen B. A., Wilson N. K. (1985) Report EPA/600/D-85/069, Order No PB85-176790 Avail. NTIS
[10] El-Bayoumy K., O'Donell M., Hecht S. S., Hofmann D. (1985) Carcinogenesis **6**: 505–507
[11] Rosenkranz H. S., McCoy E. C., Sanders D. R., Butler M., Kiriazides D. K., Mermelstein R. (1980) Science **209**: 1039–1043
[12] Abdelnasser M., Hyland M., Jespersen N. D. (1986) Environ. Sci. Technol. **20**: 145–149
[13] Dennis M. J., Massey R. C., McWeeny D. J., Knowles M. E. (1984) Food Add. Contam. **1**: 29–37
[14] Kinouchi T., Tsutsui H., Ohnishi Y. (1986) Mut. Res. **171**: 105–113
[15] Rosenkranz H. S., Mermelstein R. (1985) Biological Aspects of Nitrated Polycyclic Aromatic Hydrocarbons. In: White C. M. (ed.) Nitrated Polycyclic Aromatic Hydrocarbons. Huethig, Heidelberg, pp 267–297
[16] Tokiwa H., Ohnishi Y. (1986) CRC Crit. Rev. Toxicol. **17**: 23–60
[17] Beiji B., Müller L. (1988) Mut. Res. **196**: 177–209
[18] Sweetman J. A., Karasek F. W., Schuetzle D. (1982) J. Chrom. **247**: 245–254
[19] Quiliam M. A., Messier F., D'Agostino P. A., McCarry B. E., Lant M. S. (1984) Spectros. Int. J. **3**: 33–43
[20] Levsen K., Puttins U., Schilhabel J., Prieß B. (1988) Fresenius. Z. Anal. Chem. **330**: 527–528
[21] Arey J., Zielinska B., Atkinson R., Winer A. M. (1988) Environ. Sci. Technol. **22**: 457–462
[22] White C. M. (ed.) (1985) Nitrated Polycyclic Aromatic Hydrocarbons. Huethig, Heidelberg
[23] Posch W (1984) Inaugural-Dissertation, Karl Franzens-Universität Graz
[24] Lindner W., Posch W., Wolfbeis O. S., Tritthart P. (1985) Chromatographia **20**: 213
[25] Jaklin J., Krenmayr P. (1985) Intern. J. Environ. Anal. Chem. **21**: 33–42
[26] Jaklin J. (1987) Fresenius Z. Anal. Chem. **327**: 723–724
[27] Bredeweg A., Rothman L. D., Pfeiffer C. D. (1979) Anal. Chem. **51**: 2061–2063
[28] White C. M., Robbat A. Jr., Hoes R. M. (1983) Chromatographia **17**: 605–612
[29] Ramdahl T., Zielinska B., Arey J., Atkinson R., Winer A. M., Pitts J. N. Jr. (1986) Nature **321**: 425–427
[30] Gibson T. L. (1982) Atmos. Environ. **16**: 2037–2040
[31] Kamens R., Bell D., Dietrich A., Perry J., Goodman R., Claxton L., Tejada S. (1985) Environ. Sci. Technol. **19**: 63–69
[32] Arey J., Zielinska B., Harger W. P., Atkinson R., Winer A. M. (1988) Mut. Res. **207**: 45–51
[33] Doerffel K. (1982) Statistik in der analytischen Chemie. VEB Deutscher Verlag für Grundstoffindustrie, Leipzig

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