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List of Abbreviations

Nitro-PAH : Nitrated polynuclear aromatic hydrocarbon(s)

PUF plug : Polyurethane foam plug
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1. **Abstract**

WERNER ROLLFS. Development of a Procedure for Sampling, Fractionation and HPLC Analysis of Nitrated Polycyclic Aromatic Hydrocarbons in Air. (Under the direction of RICHARD M. KAMENS)

A procedure has been developed for sampling, fractionation and HPLC analysis of carcinogenic nitrated polycyclic aromatic hydrocarbons (nitro-PAH) in air.

A high volume sampling system was selected consisting of a Teflon coated glass fiber filter and two polyurethane foam (PUF) plugs in series downstream from the filter. Teflon coated filters were chosen because of their low catalytic potential for artifact formation of nitro-PAH during sampling. PUF was used over XAD-2 resin for sampling volatilized nitro-PAH because of better blank values and extraction efficiencies. Recoveries of simulated sampling experiments (nitro-PAH added on filters were volatilized by operating the sampling pumps) were in the range of 73.6 - 93.8 %.

Fractionations of crude sample extracts were performed using a normal phase preparative HPLC column and an 83 minute solvent program that controlled the proportions of n-hexane, methylene chloride and acetonitrile as constituents of the mobile phase.

The selected method consisted of an HPLC separation on a reversed phase column (Supelcosil LC-PAH (5 micron)) using a mixture of methanol/water (80/20) as the mobile phase. After separation the nitro-PAH were reduced on-line to the corresponding amino-PAH using a heated catalytic reducer column containing a platinum-rhodium three-way automotive catalyst. A fluorescence detector was used to quantify the amino-PAH produced. The nine nitro-PAH studied in this project could be analysed in chromatograms of 17 minutes length and with detection limits ranging from 7.6 - 90 pg/10 microliter injection.

The identity between sample and standard compounds could be tested by comparing peak height changes that occurred when non-optimal wavelengths for the emitted fluorescence light were used for detection instead of the optimal wavelengths. In this way, 1-nitropyrene was confirmed in a filter sample from a conveyor station of a carbon plant.
2. Introduction

The purpose of this Master's project is to develop a method that is capable of quantifying trace amounts of nitro-PAH in air.

Research on the analysis of nitro-PAH is necessary because a number of scientific studies demonstrated the strong mutagenic potential of these compounds in bacterial (70), 17, 86, 85) and mammalian (42) test systems. However, the major health concern is that many nitro-PAH can cause cancer in animals (74, 65, 57). The main source of these substances incomplete combustion such as diesel emissions (88, 77, 78). Since probably every incomplete combustion causes emission of PAH and potentially nitro-PAH, and also because nitrating agents such as nitrogen dioxide are widespread in our environment, nitro-PAH are commonly found in ambient air (81, 62, 61, 23, 25, 59).

However, only very few studies have tried to investigate the presence of these compounds in the occupational environment (82, 64, 26), and almost no information is currently available in terms of what concentrations can be found in industry. The majority of industrial operations with some potential for nitro-PAH emissions remain uninvestigated. These operations include incomplete combustion processes of various kinds and heating of coal and coal tar pitch.
In order to evaluate the occupational health risk posed by nitro-PAH, more research is necessary to quantify the compounds in air samples taken from potential sources in industry.

The goal of this Master’s project was to develop a method that could detect nitro-PAH in occupational air samples. It was hypothesized that already existing methods for sampling and analysis could be modified in order to meet the following requirements:

-- high sensitivity in order to allow short sampling times in cases of short source emission periods;

-- high selectivity since occupational air samples might include other organics in high concentrations;

-- collection of nitro-PAH adsorbed on particles and present as vapors in order to determine the total nitro-PAH concentration;

-- testing of the identity between sample and standard compounds during analysis;

-- use of common techniques and equipment such as HPLC and high volume sampling devices.
The approach to meet this goal was:

* to select a HPLC method for nitro-PAH analysis that is sensitive, and not too difficult to operate.

* to find a technique that is selective enough in order to avoid interferences from other sample compounds, such as fractionation of the crude sample extract and selective detection;

* to select a filter and a collector material that can retain nitro-PAH efficiently, that does not catalyze chemical reaction during sampling, and that allows good recovery of nitro-PAH during extraction;

* to devise a procedure to test the identity between a sample and standard compound by means of a specific change in the method of analysis.

The selection of the developed method was based on a review of relevant literature and on experiments performed in the laboratory.

This report documents this selection and modification process and describes the final method for sampling, fractionation, analysis and identity confirmation of semivolatile nitro-PAH in air.
3. Characteristics of Nitro-PAH

3.1 Structural Formulas and Physical Properties

Figure 1 shows the structural formulas of the nine nitro-PAH studied in this project. Table 1 presents some of the physical properties. However, the boiling points and the vapor pressures of these compounds were not found in the literature except for 9-nitroanthracene (275 °C). 6-Nitrochrysene sublimes without decomposition (2).

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>Molecular Weight</th>
<th>Melting Point °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>173.17</td>
<td>79</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>211.22</td>
<td>158</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>233.23</td>
<td>146</td>
</tr>
<tr>
<td>12-Nitroanthracene</td>
<td>223.23</td>
<td>(not published)</td>
</tr>
<tr>
<td>11-Nitropyrene</td>
<td>247.3</td>
<td>155</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>247.3</td>
<td>160</td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>247.3</td>
<td>(not published)</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>274.3</td>
<td>(not published)</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>273.3</td>
<td>209</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>297.3</td>
<td>251</td>
</tr>
</tbody>
</table>

Table 1: Physical characteristics of the nitro-PAH studied.

Also, the sensitivity of nitro-PAH to light-induced chemical decomposition is not well documented. However, it is known that 9-nitroanthracene is stable in ethanolic solution in the dark but decomposes to anthraquinone, acetaldehyde and nitric acid when exposed to sunlight (2).
Figure 1: Structural formulas of nitro-PAH
3.2 Sources and Formation

Nitro-PAH may be produced by several sources:
- primary emissions from combustion processes and other stationary sources;
- atmospheric reactions with air pollutants and oxidants;
- chemical transformation of PAH by air pollutants and oxidants adsorbed on particles or present in the vapor phase during sampling (sampling artifacts).

All of these possible sources for nitro-PAH are currently being investigated by numerous researchers (88, 19).

Early studies detected nitro-PAH on carbon black and photocopies (5, 76) which caused considerable public interest. However, much more important sources are diesel emissions (25, 78, 89) and stack gases from power plants (61) or other types of incomplete combustion.

Formation of nitro-PAH in the atmosphere or during sampling has been a concern and was described in a number of studies. Table 2 lists selected chemical reactions (in-situ) between atmospheric fixed gases and analytes adsorbed onto substrate:
Table 2: Nitro-PAH formation on substrate during sampling.

<table>
<thead>
<tr>
<th>Reactive Gas</th>
<th>Substrate/Reactant</th>
<th>Product(s)</th>
<th>Reference</th>
</tr>
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<tr>
<td>N$_2$O$_5$</td>
<td>Pyrene</td>
<td>1-Nitropyrene</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Perylene</td>
<td>3-Nitroperylene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Naphthalene</td>
<td>1-N-naphthalene</td>
<td>66</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>Perylene</td>
<td>3-N-perylene</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Benzo[a]pyrene</td>
<td>1-N-b[a]p</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-N-b[a]p</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-N-b[a]p</td>
<td></td>
</tr>
<tr>
<td>NO$_2$, HNO$_3$</td>
<td>Benzo[a]anthracene</td>
<td>10-N-b[a]p</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Benzo[a]pyrene</td>
<td>6-N-b[a]p</td>
<td></td>
</tr>
</tbody>
</table>

One has to be aware of the potential for these reactions when concentrations of nitro-PAH in air are given because the relative proportion of sampling artifacts is usually not known. When evaluating the concentrations of 1-nitropyrene in ambient air given in Table 3 this concern should be kept in mind.

Table 3: Concentrations of 1-nitropyrene in ambient air

<table>
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<tr>
<th>Location</th>
<th>Range, ng/m$^3$</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Street level near traffic in Oslo</td>
<td>0.01 - 0.22</td>
<td>81</td>
</tr>
<tr>
<td>Residential area west of Copenhagen</td>
<td>&lt;0.001 - 0.4</td>
<td>62</td>
</tr>
<tr>
<td>Rural area west of Copenhagen</td>
<td>0.009 +/-0.005</td>
<td>61</td>
</tr>
<tr>
<td>Detroit, Michigan</td>
<td>0.016 - 0.03</td>
<td>23</td>
</tr>
<tr>
<td>Allegheny mountain tunnel, PA</td>
<td>&lt;0.04 - 0.12</td>
<td>25</td>
</tr>
<tr>
<td>Industrial area in Japan</td>
<td>0.0208</td>
<td>59</td>
</tr>
</tbody>
</table>
3.3 Biological Effects of Nitro-PAH

Nitro-PAH have been tested extensively for mutagenicity in bacteria, primarily *Salmonella typhimurium* (70, 17, 86, 85, 74). Although the majority of nitro-PAH appear to be mutagenic, the range of mutagenicity varies widely: between 0 (7-nitrobenz[a]anthracene) and 254000 (1.8 dinitropyrene) revertants per nanomole (74). Also, the majority of nitro-PAH are mutagenic in mammalian cell systems (75). The mutagenic potential of a compound in such a bioassay is a rough indication of its mutagenic potential for humans. Somatic mutations can cause cancer which is the health hazard that ultimately needs to be evaluated if a compound is mutagenic in bacterial or cell test systems.

Several bioconversion schemes of nitro-PAH have been described and reviewed (74). The metabolism of nitro-PAH to their proximate mutagenic form may occur by oxidation of the aromatic ring system to form epoxides, or by enzymatic reduction of the nitro group with subsequent formation of aryl hydroxylamines (10). It has been shown that lung cells and tissue of rabbits are capable of binding 1-nitropyrene or its metabolites to DNA and protein. Also, macrophages as well as lung and tracheal tissue metabolize 1-nitropyrene by both oxidative and reductive pathways (42). It is not known whether the carcinogenicity of nitro-PAH (discussed later in this chapter) has the same structural basis as the mutagenicity,
i.e. nitroreduction, or whether it can be initiated by the formation of other DNA adducts, i.e. via epoxides (74).

About 80 nitro-PAH have been tested for their mutagenicity in a bacterial test using *Salmonella typhimurium* (TA98, +/- S9) (74). Table 4 shows the data given for the nitro-PAH studied in this project.

<table>
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<tr>
<th>Nitro-PAH</th>
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<tr>
<td></td>
<td>+S9</td>
</tr>
<tr>
<td>2-Nitronaphthalene</td>
<td>26.4</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>2.0</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>58.4</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>3-Nitrofluoranthenene</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>22.4</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>141</td>
</tr>
</tbody>
</table>

Table 4: Mutagenicity of nitro-PAH

It is evident that metabolic activation markedly influences the mutagenic potential of nitro-PAH (10). The findings shown in Table 4 indicate that some nitro-PAH are so mutagenic that a biological test system can be of similar detection sensitivity as modern instrumental analysis such as GC-FID or HPLC-UV. The major public health concern, however, is caused by findings of carcinogenicity studies. All of the 17 nitro-PAH (except 1-nitronaphthalene) tested for carcinogenicity in animals caused tumors. Some such as
6-nitroanthracene are powerful cancer-causing agents (74). Among the compounds studied in this Master's project, the following nitro-PAH have been shown to cause tumors in animals:

2-nitronaphthalene, 2-nitrofluorene, 1-nitropyrene,
3-nitrofluoranthene, 7-nitrobenz[a]anthracene,
6-nitrochrysene, 6-nitrobenzo[a]pyrene (74, 65, 85, 57, 42).

For the remaining three of the nine nitro-PAH of this project, no data are available from the literature.

However, there are many other factors that need to be evaluated in order to estimate the magnitude of the health hazard caused by nitro-PAH, such as the absolute amounts present in the environment and the bioavailability to organisms. A high concentration of nitro-PAH does not necessarily correlate with a high health risk. For example, the relative high exposure to PAH in aluminum smelters is not correlated to a great extent in epidemiological studies with cancer among workers in the aluminum industry (8). A reason for this observation might be that only a small fraction of the airborne PAH is taken up by humans.

Similarly, isotopic exchange studies and tissue culture media extraction studies indicate that only a small amount (10%) of 1-nitropyrene is extracted from fly ash in tissue media (60). Also, it has been shown that the physical state of nitro-PAH (crystalline versus adsorbed onto a particle) influences its biodistribution as well as its retention (74). It is also reasonable to assume that the relative distribution between solid and vapor phase is
important in terms of evaluating the bioavailability of nitro-PAH. The available information is not sufficient to permit a risk assessment for humans given our lack of knowledge regarding exposure levels and doses of nitro-PAH to the susceptible organ.

3.4 Industrial Relevance

Nitroaromatics are used to make several thousand consumer products, which account collectively for nearly 10% of the chemical industry sales. Aniline and toluene diisocyanate are the two major nitroaromatic compounds. Slightly more than one billion pounds of benzene, toluene and xylene are used annually to make nitroaromatic compounds worth nearly $2 billion (71).

However, practically all nitroaromatics of economic relevance are one-ring compounds used as raw material for production of polymers, rubber chemicals, dyes & pigments, pharmaceuticals, pesticides, etc. (71).

Nitrated polyaromatics are only rarely produced, usually only for research purposes and in very low quantities. Only 1-nitropyrene had been produced in industry by a Japanese company in very small quantities. It was used for the production of 1-azidopyrene, which was used in photosensitive printing (2).
However, it appears reasonable to assume that nitro-PAH are generated as side products in many industrial processes that include incomplete combustion or heating of material that already contains nitro-PAH. Unsubstituted PAH are produced under such conditions and can be nitrated either during or after their formation.

In an occupational environment, nitroaromatics with two or more rings were found in only three studies, which all evaluated indoor air samples in aluminum plants (82, 64, 26). Among the compounds found were 2-nitronaphthalene, 2-nitrofluorene, 9-nitroanthracene, and 1-nitropyrene, which were all studied in this project.

A concentration of a nitro-PAH was given in only one study (26): 1-Nitropyrene was found in the potroom of a Soderberg aluminum plant in a concentration of 64 ng/m³, which compares to concentrations of unsubstituted pyrene in a similar plant of about 35000 ng/m³ (volatile and particulate matter).

Aluminum smelters in general are significant emitters of PAH. For example, the emission of benzo[a]pyrene from an aluminum smelter in Sundsvall, Sweden, has been estimated to be 0.26 kg/hour (44). In an other estimation of a Norwegian horizontal Soderberg aluminum plant, the PAH output per ton of aluminum produced was 4.4 kg., most of them volatile (3). This particular process in aluminum production is practically no longer used in the U. S.. The amount
of PAH emitted by aluminum smelter techniques employed in the U. S. is generally less, but still significant.

Even though the concentration of 1-nitropyrene appears to be less than 1/500th of that of unsubstituted pyrene in the Soderberg process, it is still more than 100 times higher than the concentrations of 1-nitropyrene found in ambient air (see Table 3 in Chapter 3.2).

The threshold limit value in the Federal Republic of Germany for 1-nitropyrene is 5 mg/m$^3$ in the workplace atmosphere. The 30 min average standards in ambient air are 0.85 and 0.3 mg/m$^3$ for short and long time exposure, respectively (1).

In general, the presence of nitro-PAH in industry (as contamination or side product) is likely. However, due to a lack of published sampling data a risk assessment is impossible to perform at this time.
4. Apparatus and Chemicals

HPLC System Used for Platinum/Rhodium Method:

HPLC: Varian Ass., Varian 5000 Liquid Chromatograph;
Recorder: Fisher Recordall, Series 5000;
Fluorescence Detector System:
   Power Supply Unit: Perkin Elmer, Model 150;
   Detector Unit: Perkin Elmer, Model 650 - 10S Fluorescence
   Spectrophotometer;
Ultraviolet Detector: Waters Ass., Model 440 Absorbance Detector;
Analytical Column: Supelco, Reversed Phase, Supelcosil LC-PAH (5
micrometer), 15 cm x 4.6 mm ID., col. #: 30386
Catalyst Column: 4.0 cm x 2 mm ID., see Chapter 5.2.2.1 for
   packing;

HPLC System Used for Zinc Method:

Injector: Waters Ass., Model UGK Universal Liquid Chromatograph
   Injector, 2 ml injection loop;
Recorder: Linear Instruments, Delux Laboratory Chart, triple
   channel, overall limit of error from all sources less than
   0.5 %;
Solvent Programer: Waters Ass., Model 660;
Pumps: Waters Ass., Model 6000 A Solvent Delivery System, (two pumps);

Fluorescence Detector System:

Power Supply Unit: Perkin Elmer, Model 150;
Detector Unit: Perkin Elmer 650 - 10S Fluorescence Spectrophotometer;

Separate Fluorescence Detector System used only for Analysis of NBS Diesel Soot Standard:
Varian Ass., Aerograph Fluorichrome Detector, single channel, filters used: excitation: 340 - 380 nm,
Raman cutoff*): 460 nm,

Ultraviolet Detector: Waters Ass., Model 440 Absorbance Detector;
Columns: See Chapter 5.2.1.2 for details.

Apparatus used for Fractionations of Sample Extracts:

The apparatus had been used for fractionation in a number of studies (5, 39, 38, 37, 40) and is identical to the one used for this project. It consisted of a Tevnary Spectra Physics 8700 high pressure liquid chromatograph and a Waters Semipreparative M Porasil (300 x 7.8 mm ID) column.

*) Raman cutoff wavelength: shorter wavelengths than the one given are filtered out, longer wavelengths pass through the filter with intensities up to 85 % of those before the filter.
Apparatus for Air Sampling:

The Apparatus as shown in Figure 8 in Chapter 12.2.2 for air sampling was designed and provided by Professor Richard M. Kamens. Glass fibre filters: MSA Glass Fibre Filter WEB, Cat. #: CT-75428, Mine Safety Appliances Co., Pittsburg, PA. Teflon coated glass fiber filters: supplied by Richard M. Kamens. High volume pump: supplied by the Department of Environmental Sciences and Engineering.

Apparatus for Extraction and Sample Handling:

Rotary Evaporator: Buechi, Buechi HP-140; Shaker: Lab - Line Junior Orbit Shaker, Lab - Line Instruments, INC., Melrose Park, Illinois; Petri dishes for filter extraction: diameter 14.8 cm, 1.8 cm depth; For PUF plug extraction: - beaker, 1 liter graded, diameter 10.3 cm, - plunger for squeezing PUF plugs, 25 ml graded measurement glass cylinder with bottom of 5.5 cm diameter; Sample vials: volume 4.5 ml, Teflon sealed cap;
### Chemicals:

<table>
<thead>
<tr>
<th>Name</th>
<th>Purity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitro-PAH</td>
<td>---</td>
<td>Prof. Richard Kamens</td>
</tr>
<tr>
<td>Silica Gel 60 G</td>
<td>For thin layer chromato-</td>
<td>E. Merck</td>
</tr>
<tr>
<td>Water</td>
<td>Distilled in glass</td>
<td>University of NC, Dept.</td>
</tr>
<tr>
<td>Platinum/Rhodium Catalyst</td>
<td>---</td>
<td>ESE, Room 148</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>HPLC grade</td>
<td>Mr. S. B. Tejada</td>
</tr>
<tr>
<td>Methanol</td>
<td>HPLC grade</td>
<td>EM Science</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td>HPLC grade</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Hexane</td>
<td>UV grade</td>
<td>EM Science</td>
</tr>
<tr>
<td>Toluene</td>
<td>Distilled in glass</td>
<td>BJ</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Glacial, min. 99.7%</td>
<td>BJ</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>Fused-anhydrous</td>
<td>EM Science</td>
</tr>
<tr>
<td>Zinc, granular, 20 mesh</td>
<td>A. C. S. reagent</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Zinc, 7.1 micrometer</td>
<td>---</td>
<td>Aldrich Chem.</td>
</tr>
<tr>
<td>Olympic 3415 polyurethane -</td>
<td>Free of silicon density</td>
<td>Alfa Products</td>
</tr>
<tr>
<td>polyether flexible foam</td>
<td>24 kg/m³</td>
<td>Olympic Prod. Comp.</td>
</tr>
<tr>
<td>pH - paper</td>
<td>---</td>
<td>Franc Sasser</td>
</tr>
<tr>
<td></td>
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<td>Senior Chemist</td>
</tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>E. Merck</td>
</tr>
</tbody>
</table>
5. Analysis of Nitro-PAH by High Performance Liquid Chromatography

5.1 Principal Methods and Considerations

After gas chromatography, high pressure liquid chromatography is most frequently used for analysis of extracts of airborne particulate matter (19).

There are several applications of reversed phase HPLC in the analysis of nitrated PAH. Most researchers employ a standard HPLC system with column diameters typically about 4 - 5 mm and generally less than 20,000 effective plates. This is considerably less than the more than 100,000 plates that can routinely be generated with capillary gas chromatography (52).

Several methods are possible to overcome this short separation power in HPLC-technique. One is to employ a multi-column system (frequently also referred to as column-switching or multi-column chromatography). The idea is to have several columns of different selectivities in a row. Such a two-dimensional column switching HPLC system was used to analyse nitro-PAH in diesel extracts (52). This method showed good specificity and a relatively quick analysis time (15 - 20 min). However, the detection of several nitro-PAH was
confounded by coeluting peaks, making quantitation of these components difficult or impossible.

Reversed phase column material was also used for separating the reaction products of a nitration of 10 PAH. The nitrated PAH were detected in a UV detector at 260 nm. However, a complete peak separation could not be established with this technique (16).

Another method to deal with insufficient separation capacity in HPLC-technique is the use of highly specific detectors rather than an UV-detector at wavelengths such as 254, 260 or 280 nm which can be used to detect many classes of organic compounds, especially all aromatics. The selectivity of an UV detector can be improved by making measurements at 400 nm, but even at this wavelength interference with other components in environmental extracts is quite common. The limit of detection using UV detection is generally in the range of 1 - 10 ng/injection (88).

One of the more specific detectors is the electrochemical detector which measures (by an amperometric controller) the electric current in a cell to which a certain potential difference (between a "working" and a reference electrode) is applied. The amount of current is proportional to the amount of electrochemically active substance in the cell that causes the current.

In case of nitro-PAH, a reduction to amines is caused by the application of -0.5 to -0.7 Volts vs. a Ag/AgCl-reference electrode
(70, 56). Although the sensitivity was good (10 - 100 pg for most nitro-PAH (70)), several disadvantages also have to be considered:

- The HPLC system required periodic maintenance such as removal of nonpolar residues from the column, and repolishing of the working electrode.
- Efforts were needed to prevent traces of atmospheric oxygen from coming into the solvent system (even the sample needed to be purged of dissolved oxygen with nitrogen for about 10 min.). This strict requirement has limited the widespread application of reductive electrochemical LC detection. However, a promising oxygen removal method via a zinc oxygen scrubber column has recently been published (55).
- Several nitro-PAH eluted as a single peak.
- Quinones and ketones were detected with sensitivities comparable to those of the nitro-PAH since they are reduced at potentials below -0.7 volts. They can, however, possibly be differentiated from nitro-PAH by their hydrodynamic voltamograms (56).

A reduction to amines is also performed with a different, very sensitive and selective detection technique: fluorescence detection. Unlike nitro-PAH and many other components present in extracts of airborne particulate matter, amino-PAH are strong emitters of fluorescence light and can be detected in even low picogram levels.
The first application of this detection principle for nitro-PAH in air pollution analysis was published in 1978 with a fluorescence quenching technique on thin-layer plates. In this case the reducing agent consisted of potassium borohydride, copper(II)chloride, methanol and water. The detection limits, however, were very high and ranged from 1 - 500 ng (34).

Three procedures have been proposed in the literature for this type of detection in HPLC applications: one pre-injection reduction and two on-line reduction methods.

In the pre-injection method (23, 79, 24), an aliquot of a sample extract is treated for 3 - 16 hours at room temperature with a reduction solution consisting of sodium borohydride in a mixture of methanol, deionized water and cupric chloride. The reducing agent was freshly prepared for each use. The mobile phase consisted of acetonitrile and water buffered with 0.2 % ammonium hydroxide or t-butylammonium phosphate. Retention times, wavelengths used for detection and the corresponding detection limits are given in Table 5. It should be noted that these detection limits are the lowest of all current analytical methods for nitro-PAH.
Table 5: HPLC-fluorescence analysis of PAH and amino-PAH

<table>
<thead>
<tr>
<th>Compound</th>
<th>TR min</th>
<th>Detection</th>
<th>Minimum Detection Limit pg/injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Excit.</td>
<td>Emiss.</td>
</tr>
<tr>
<td>2-Aminofluorene</td>
<td>8.3</td>
<td>290</td>
<td>365</td>
</tr>
<tr>
<td>3-Aminofluoranthene</td>
<td>11.1</td>
<td>415</td>
<td>518</td>
</tr>
<tr>
<td>1-Aminopyrene</td>
<td>12.1</td>
<td>365</td>
<td>430</td>
</tr>
<tr>
<td>Aminobenz[a]anthracene</td>
<td>15.7</td>
<td>310</td>
<td>470</td>
</tr>
<tr>
<td>6-Aminochrysene</td>
<td>16.1</td>
<td>345</td>
<td>430</td>
</tr>
<tr>
<td>6-Aminobenzo[a]pyrene</td>
<td>24.0</td>
<td>430</td>
<td>498</td>
</tr>
<tr>
<td>Aminobenzo[k]fluoranthene</td>
<td>24.5</td>
<td>310</td>
<td>495</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>7.2</td>
<td>250</td>
<td>365</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>8.7</td>
<td>280</td>
<td>500</td>
</tr>
<tr>
<td>Benzo[c]fluoranthene</td>
<td>9.5</td>
<td>305</td>
<td>355</td>
</tr>
<tr>
<td>Pyrene</td>
<td>9.9</td>
<td>325</td>
<td>389</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>15.5</td>
<td>310</td>
<td>404</td>
</tr>
<tr>
<td>Perylene</td>
<td>18.8</td>
<td>430</td>
<td>468</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>18.0</td>
<td>383</td>
<td>430</td>
</tr>
</tbody>
</table>

Fluorescence detection offers another advantage in terms of increased selectivity; as can be seen from Figure 2 - using PAH as an example - the specific combination of two wavelengths for each component permits a selective detection even if several compounds are not well separated. For example, benzo[k]fluoranthene and perylene were not resolved chromatographically, but at 310 nm excitation and 404 nm emission, only benzo[k]fluoranthene was detected; and at 430 nm excitation and 468 nm emission, perylene could be quantified free of interference.
Figure 2: Series of liquid chromatograms for a single diesel sample at wavelengths selected for optimum analysis. The time scale in minutes, the level of each compound in ng/mg of diesel particulate, and the fluorescence wavelengths (excitation in nm, emission in nm) used are shown for each chromatogram. (reprint from reference 24)

Potential problems of this method include poor photolytic and oxidative stability of the derivatives, poor resolution of amino-PAH isomers on reversed phase columns, and peak tailing/sample adsorption on some column packings at low amino-PAH concentrations (88).
On-line reduction of the nitro-PAH to amino-PAH means that this chemical reaction takes place in a specific reduction column which is located after the analytical column during the chromatographic run. The principal advantage is significantly reduced sample handling. Therefore the analysis is easier and faster as well as more precise. The major disadvantage is the requirement of a oxygen-free mobile phase.

The first method of this kind was published by Tejada et. al. in 1982 (83). This method was later selected for nitro-PAH analysis in this project.

In this method, the conversion of nitro-PAH to the corresponding amino-PAH takes place in a reduction column that is placed after the first of two analytical columns. The reduction column consists of a 5 * 0.46 cm (i. d). stainless steel tube packed with platinum-rhodium on 5 micrometer alumina.

Dissolved oxygen was removed from the mobile phase prior to the analytical and reduction columns by a stainless steel tube (3 inch * 0.46 cm (i. d.), packed with ground (60 - 100 mesh) three-way automotive catalyst.

The chromatographic separation was performed by two analytical columns, individually controlled by the LC's microprocessor. Methanol and water spiked to pH 8 with dilute sodium hydroxide, were used as the mobile phase. Reduction of the nitro-PAH was both
efficient (greater than 99%) and fast (less than one minute). The analytical system did not require the addition of consumable derivatization agent. The catalyst generated the reducing agent from the solvent used in the chromatography. Due to solvent programming and a column switching technique it was possible to achieve a good separation of a diesel extract and a sensitivity of below 10 pg for 1-nitropyrene in standard solution (83).

Two other methods of this kind were published in 1983. In one application (78), an unspecified on-line catalytic procedure was used for fluorescence HPLC. No conversion efficiencies were presented in that work.

The other application (56) used zinc and an acidic mobile phase to reduce the nitro-PAH. The injected nitro-PAH were reduced after chromatographic separation on the analytical column. The reduction column was dry-packed (with vibration) with one part zinc dust (7 μm, about 200 mg) and two parts silica (20 micrometer, about 400 mg). Approximately one second of peak broadening was observed for a chromatographic peak of 15 seconds half-width (56).

As a general view of all HPLC techniques described so far, it has not been demonstrated that isomeric mixtures of nitro-PAH can be separated adequately by HPLC. This is a major disadvantage of this technique because a separation of isomers may be essential for assessing the true health effects aspects of the sample. Moreover, selectivity and sensitivity of most applications (all UV and
electrochemical detection methods) are not adequate for quantifying nitro-PAH in environmental samples. Only the fluorescence detection technique appears to be a promising technique, although only a few groups of researchers published papers using this method up to now, including Tejada (83), Kamens (39), MacCrehan (55, 56), Greensberg (27), Schuetzle (78) and Gibson (23, 22, 79, 24).

The objective of this Master's project was to implement an analytical procedure capable of detecting trace levels of nitro-PAH in environmental samples. From the literature review, the fluorescence detection method appeared to be promising due to its superior sensitivity and selectivity. An on-line reduction method appeared to be appropriate for its convenience during sample handling. Of the two methods described, the zinc reduction method was tried first.
5.2 Application of Two On-Line Reduction Methods for Fluorescence Detection of Nitro-PAH

5.2.1 Zinc Reduction Method

5.2.1.1 Chemical Considerations

Reduction of nitro-PAH by metals in mineral acids always produce the amine and cannot be stopped at an intermediate stage. The mechanisms of these reductions have been studied very little, though it is usually presumed that, at least with some reducing agents, nitroso compounds and hydroxylamines are intermediates. The reaction is in short (58):

\[ \text{NO}_2\text{-PAH} + 6 \text{H}_3\text{O}^+ \xrightarrow{\text{Zn}} \text{NH}_2\text{-PAH} + 8 \text{H}_2\text{O} \]

However, under neutral conditions the reduction of nitro-PAH is reported to stop at hydroxylamines (58).

This is not quite in agreement with the findings in (56) that for 1-nitropyrene the reduction efficiency to 1-aminopyrene was pH-independent in the range of pH 3.5 - 7.0.
Hydroxylamines, however, are readily reduced to amines in the presence of an acid and zinc (58) which indicates that the mobile phase probably has to be only mildly acidic in order to achieve complete reaction towards the amines.

The zinc of the short reducer column can be consumed via dissociation into the mobile phase due to traces of oxygen (55, 88):

\[
\text{Zn} + \text{O}_2 + 2 \text{H}^+ \rightarrow \text{Zn}^{2+} + \text{H}_2\text{O}_2
\]

\[
\text{Zn} + \text{H}_2\text{O}_2 + 2 \text{H} \rightarrow \text{Zn}^{2+} + 2 \text{H}_2\text{O}
\]

Due to these traces of oxygen the conversion efficiency of the reducer column rapidly decreased to zero after passage of only a few hundred milliliters of mobile phase (55, 88). Moreover, the fluorescence signals of several PAH components show sensitivity to the quenching effects of oxygen (55). To prevent a rapid deterioration of the reducer column and to eliminate oxygen fluorescence quenching, a large sacrificial "oxygen scrubber" column was placed between the pump and the injection valve. The oxygen scrubber column was designed to significantly reduce the oxygen content in the mobile phase and thus to extend the lifetime of the reducer column to more than 40 hours of use and to guarantee full sensitivity at fluorescence detection (56, 55). The conversion efficiency for several nitro-PAH was greater than 99.5 % (56).
The following HPLC columns were evaluated because they were available in our laboratory and could potentially separate nitro-PAH:

- Micro Bondapak, reverse phase
- Micro Pak, reverse phase
- Particle 10 DDS 2
- Micro CN (PIN 8420)
- Supelcosil LC - PAH (# 80163)
- Supelcosil LC - PAH (# 80259)

The columns were tested using an UV-detector and mixtures of methanol/water and acetonitrile/water as the mobile phase without the catalytical column.

The best results were obtained with Supelcosil LC-PAH (# 80163). Figure 3 shows an example of these results.
Figure 3: Chromatogram of nitro-PAH obtained by the zinc reduction method and list of analytical conditions used
UV detection: 254 nm, 0.05 A.U., 1.5 ml/min., acetonitrile/water (75/25)
The method of packing the catalytical column significantly determined the performance of the chromatographic system, especially the peak width. Broad peaks were a central problem with all reducer columns used. Attempts to separate the corresponding amino-PAH rather than the original nitro-PAH were not successful. Also, a gradient program of methanol/water were used to improve resolution but a strong increase in baseline limited this approach. Peak broadening by the catalytical column could not be decreased by using a mixture of one part silica and 0.73 parts zinc dust (by weight) instead of pure zinc dust. It was not possible to analyze the nitro-PAH under study when an unfractionated NBS direct soot extract in methylene chloride was injected that contained certified amounts of four of the nine nitro-PAH studied in this project.

At this point the work on the zinc reduction method was stopped. A brief list of some problems that occurred during this work follows:

1) Zinc in the reducer column dissociates into the mobile phase due to traces of oxygen and elutes out of the HPLC system as zinc ions (55). Even if an oxygen scrubber column is used there are still traces of oxygen left that cause slow consumption of the zinc in the reducer column.

In this work zinc precipitation was observed in the UV detector, causing an irregular decrease in sensitivity and a danger of clogging. A few times upon cleaning the detector cell, a grey layer
of precipitated zinc could be seen. Sometimes, parts of this precipitated zinc detached from the cell surface and
- either agglomerated within the detector cell to a bigger zinc particle (identifiable by a sudden and stable rise in the baseline of one of the two UV detector cells
- or were flushed out of the system (identifiable by a very sudden and short peak in the downstream UV detector cell).

Moreover, sometimes capillary connections downstream to the reducer column clogged although no mechanical changes were performed immediately before. Once, a capillary downstream to the fluorescence detector cell clogged, causing a pressure buildup in the system which destroyed the glass cell of the fluorescence detector, a very expensive part. Most likely, these blockages were caused by either precipitated zinc or by zinc particles escaping the reducer column.

2) Several times the pressure drop at the reducer and oxygen scrubber column increased substantially, reaching the selected maximum allowable pressure (4000 psi) of the HPLC-system. This pressure drop could be lowered to normal values by turning the columns around. This measure worked only two or three times for a particular column. Afterwards, the column needed to be repacked.

3) When a mixture of zinc and silica was used for the reducer column, the mixture occasionally separated during use.
4) Sometimes upon emptying an oxygen scrubber or reducer column, it was found that the packing material had become very hard and difficult to remove. Once even an electric drill had to be employed in order to empty an oxygen scrubber column, because the packing material seemed to have "melted" together. This effect made it much more time consuming to empty than to pack a column.

5) The peak resolution potential of the analytical column decreased during prolonged use of the acidic mobile phase at pH 4.7. In order to prevent or at least to slow down this process, a less acidic mobile phase (pH 5.5) was used as suggested (56). Nevertheless, such an acidic mobile phase may not be applicable to some packing materials of analytical columns.

6) Sometimes leakages in the UV detector occurred, causing precipitation of sodium acetate used in the buffer solution on the lense and glass surfaces of the detector cell that were difficult to remove even by ultrasonic treatment in distilled water. Since acetic acid is a liquid under ambient conditions, this substance was used rather than the buffer (acetic acid/sodium acetate) to acidify the mobile phase and to avoid such precipitations in case of another leakage.
As discussed in Chapters 5.1 and 5.2.1.1, applications of the zinc reduction method described in the literature suggested this method was a relatively selective and very sensitive analytical procedure, potentially capable of even direct analysis of unfractionated extracts of airborne particles. When this method was used, however, the resulting problems suggested that this method was rather unreliable and time consuming. These problems were:

- deterioration of the analytical column due to acidic mobile phase;
- difficulty of packing the reducer column reproducibly and free of dead volume;
- "hardening" of the zinc in the columns during use;
- pressure drop increase in zinc columns during prolonged use;
- frequent maintainance of zinc columns;
- zinc precipitation in the UV detector (and probably also other parts downstream the zinc columns), causing a decrease in sensitivity;
- risk of clogging capillaries during chromatographic runs due to zinc precipitation or zinc particles escaping the reducer column.

The HPLC-on-line-reduction method described by Tejada (83) was used instead of the zinc reduction method because of these problems.
5.2.2 Platinum-Rhodium Method

The literature does not give as much information about this method as it does for the zinc reduction method. The original article (83) described it only rather briefly so that several important parameters and procedures needed to be determined before the method could be used for nitro-PAH analysis in other experiments. The following pages describe the efforts made to determine those.

5.2.2.1 Packing of the Catalyst Column

This procedure was expected to require less than an hour, but turned out to be a rather difficult task. One week of effort was necessary in order to deal with problems of:

- poor chromatographic behavior (peak broadening)
- low catalytic action (small peaks)
- high pressure drop.

The following packing procedure resulted in a working catalyst column:

Two particle size fractions of the platinum/rhodium catalyst were mixed:
The column (4 cm length, 2 mm ID) was closed on one end and filled with a mixture of methanol/water (80/20). A funnel was placed on the column opening and the catalyst was slowly added until the column was full. The material was stirred slowly with a fine needle to create a homogeneous mixture and to eliminate bubbles. During this procedure the level of catalyst material in the column dropped. More material and solvent were added until full, sometimes stirring the material again with a needle.

The column was then heated to about 60 °C for a few minutes to evaporate the free methanol/water mixture on top, leaving the material on top moist but not wet. Slight pressure was applied by a thick wire, and some more material was added until the column was full. The column was closed and used thereafter for all the experiments described here. However, the pressure drop across this column increased about 40 atmospheres over 150 hours of operation.

5.2.2.2 Effects of Catalytic Column Temperature

The catalyst of platinum/rhodium needs to be heated in order to activate the reduction of the nitro-PAH to the amino-PAH (83). However, it was not known which temperatures were to be selected
for best system performance and safety. Two criteria had to be considered:

- effect of temperature on reduction efficiency
- effects of high solvent temperature on detector cell safety due to expansion of the cell material.

In order to determine these effects, the following experiment was conducted: standard solutions of nitro-PAH were injected at different temperatures and the peak response measured.

Figure 4 shows the results, normalized to a peak height of 1 at the lowest temperature. There is an exponential relationship between temperature and peak height which levels off at higher temperatures of about 130 °C.

The curves also reflect the specific detector response per unit mass of each compound. For example, at 135 °C a peak of ng 1-nitropyrene would be about 20 times longer than a peak of 1 ng 2-nitroanthracene.

Since the detector response at a certain temperature per ng is substance specific, and since the dependence between temperature and peak height shows a substance-specific exponential relationship, the ratio of peak heights must be temperature dependent. Using a peak height ratio for calculating expected peak
heights therefore requires a constant temperature of the catalytic column during analysis.

For this project, a temperature of about 135 °C was chosen because of the following reasons:

1) The curves of Figure 4 are relatively flat at that high temperature. If the parameter temperature were to show some variability the peak height differences caused by such variability would be smaller at a high temperature of 130 °C than at a lower one.

2) Higher temperature provided a higher sensitivity.

3) The capillary between the heated catalytical column and the detector cell had a big enough surface to allow the heated solvent to cool down to a temperature a little above 36 °C at the cell, which did not cause a very significant expansion of the cell material.
1 : 2-Nitroanthracene
2 : 6-Nitrochrysene
3 : 2-Nitrofluorene
4 : 2-Nitronaphthalene
5 : 1-Nitropyrene

Figure 4: Relative increase of peak height with temperature.
5.2.2.3 Conditions used for the Nitro-PAH procedure in this work

The HPLC system was allowed to run for about 30 minutes in order to reach equilibrium. Sample and standard vials were moved from the deep-freezer (-60 °C) and stored in the refrigerator (+4 °C) until the actual injection. To prevent carry over from a previous injection, the injector was flushed with 0.5 ml methanol in the load and inject position prior to sample injection. Additionally, two blank injections of pure methanol were done, before approximately 0.013 ml of the sample were injected in order to fill the injection loop with a volume of 0.01 ml. The catalyst column was kept at a constant temperature of about 135 °C. Detection wavelengths and sensitivities were changed manually for each compound of a chromatogram. The slit width of the monochromator was 10 nm for both excitation and emission. The mobile phase consisted of a mixture of methanol/water in the ratio 80/20 and was constantly degassed by a small flow of helium through the solvent container solution. A flow rate of 1.3 ml/min was used for all experiments. In order to prevent boiling of the HPLC eluent in the catalytic column, a pressure drop downstream from the fluorescence detector was caused by an HPLC capillary (about 1.5 meter long) and a pressure pulse damper. The HPLC was operated in a recycling mode, i.e. the eluent was led back to the solvent reservoir, thus saving considerable amounts of solvent. Wavelengths used for detection are given in Table 9 of Chapter 6.3.
No special solvent reductive column was placed before the sample injection valve. A solvent reduction column filled with automotive catalyst reduced quenching of PAH fluorescence signals presumably due to removal of dissolved oxygen (83). In this project, however, no significant difference in peak response could even be observed between degassed and non-degassed HPLC solvent. Thus no attempt was made to pack an additional solvent reduction column.

No pH modification of the HPLC solvent were performed because
* typical detection limits of this method could be obtained without changing the pH value: 5 pg of 1-nitropyrene per injection with this procedure versus 3 pg according to (83).
* a mobile phase of pH 8 as described in (83) is likely to cause some column deterioration over time.

5.2.2.4 Determination of Variability between Injections

The variability of injection during HPLC analysis was checked by calculating standard deviation and relative deviation of peak heights obtained from the same compound in the same sample during a set of injections. Table 6 lists typical results:
Table 6: Consistency of peak heights in a set of injections.

As can be seen, the relative deviation varied considerably. Therefore several injections were required to average out this random variability, which was most likely due to a technical problem of the injector: a small leak between the syringe needle and a Teflon seal.

An internal standard was not used because:

* an internal standard would have to have very similar properties to the nitro-PAH under study if it were to be used prior to fractionation. It also would have to be fluorescence active.
* an internal standard should not be present in a real sample of a field study.

To find such a compound would be very difficult.

Even if the internal standard were to be used after fractionation, its retention characteristics would have to be such that it would not interfere with the peaks of nitro-PAH and also would not extend the total run time. Initially the technical problem in the injector was not expected to persist so that the experiments were performed without using an internal standard, avoiding the extra work of
finding such a substance. However, it should be noted that the use of an internal standard would considerably reduce injection variability and time required for the analysis of a sample, since only 1–2 injections would be necessary. It is therefore recommended that future potential users of the method described in this project find an appropriate substance as internal standard.

5.2.2.5 Determination of the Stability of Peak Height Ratios

The stability of peak height ratios between several chromatograms of the same sample was studied in order to improve peak height analysis of chromatograms that include 7 or less of the 9 nitro-PAH under study. The following experiment was conducted:

In each of the chromatograms of eight injections of a standard solution, the peak height ratios relative to 1-nitropyrene were determined. The ratios of each nitro-PAH over the eight injections were averaged and the standard deviation and relative deviation were calculated. The relative deviation was defined as

\[
\text{Rel. dev.} = \frac{(\text{Std. dev.}) \times 100}{(\text{average})}.
\]

The relative deviation of the nine compounds ranged from 1.15%–5.8% with an average of 3.35%. Therefore the average error that one would expect to occur after calculating the expected peak heights
versus measuring the true peak heights would be about 3.35% of the true peak height. This error appeared to be acceptable.

5.2.2.6 Analysis of Chromatograms

The peak heights of standards and samples were measured and standardized mathematically to the same detector conditions. Concentrations in the sample were determined by comparison with a standard analyzed before and after the sample injection series. In this way, an analysis of a single sample required up to about 12 injections. If several samples were to be analyzed, a sequence of "standard - sample - standard - sample" was used, thus requiring about 8 injections per sample analysis. No carry over between standards and blanks was observed if the precautions prior to an injection were followed that are described in Chapter 5.2.2.3.

Since in a sample analysis the peak height ratios of different nitro-PAH in a chromatogram remained almost constant between several injections, it was also possible to calculate the expected peak height of a compound in an injection where that particular compound was not directly determined. Due to differences between injections, the absolute peak heights showed significant variability (about 15%) from one injection to another. However, the ratio of peak heights within each injection did not change much. Using one of the nine nitro-PAH as a reference, this
phenomenon was used for calculating expected peak heights of components that were not directly measured in one or two of a set of injections. Obviously, these ratios were different in each sample and needed to be calculated within each set of injections of a sample or standard. An example in Table 7 shows the procedure:

<table>
<thead>
<tr>
<th>Injection</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>19.1</td>
<td>20.4</td>
<td>15.0</td>
<td>unknown</td>
<td>18.0</td>
</tr>
<tr>
<td>B</td>
<td>3.9</td>
<td>4.0</td>
<td>3.0</td>
<td>3.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Ratio A/B</td>
<td>4.9</td>
<td>5.1</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Average ratio = \((4.9 + 5.1 + 5.0) / 3 = 5.0\)

Expected peak height of compound A in injection 4 = \(3.5 \times 5.0 = 17.5\)

Table 7: Example of calculation of expected peak heights

5.2.2.7 Determination of Detection Limits

The detection limit is an important factor in the evaluation of an analytical system because of its potential application in the analysis of trace concentrations of organic compounds. The peak height of HPLC fluorescence detectors depends mainly on two parameters: the compound specific fluorescence spectra and
technical features of the fluorescence detector such as cell volume and geometry, intensity of the light beam from the lamp, sensitivity of the actual detector and whether a monochromator or a filter is used for wavelength selection. Detection limits for fluorescence detection are therefore principally also a function of the apparatus used.

The detection limit was determined using the chromatographic system and method as described in Chapter 5.2.2.3. Standards were prepared by diluting aliquots of the NBS standard solution with methanol. The detection limit was defined as being a peak of 3 times the noise width. As an example, Figure 5 shows the detection limit of 2-nitrofluorene. The detection limits are listed in Table 8.

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>pg/injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitrofluorene</td>
<td>7.6</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>54</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>5</td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>74</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>40</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>90</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 8: Detection limits of nitro-PAH (injection volume 0.01 ml, in methanol, temperature of catalyst column 135 °C)
Figure 5: 7.64 pg 2-nitrofluorene in 10 microliters of methanol,
diluted from NBS standard

As can be seen, there are significant differences between the
various detection limits, which range from 5 to 90 pg per injection
of 0.01 ml. These values are in the same range as values published
in the literature (24), using zinc as catalyst material.
5.2.2.8 Conclusions

The platinum – rhodium method for HPLC analysis of nitro-PAH was successfully implemented for this work. The detection limit obtained with this system appeared to be adequate. However, several difficulties are associated with this technique:
- packing of the catalyst column is time consuming;
- catalyst material needs to be heated well above the boiling points of the HPLC solvents which requires a sufficient pressure drop downstream the catalyst column;
- if the use of an internal standard were to be desired a compound would have to be found for this purpose that is fluorescent, does not interfere with the compounds of interest and is stable at the high temperature of the catalyst column.

In general, once the equipment is set up correctly this technique is reliable and allows sensitive analysis of nitro-PAH.
Development of a Method for Testing the Identity between Sample and Standard Compounds during HPLC Fluorescence Analysis

6. 1 General Considerations

Organic extracts of airborne particulate matter and semivolatile components are usually very complex mixtures containing up to hundreds of substances. Even though a fractionation prior to analysis will usually help prevent interferences from other compounds, there is no guarantee of an "easy" analysis. Interference might be due to other nitro-PAH than the ones of interest or due to compounds of different chemical nature. The identity of the observed peak with the one obtained by a known standard needs to be established.

In general, it is common practice to prove the identity of a sample compound with a known standard by two different analytical procedures that use different compound characteristics for analysis. Since it is very time consuming to implement two instrumental analytical methods for nitro-PAH analysis (such as HPLC with fluorescence detection and gas chromatography with nitrogen sensitive detection), a more convenient way of peak confirmation was examined:
Many organic compounds are not fluorescence at all. Those which are have unique excitation and emission spectra. It is therefore extremely unlikely to find two compounds that show the same change in fluorescence light after a change in the combination of excitation and emission wavelengths.

It was therefore assumed that the identity of a sample and a reference compound is proven if all three of the following criteria are met:

1) Both compounds are collected in the nitro-PAH fraction during the fractionation step.

2) Both compounds have the same retention time on the analytical HPLC.

3) Both compounds show the same response ratio if the emission wavelength used for detection is changed from the optimum to a non-optimum wavelength.

If conditions one and two were met, both compounds would have at least very similar polarities and chemical structures because they would have the same distribution coefficient between solid and liquid phases in two different chromatographic systems. Condition three would require an identity of the fluorescence spectra of the two compounds. It appears to be almost impossible that all three
criteria can be met by two different compounds. The main advantage of this approach is its

* convenience: no additional analytical method needs to be implemented.

* sensitivity: the most sensitive fluorescence detector can be used.

The main disadvantage is of conceptual nature: the identity of two compounds is only concluded, not directly measured (as it would be possible using mass spectroscopy). However, in most cases an identification by these criteria would be considered sufficient.

6.2 Experimental Methods

In order to apply the concept developed in the previous chapter it was necessary to determine the wavelength combinations that were to be used other than the optimal one used for first detection.

It was decided always to use the optimal excitation wavelength of the specific nitro-PAH of interest, but to change the emission wavelength both to a shorter and to a longer wavelength.
Several emission wavelengths of each compound were tried (+15 nm, +30 nm, +40 nm, +60 nm and -15 nm and -30 nm) and the corresponding peak responses were measured.

6.3 Results

Tables 9 and 10 list the wavelength best suitable for identification purposes along with the corresponding peak height ratios.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>optimal wavelength</th>
<th>sub-optimal, longer wavelengths</th>
<th>Ratio of peak heights, opt/i sub.- opt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>exit.</td>
<td>em.</td>
<td>exit.</td>
</tr>
<tr>
<td>2-Nitronaphthalene</td>
<td>234</td>
<td>403</td>
<td>234</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>260</td>
<td>495</td>
<td>260</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>360</td>
<td>430</td>
<td>360</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>300</td>
<td>475</td>
<td>300</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>273</td>
<td>437</td>
<td>273</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>420</td>
<td>495</td>
<td>420</td>
</tr>
</tbody>
</table>

Table 9: Sub-optimal, longer wavelengths and peak height ratios

The sub-optimal wavelengths were selected such that a peak height ratio of approximately 1.5 was accomplished. This ratio was sufficiently big to be noticeable during peak evaluation, but not too big to prevent a sensitive detection of the compound of interest.
Table 10: Suboptimal, shorter wavelengths and peak height ratios

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sub-optimal, shorter wavelengths</th>
<th>Peak height ratios, (opt.)/(sub.-opt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>234  388</td>
<td>1.97</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>285  355</td>
<td>2.01</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>263  490</td>
<td>1.33</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>260  480</td>
<td>1.65</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>360  415</td>
<td>1.95</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>300  460</td>
<td>1.76</td>
</tr>
<tr>
<td>16-Nitrochrysene</td>
<td>273  422</td>
<td>1.92</td>
</tr>
</tbody>
</table>

Table 10: Suboptimal, shorter wavelengths and peak height ratios

6.4 Application Using a Field Sample from a Carbon Plant - Identification of 1-Nitropyrene

The concept outlined above was applied in the analysis of a filter sample from a conveyor station of a carbon plant where coal tar pitch volatiles were measured. The sample was taken by plant personnel, extracted with benzene and mailed to our laboratory.

After fractionation and analysis, two of the nine examined nitro-PAH were suspected to be present in the sample: 9-nitroanthracene and 1-nitropyrene because of practically identical retention times with those of the standards. The sample was analyzed at optimal and suboptimal emission wavelengths. Table 11 shows the results.
Table 11: Peak height ratios of compounds in a carbon plant air filter sample

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Observed ratio at shorter wavelengths</th>
<th>Observed ratio at higher wavelengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-Nitroanthracene</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>1.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

As can be seen, 9-nitroanthracene could not be confirmed because instead of a decreasing response (expected for this compound) the response actually increased about threefold.

However, 1-nitropyrene was confirmed because the peak height ratios at the shorter wavelength were identical for sample and standard. Peak identity was concluded although the ratio at the longer wavelength is different from the one obtained from the standard. This difference can be explained by loss of solvent due to evaporation during the last hour of analysis. Since the peak heights showed considerable variability, many injections (4-6) were necessary to obtain reliable average values. Moreover, several injections of this sample had been performed several weeks prior to this experiment. Therefore, not much sample solution was left for the last detection series at the longer wavelength. Since a small solvent volume is subject to a large evaporation error, the smaller ratio was probably caused by a concentration of the sample due to evaporation of solvent during the last part of the sample analysis.
Technical problems of the HPLC injector caused peak height variations between injections. Therefore, the attempt to confirm the peak identity of these two potential nitro-PAH in a filter sample extract resulted in a rather time consuming procedure. However, this approach appears in theory to be an elegant way to solve the problem of peak identity confirmation. Ideally, for a HPLC system in perfect working condition this procedure would require only two additional injections to the initial one at optimal detector settings, thus providing confirmed identification and quantification of nitro-PAH in less than an hour. However, this procedure might require several hours per sample if any one of the HPLC system components shows some variability.
Fractionation of Crude Extracts by Preparative High Performance Liquid Chromatography

7.1 General Considerations Regarding the Chemical Composition of Organic Extracts from Airborne Samples

Since samples from airborne particulate matter and vapor usually contain a large number of different compounds, a fractionation step prior to analysis of individual compounds is necessary to prevent interference during subsequent analysis.

Several fractionation methods have been described, including fractionation according to the acidity of the compounds into acids, neutrals and bases (73) and according to the polarity (39).

It has been found that the largest portion (about 80%) of the total mass of organic compounds adsorbed on ambient particles from Berlin, West Germany, was found in the neutral fraction of the extract (73). Since nitro-PAH are chemically neutral, a separation into acids, neutrals and bases would probably not be very effective. They would still be included in the fraction with the highest mass.
On the other hand it has been found that a great portion of organics (about 44% of the total organic mass) adsorbed on urban particulate matter is of rather non-polar nature, such as aliphatics and unsubstituted PAH (73). However, most of the organic mass on wood smoke particles are of rather polar nature and includes more oxygenated aromatics such as phenols or quinones (9).

Compounds of medium polarity such as most nitro-PAH do not appear to be present in high proportion in extracts of wood smoke particles or in ambient air. Therefore, a chromatographic approach for separation was selected.

7.2 Description of the Fractionation System Used

An HPLC fractionation system was already developed, characterized and in use in the laboratory. That system had been used for fractionation in a number of studies (5, 39, 38, 37, 40) and is identical with the one used for this project. It consisted mainly of a Tevnary Spectra Physics 8700 high pressure liquid chromatograph and a water semiprep M Porasil (300 x 7.8 mm ID) column. Different solvent gradient programs were used, all including n-hexane, methylene chloride (MeCl2) and acetonitrile (ACN).
Table 12: Fractionation program used by laboratory co-workers

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>% Hexane</th>
<th>% MeCl₂</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>31</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>67</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>69</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>79</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>81</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>97</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

For research performed by other laboratory co-workers during this project, a 97 minute program was used which is presented in Table 12. This program was developed by Professor Richard Kamens for fractionation and collection of non-polar and polar aromatics, and provided a good resolution of even the most polar compounds.

However, since it was not necessary for this project to investigate the most polar fraction, the program was shortened.

7.3 Modification Experiments - Results and Discussion

It was hypothesized that a quick ramp of the solvent program to 100% acetonitrile, which elutes polar compounds most effectively
after elution of the nitro-PAH fraction, would elute the remaining polar compounds quickly from the column.

Table 13 shows the gradient program that was tried first. The chromatogram obtained from a standard solution containing several unsubstituted PAH, nitro-PAH and oxygenated PAH showed poor resolution and broad peaks. Also, it was not directly compatible with the program of Table 12 which was used routinely by other co-workers in the laboratory. To return the system to its original conditions a full blank run was required between the two programs in order to equilibrate the column. Therefore a net savings of time was lost.

These disappointing results may be partially explained by two factors:

1) Normal phase column packing material is usually rather sensitive to changes in the polarity of the mobile phase, especially if these changes occur rather rapidly or include a big change in the polarity of the solvent composition. It takes time to equilibrate HPLC columns packed with this material.

2) Acetonitrile and hexane are immiscible. This program probably does not provide a long enough time to completely elute the acetonitrile from the column before hexane is delivered.
Table 13: First fractionation program used, flow rate 2 ml/min.

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>% Hexane</th>
<th>% MeCl2</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>31</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>33</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>44</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>47</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 14: Final fractionation program used, flow rate 2 ml/min.

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>% Hexane</th>
<th>% MeCl2</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>31</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>33</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>53</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>55</td>
<td>0</td>
<td>100</td>
<td>0</td>
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<tr>
<td>65</td>
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<td>100</td>
<td>0</td>
</tr>
<tr>
<td>67</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>83</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

The next program tested did not change solvents as rapidly as the first one and especially allowed the acetonitrile in the column to be completely eluted before hexane was delivered. Also, some time was spent to equilibrate the column under initial conditions. Table 14 shows the program used (flow rate: 2 ml/min). The chromatograms obtained from standard injections showed a good
resolution of the nitro-PAH. The program was compatible with the one used by co-workers. The method was capable of completely eluting the polar compounds of sample extracts of later experiments; no "carry over" of polar compounds from a previous injection to the next one was observed. The program was still 14 minutes shorter than the one used otherwise in the lab and thus saved time and solvents. As an example, Figure 6 shows a chromatogram obtained by injection of a solution of 7 nitro-PAH: 2-nitrofluorene, 9-nitroanthracene, 3-nitrofluoranthene, 1-nitropyrene, 7-nitrobenz[a]anthracene, 6-nitrochrysene and 6-nitrobenzo[a]pyrene.

It was observed that the injection volume affected the peak width of the nitro-PAH. The peak shape is important for accurately determining the beginning and end of the desired fraction. In order to have the same injection conditions for standards and samples, both were injected in a volume of 0.25 ml.

7.4 Conclusion

The selected fractionation program showed encouraging results in terms of run time and separation of nitro-PAH into a relatively small fraction. Therefore this program was used for all further experiments that required fractionation.
Figure 6: UV-chromatogram of a nitro-PAH standard using the selected fractionation procedure and solvent program.
8. Determination of Losses during and after Fractionation

In order to determine where most of the losses occur, the sample handling procedure during and after the fractionation procedure was studied.

8.1 Experimental Methods

Two standard solutions of 2% methanol and 98% methylene chloride containing the nitro-PAH given in Table 15 were fractionated according to the procedure described in Chapter 7.3 and analyzed using an unfractionated nitro-PAH standard as reference.

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>ng/0.25 ml injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>35.6</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>38.2</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>19.8</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>ca.30</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>35.35</td>
</tr>
<tr>
<td>3-Nitrofluoranethene</td>
<td>36.5</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>36.6</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>32.1</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>24.0</td>
</tr>
</tbody>
</table>

Table 15: Nitro-PAH injected in fractionation HPLC for recovery experiments
8.2 Results and Discussion

The recoveries (percent) are given in Table 16. The data revealed good recoveries of about 93% with only little variation (range: 88-98%). Therefore the fractionation procedure caused only few losses of about 7%. This appeared to be acceptable.

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>% recovery</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>88</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>89</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>89</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>92</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>96</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>97</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>94</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>92</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>95</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

Table 16: Recoveries of nitro-PAH (percent) after fractionation and subsequent sample handling

8.3 Determination of Losses by a Short Concentration to Dryness for Solvent Exchange Purposes

During the sample preparation procedure there are several concentration steps. All required that the sample is not allowed to become "dry" because usually losses of semivolatile compounds tend to occur especially in cases when such compounds are no longer
protected by the solvent from a temperature raise above the solvent's boiling point and from oxidants in the gas phase.

Since for solvent exchange purposes it was necessary to evaporate once to dryness for a short time, and because there is always a chance of involuntarily blowing off the solvent completely during nitrogen stream concentration, it was felt to be important to know the losses that occur during this procedure.

8.3.1  Experimental Methods

Two standard solution were prepared, all containing the same concentrations of the nine nitro-PAH in 0.25 ml. In one standard, the compounds were dissolved in methanol and the vial was immediately stored at -60 °C as reference for subsequent analysis of this experiment.

The other two vials contained the same masses as the reference but were dissolved in methylene chloride. They were blown down to dryness using the same procedure as described in Chapter 11. (See: On the day of analysis...).
8.3.2 Results and Discussion

The recoveries were very good, on the average 99%, with a range of 97-100%. It appears that a short concentration to dryness does not significantly contribute to the losses of the overall procedure as described in Chapter 11. Table 17 shows the results.

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>2-Nitronaphthalene</td>
<td>97</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>98</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>99</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>99</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>100</td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>100</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>100</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>100</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 17: Recoveries of nitro-PAH (percent) after a short concentration to dryness
Filter Selection for Sampling of Nitro-PAH Adsorbed on Airborne Particulate Matter

9.1 General Considerations

The traditional procedures for collection of PAH in work-room air have centered on filtration. These filters (usually glass fiber) were usually Soxhlet extracted with hot benzene, and the resulting weight loss was indicated as "benzene soluble matter" (BSM). In 1967 it was suggested that silver membrane filters should be used instead of glass fiber because of better weight stability. The silver membrane filter, however, tended to plug easily (6). Accordingly, a glass fiber filter was placed in front of the silver membrane. This filter arrangement is at present time the standard system for monitoring coal tar pitch volatiles, as described by OSHA's 1976 Standard for Coke Oven Emissions. However, glass fiber filters are still widely used and are furnished by EPA for use by air pollution regulatory agencies including comprehensive specifications for various materials (54).

Liquid absorbers have been used to back up filter systems, but they are not suited for personal sampling (6). For this purpose, solid sorbents such as Tenax GC, XAD-2 and Chromosorb 102 are preferable and commonly used (77, 36). Moreover, impinger methods have proven not to be as efficient as adsorbent sampler methods (36). However,
very high relative humidity (>80%) can cause some loss of adsorption efficiency during collection of organic air pollutants by silica gel, activated charcoal and Tenax GC (54).

Porous polymers, such as Tenax GC are efficient collectors, but sometimes do not permit a sufficiently fast air flow through a high volume samplers (84). For the same reason absorption bottles containing organic solvents cannot be used for samples with low concentrations (13). An alternative is polyurethane foam (PUF) as adsorbent. Both PUF and - in some sampling systems - granular sorbents can be employed for sampling air at flow rates of 200 - 250 L/min. (48).

Although activated charcoal is an excellent sampling sorbent for many air pollution monitoring applications (54) it can not be used for sampling PAH. This type of material quantitatively retains only PAH-compounds less volatile than fluoranthene (4) but retains them so strongly that extractions with organic solvents yield only poor recoveries (69).

Reviews of the different methods of monitoring organic constituents in air have been published (54); more specific methods for nitrated and oxygenated PAH are also available (88, 19).
9.2 Sampling of PAH with Different Filter Systems

This chapter will present more specific considerations important for the selection of a filter system for nitro-PAH measurements in air.

Nitro-PAH may be produced as artifacts during sampling by chemical transformation of PAH by air pollutants and oxidants adsorbed on particles or present in the vapor phase (88).

The heterogeneous reactions between
* substrate-adsorbed and filter-deposited PAH and
* pollutant gases
are a complex function of parameters such as the nature of the substrate, the PAH concentration, the gaseous pollutant level, the exposure time, etc. (15, 43, 68). "Losses" of PAH are an indication of these reactions.

Losses of PAH during sampling are very common and can be severe: It was found that up to 99% of the lower molecular weight PAH such as phenanthrene and anthracene are not retained by quartz fiber filters (21).

Two loss processes, namely volatilization and chemical reactions, may be of importance when sampling airborne PAH and when evaluating the occurrence of nitro-PAH on glass filters:
1) Vapor pressure and adsorption considerations indicate that at least for the lower molecular weight PAH, there is a measurable temperature-dependent partition between gaseous and particulate phases. This partition may contribute to PAH losses by volatilization during filter sampling when the gas phase concentration of the PAH of interest is lower than the equilibrium concentration (29). Simultaneously, there is a pressure drop behind the filter during high volume sampling so that the more volatile compounds may be lost into the gas phase (blow off) (4). The incomplete recovery of PAH from highly adsorptive substrates such as ambient particulate matter, soot, fly ash etc. when using solvent extraction is an additional reason for losses of PAH (30).

2) Filter deposited PAH may be depleted by a number of photochemical and thermal reactions, including PAH reactions with major pollutant gases such as O₃, NO₂, and SO₂. It has been shown in a number of laboratory studies that PAH and gaseous pollutants interact readily on glass filters to form a variety of so-called "filter artifacts" (29). For example, it has been reported that gaseous oxidants such as sulfur oxides and nitrogen oxides may be converted to sulfate and nitrate on the glass fiber filter surface. It is possible that these converted oxides in turn cause the oxidation of PAH (45). These interactions are not completely understood but probably depend on a variety of parameters, some of which are discussed below.
The accessibility of reactive gas molecules to finely divided PAH over a particle surface depends on specific surface area, and particle size and shape. While these physical parameters are more or less constant for glass fiber filters, they can vary considerably for reactions on particles. These reactions of particle adsorbed PAH may be a result of gas-solid and liquid-solid reactions, or a combination of both (69). The physical and chemical nature of the surfaces onto which the PAH are adsorbed can result in large variations in the reactivities of PAH towards oxidation (45). The hypothesis has been suggested that air oxidation of PAH requires an active surface, and thus can be minimized when an inert surface such as Teflon is used for collection (45). Active surface properties may include several complex and interrelated factors such as catalysis, specific surface area, affinity for water and other vapors, surface pH, or surface polar functional groups. Although the mechanisms are not understood, it is interesting to note that in parallel sampling experiments, the observed order of increasing BaP recovery (Teflon membrane filters (Fluopore, Zefluor) > Teflon impregnated glass fiber filter (T60A20) > glass fiber (Gelman A) or silica fiber (2500 QAO) filters) is in accordance with the order of decreasing specific surface area of these five commonly used filters (45). Other results in the same study suggest that the surface area or some commensurate surface properties such as number of reaction sites (eg.: the ratio (amount of BaP/filter surface area)), rather than the volume of air exposure, governs the extent of BaP degradation.
In another study (29) levels of ambient particulate PAH have been measured using quartz, glass and Teflon filters operating in parallel at the same sampling velocities. On the average, PAH concentrations measured on glass and quartz filters were lower in 85 - 90% of all observations than those measured in samples collected on Teflon filters. Glass/Teflon (G/T) and quartz/Teflon (Q/T) ratios ranged from 0.25 for pyrene to 0.8 for coronene. These ratios exhibit distinct seasonal and diurnal variations. For most PAH, G/T and Q/T ratios were lower during the summer than during the winter.

However, there are also studies that contradict the hypothesis that the type of filter is an important factor for the chemical integrity of the sample. One of the most comprehensive studies is presented below (31).

Three PAH (BaP, perylene, 1-nitropyrene) deposited on - glass fiber (Gelman A/E) and - Teflon filters in quantities of 0.05, 0.25, 4.1 ug/cm², respectively as well as on - glass and Teflon filters loaded with - fly ash, - diesel exhaust and - ambient particles were exposed in the dark to - pure humid air, - particle-free ambient air
- 100 ppb ozone,
- 100 ppb SO₂ and
- 100 ppb nitric acid-free NO₂

for three hours at sampling face velocities equivalent to those typical of high volume sampling. Under these conditions, there was no evidence for chemical or physical transformation of the PAH irrespective of filter type, carrier particle and pollutant gases. Also, no new peaks indicative of reaction products were recorded in either UV or fluorescence chromatograms. Particularly, 1-nitropyrene was found to be resistant to oxidation, further nitration, or other chemical reaction under the conditions used. It was strongly suggested that nitric acid, rather than NO₂, should be considered in studies of PAH-nitration (31).

In the absence of NO₂ or HNO₃, pyrene and BaP undergo photochemical decomposition at a detectable rate when adsorbed on some substrates (some fly ashes, silica, alumina) but not on others (some fly ashes). There is no evidence that NO₂ accelerates these photoreactions. These photoreactions seem to be a significant process only if nitric acid, or perhaps strong oxidants such as ozone, are also present (91).
9.3 Conclusions

The degree to which the type of filter is of significance in terms of catalyzing artifact formation during PAH sampling is still not known. However, in order to be on the safe side and to prevent potentially occurring nitro-PAH formation on the filter surface, Teflon or Teflon coated glass fiber filters appear to be the filter matrix of choice. Teflon coated glass fiber filters were available in the laboratory and were used for all experiments of this project.

9.4 Filter Extraction Procedure

The filter was placed on a petri dish of 14 cm ID and extracted four times according to the following procedure:

1. 50 ml methylene chloride were added to give a layer of solvent about 2 - 3 mm thick. The petri dish was capped by another one to prevent excess solvent evaporation. The petri dish was shaken mechanically at about 100 revolutions per minute on a standard laboratory shaker for about 12 minutes. Afterwards the solvent was decanted into a round bottom flask for future handling.
10. Comparison of Two Adsorbent Media for Sampling Nitro-PAH Vapors

10.1 XAD-2 Resin

10.1.1 General Considerations

The XAD series of resins are undoubtedly the most widely used solid phases for the extraction of organic compounds from aqueous media, but also many applications in air sampling have been described (6, 28). The XAD resins are generally available as four different materials, XAD-2, -4, -7 and -8. The basis for sampling with XAD resins is adsorption on the surface; no pore exclusion mechanisms are involved (28). XAD-2 is a polymer (polystyrene) and is essentially nonpolar. It has been evaluated for air sampling of polychlorinated biphenyls and naphthalenes, for polychlorphenols in workroom air (7) as well as for the sampling of unsubstituted PAH in several industries (6).

There are several good reasons for these applications of XAD-2 in air sampling:

The recoveries of these compounds were approximately 80 % or
higher, showing this adsorbent material to be an excellent medium for sampling aromatics with or without polar adducts. Moreover, after adsorption of unsubstituted PAH on XAD-2 the reactivity of these PAH with an reactive gas mixture (diesel exhaust) has been shown to be greatly reduced, suggesting a protection effect against sampling artifacts. Similarly the chemical conversion of PAH to nitro-PAH has been only a minor problem during dilution tube sampling of vehicle emissions using Teflon filters and XAD-2 resin with short sampling times (46 min.) and low temperature (42 °C) (77).

However, XAD-2 resin also has some disadvantages that have limited its use:

1) It undergoes some reactions with exhaust gases to form quinones (77). Therefore it might not be suitable for studies in which quinones are not separated from the compounds of interest during sample cleanup.

2) Methylene chloride extracts of XAD-2 exposed to about 100 ppm of NO or NO₂ were mutagenic and precluded the use of this material for the detection and bioassay of vapor-phase organics in effluent streams. Several of the decomposition products were identified (32). However no degradation upon SO₃-exposure occurred on XAD-2 (53).

3) Long Soxhlet extraction times using methylene chloride resulted in high background spectra in GC/MS analysis, owing to extraction of organics inherent in the resin itself or to resin breakdown.
However, this problem could be solved by two 0.5 hour Soxhlet extractions with methylene chloride (20).

4) The total volume of air filtered through the XAD-2 during sampling affects the percentage of recovery. Smaller total volumes of air samples were found to improve recovery (20).

From this review it can be expected that XAD-2 probably is an effective adsorbent for nitrated aromatics in air if a few limitations are considered:
- no long-term sampling,
- no sampling at high NO or NO$_2$ levels,
- no long Soxhlet extraction with methylene chloride.

10.1.2 Implementation Experiments - Results and Discussion

The XAD-2 resin beads were Soxhlet extracted prior to first use. A series of solvents was used for extraction:

- methanol: 48 hours
- acetonitrile: 50 hours
- hexane: 43 hours
- methanol: 55 hours

The precleaned resin was stored in methanol in Teflon sealed glass jars as already described in the literature (12).
Next, four 16-hour Soxhlet extractions of the precleaned resin were performed in parallel using methanol in order to determine whether blank values suitable for further experiments could be obtained. After this procedure, the sample solution was concentrated to about 5 ml in a rotary evaporator with a water bath temperature of 90 °C, using no vacuum.

The samples were fractionated and analyzed by HPLC according to the procedures described in chapters 7.3 and 11.

The chromatograms of the analytical HPLC were not "clean". At the retention times of the first three eluting nitro-PAH impurities would have yielded interference with the analysis of the corresponding nitroaromatics. 2-Nitronaphthalene would have been confounded in all four samples, 9-Nitroanthracene in three cases and 2-nitrofluorene in one of the four samples. The biggest peak was observed at a retention time typical for 2-nitronaphthalene. No peaks were recorded at retention times of the other six nitro-PAH of interest.

Even though three compounds were confounded by impurities, a recovery experiment was performed in order to find out whether this material should be used for further experiments of this project.

A 0.05 ml injection containing a standard of about 35 ng of each of the nine nitro-PAH was injected into 650 ml XAD-2 resin already
placed into two Soxhlet extractors. Methanol was used for the 16-hour extractions. The remaining sample handling procedure was the same as indicated above. The results are shown in Table 18.

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>confounded</td>
<td>confounded</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>20.3</td>
<td>confounded</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>confounded</td>
<td>71.3</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>18.2</td>
<td>46.6</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>79.3</td>
<td>61.4</td>
</tr>
<tr>
<td>3-Nitrofluoranthenene</td>
<td>42.9</td>
<td>53.6</td>
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<td>7-Nitrobenz[a]anthracene</td>
<td>46.0</td>
<td>30.0</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>65.3</td>
<td>41.7</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>81.1</td>
<td>24.8</td>
</tr>
</tbody>
</table>

Table 18: Recoveries of nitro-PAH (percent) from XAD-2 resin

Poor recoveries ranging between 18.2 and 79.3% were obtained. The data showed a large variability from compound to compound and also between the two samples. The first 3 nitro-PAH in Table 18 were difficult or impossible to analyze due to impurities of the resin.

Reasons for these low recoveries might be:

--- a high specific adsorptivity of the resin for nitro-PAH.

--- major losses of nitro-PAH during concentration at 90 °C in the rotary evaporator.

--- breakdown phenomena of the resin during extraction.

Of these possible explanations, only the concentration temperature appeared to be a parameter that could be changed in a controlled
manner. However, methanol and methylene chloride are the two solvents most often used for nitro-PAH extraction (88). Methylene chloride offers a much lower boiling point than methanol (40.1 °C versus 64.5 °C) but unfortunately causes even further XAD-2 breakdown as described in (20). Therefore a solvent change from methanol to methylene chloride did not appear to be a promising alternative.

10.1.3 Conclusion

In view of these results and considerations it was decided not to investigate any further the appropriateness of XAD-2 resin for nitro-PAH sampling. Instead, polyurethane foam plugs were tested for their potential use in collection of nitro-PAH in air.
Polyurethane foam (PUF) can be used for trapping contaminants in air and water samples. However, the applications of this material described in the literature have been limited —
- to air samples (only a few researchers used it for water samples); and
- to mostly chlorinated pesticides, polychlorinated biphenyls or naphthalenes and other chlorinated hydrocarbons (84, 63, 49, 21).

However, within these groups of compounds, PUF is adequate only for those substances that are either not very volatile or are rather polar (49).

A relatively high polarity of a compound increases its collection efficiency on polyurethane foam. Therefore the greater polarity of some pesticides (organo-phosphates) assists in their collection, so that acceptable trapping efficiencies (about 75 %) are feasible for many of these compounds despite their higher vapor pressure (49).

Not many applications can be found for less polar organic material. Only a few studies used polyurethane foam for unsubstituted PAH (4, 84, 90). Polyurethane foam has been proved to be unsuited for most
volatile PAH, such as naphthalenes and biphenyls (4), whereas 3–5 ring PAH in the vapor phase can be efficiently trapped (90).

**Advantages** of polyurethane foam over resin based adsorbents are:
- a large surface area due to the myriad of small passages available to the air flow;
- low costs;
- low flow resistance; and
- reasonable blank values.

Corresponding **disadvantages** of PUF are:
- poor collection efficiency of non-polar, two-ring compounds; and
- PUF is not a well defined material (see below). Therefore published blank values and collection efficiencies show some variability.

Potential reasons for the last disadvantage might be the following:

1) PUF can be produced by the reaction of toluene diisocyanate (a mixture of 2,4-TDI and 2,6-TDI) with a variety of polyols, which determine the characteristics of the polyurethane foam generated. Therefore the composition of PUF from different suppliers may vary (63).

2) The available surface area of PUF is probably also related to its density which typically ranges from 20–25 kg/m³ (48, 84, 63).
The density might affect the amount of available surface area and flow resistance.

PUF foam was selected for further evaluation of its sampling characteristics for nitro-PAH because:

- PUF plugs have already been used for qualitative nitro-PAH sampling in industry (64),
- PUF plugs' good trapping efficiency for polar pesticides may correspond to a sufficient collection efficiency for moderately polar Nitro-PAH,
- the advantages described above suggested it to be a promising alternative to resin-based adsorbents.

In this report PUF (product no. 3014) from Olympic Products Co. in Greensboro, NC, was selected because foam from this supplier has already successfully been applied for air sampling of different compounds in several other studies (18, 48, 47, 80, 14, 11).

10.2.2 Precleaning of PUF-Plugs

PUF needs to be carefully cleaned prior to first use because it contains contaminants that would otherwise interfere with the analysis of the compounds of interest. After cleaning the plugs can be used several times (84).
Many different procedures for precleaning have been described in the literature. Most of them include separate Soxhlet-extraction with either
- two non-aromatic solvents of different polarity such as
  acetone - cyclohexane (90, 21), or
  acetone - n-hexane (49, 93);
- or a single extraction by a non-aromatic solvent mixture such as
  diethylether in n-hexane (47).
Tetrahydrofurane is not appropriate for PUF extraction because of permanent structural changes of the material causing loss of flexibility (51).

There are also some studies that use only a single aromatic solvent for preextraction, either
  benzene (51) or
  toluene (18);
or they use toluene at 100 °C to squeeze the PUF-plugs prior to Soxhlet extraction in either
  acetone and n-hexane (63); or in
  cyclohexane (84).

Although most of the studies used Soxhlet extraction there is also one study showing that Soxhlet-extraction with acetone and petroleummether and even with toluene might not be effective in removing electron-capturing contaminants from PUF (18). However,
background contaminations could be reduced to acceptable levels by five 10 min. extractions with toluene at 100 °C by squeezing of the plug in a beaker using the bottom of an Erlenmeyer flask (18).

For this project the squeezing procedure (18) was selected and applied because
- this procedure has been found to be optimal for removal of electron-capturing contaminants from PUF. These contaminants include a polar part in their molecular structure that might determine similar chromatographic characteristics to those of the Nitro-PAH. Therefore these contaminants may potentially interfere with the HPLC determination of nitro-PAH,
- this procedure is much faster and more efficient than Soxhlet extraction (18),
- it uses less expensive glassware than Soxhlet extraction,
- it is easier to perform for big PUF-plugs.

10.2.3 Selected Procedure for PUF Plug Extraction

One plug was placed in a 1 liter beaker, and 300 ml of methylene chloride were added. For four minutes the plug was repeatedly squeezed using the bottom of a measuring cylinder, then turned over and squeezed again for another four minutes. The methylene chloride was then decanted into a round bottom flask with the plug in place.
Much of the solvent still remaining in the plug was recovered by pressing the plug with the measuring cylinder bottom during decanting. While the volume of the methylene chloride solution was reduced in a rotary evaporator at 57 °C, the extraction procedure was repeated two more times. However, now only 200 ml methylene chloride were added each time and — after extraction — decanted into the round bottom flask of the first extraction. The volume was reduced in the rotary evaporator from about 585 ml to about 5 - 10 ml, transferred into sample vials and further concentrated using a stream of nitrogen and a water bath of about 30 °C.

10.2.4 Storage of PUF Plugs

PUF plug blanks sometimes showed peaks that eluted very close to 2-nitronaphthalene and 2-nitrofluorene/9-nitroanthracene. Of these three, however, 9-nitroanthracene was not affected because its excitation and emission spectra were different enough from those of the confounding compounds.

The interferences could originate from the foam itself (breakdown products) or from contamination from indoor air during storage. Since the impurities eluted in the beginning of the chromatogram they might be of low molecular weight, therefore possibly of high volatility. This could explain contamination during storage due to diffusion of room air organics. Most blanks were obtained with no confounding peaks, therefore the possibility of breakdown effects
of the PUF matrix seemed to be unlikely. A more likely explanation was contamination by indoor air organics after some weeks of storage in the laboratory when the plugs were only wrapped in aluminum foil.

Therefore the method of storage was changed:

Freshly cleaned PUF plugs were wrapped in aluminum foil and subsequently in a plastic bag; afterwards they were stored in a refrigerator to slow down diffusion processes.

10.2.5 Determination and Calculation of the Extraction Efficiency of PUF Plugs

10.2.5.1 Experimental Methods

In order to determine whether the extraction procedure chosen from literature yielded a good recovery of nitro-PAH the following experiment was performed: Four clean PUF plugs were spiked with standard nitro-PAH dissolved in 0.05 ml methanol by injection into the center of the plug. (See Table 19) The plugs were extracted twice as described in Chapter 10.2.3 using first 300 ml, then 200 ml methylene chloride. Two plugs were extracted a third time using 200 ml methylene chloride. The volume of methylene chloride
recovered from each extraction was measured. The extracts of the first two extractions were combined, the third extracts were processed individually. The solutions were concentrated in the rotary evaporator using a water temperature of 52 °C, with no vacuum, until a final solvent volume of about 5 ml was reached. The samples were then transferred to vials, filtered, and further concentrated using a gentle stream of nitrogen and warm water (approx. 20 – 35 °C) until the volume was about 0.1 – 0.2 ml. The vial was stored until analysis at -60 °C. Before analysis this volume was measured by a 1 ml syringe. Using the same syringe, methanol was now drawn up into the syringe and injected into the vial to give a total volume of 0.5 ml.

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>ng injected in each plug</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>33.0</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>38.2</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>19.8</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>ca.30</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>35.36</td>
</tr>
<tr>
<td>3-Nitrofluorantheine</td>
<td>36.5</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>36.6</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>32.1</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>24.0</td>
</tr>
</tbody>
</table>

Table 19: ng of nitro-PAH in 0.05 ml methanol injected in center of each PUF plug

The samples were analyzed and peak heights compared to those obtained by injection of a standard solution. The recoveries of each substance was calculated.
10.2.5.2 Calculation of Recoveries

In order to calculate the true recoveries of nitro-PAH the volumes of solvent were measured that were "recovered" from each manual extraction and decanted into a measuring cylinder. If one assumes that the nitro-PAH are completely dissolved in the 300 ml methylene chloride added at the first extraction, and if one further assumes that nitro-PAH show a behavior similar to the solvent, the recovery of the initial methylene chloride could be used as an approximation of the recovery of nitro-PAH.

The percent loss of methylene chloride due to evaporation or due to absorption by the plug were calculated according to the following relationships:

\[
\frac{300 \text{ ml}}{100\%} = \frac{A \text{ ml}}{X\%}
\]

\[X\% = \% \text{ recovered after 1st extraction}\]

\[
\frac{100\% - X\%}{500 \text{ ml} - A \text{ ml}} = \frac{Y\%}{500 - (A+B) \text{ ml}}
\]

\[Y\% = \% \text{ total loss after 2nd extraction}\]

\[
\frac{Y\%}{700 - C \text{ ml}} = \frac{Z\%}{700 - (C+D) \text{ ml}}
\]

\[Z\% = \% \text{ total loss after 3rd extraction.}\]

A = ml of methylene chloride recovered after 1st extraction
B = ml of methylene chloride recovered after 2nd extraction
C = sum of A and B
D = ml of methylene chloride recovered after 3rd extraction
10.2.5.3 Results and Discussion

The results of these calculations as well as the measured recoveries of methylene chloride are shown in Table 20 and Table 21. They will be discussed together with the recovery results of the nitro-PAH in Table 22 and Table 23.

<table>
<thead>
<tr>
<th>Extraction volume (ml)</th>
<th>Volume recovered (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name Added</td>
<td>Plug 1</td>
</tr>
<tr>
<td>A 300</td>
<td>200</td>
</tr>
<tr>
<td>B 200</td>
<td>185</td>
</tr>
<tr>
<td>C 500</td>
<td>385</td>
</tr>
<tr>
<td>D 200</td>
<td></td>
</tr>
<tr>
<td>Sum 700</td>
<td></td>
</tr>
</tbody>
</table>

Table 20: Measured recovery of solvent (percent) from PUF plug extraction

A = ml of methylene chloride added for 1st extraction
B = ml of methylene chloride added for 2nd extraction
C = sum of A and B
D = ml of methylene chloride added for 3rd extraction

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Plug 1</th>
<th>Plug 2</th>
<th>Plug 3</th>
<th>Plug 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>X%</td>
<td>66.7</td>
<td>66.7</td>
<td>66.7</td>
<td>61.7</td>
</tr>
<tr>
<td>Y%</td>
<td>12.8</td>
<td>13.3</td>
<td>13.3</td>
<td>16.4</td>
</tr>
<tr>
<td>Z%</td>
<td></td>
<td>5.6</td>
<td>5.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 21: Calculated losses (percent) of solvent from PUF plug extraction

X% = % recovered after 1st extraction.
Y% = % total loss after 2nd extraction.
Z% = % total loss after 3rd extraction.
<table>
<thead>
<tr>
<th>Nitro PAH</th>
<th>% Recovery, 3rd extraction</th>
<th>% Total Recovery, all three extractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plug 1</td>
<td>Plug 2</td>
</tr>
<tr>
<td>2-Nitronaphthalene</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>7.3</td>
<td>4.5</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>2.3</td>
<td>16.8</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>4.1</td>
<td>3.7</td>
</tr>
<tr>
<td>3-Nitrofluoranthen</td>
<td>9.5</td>
<td>2.9</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>7.3</td>
<td>5.9</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>2.6</td>
<td>5.2</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>1.5</td>
<td>7.0</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>4.3</td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Std. dev.</strong></td>
<td>3.0</td>
<td>4.6</td>
</tr>
<tr>
<td>(Std. dev.) x (100) %</td>
<td>69.4</td>
<td>83.6</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>9.5</td>
<td>16.8</td>
</tr>
</tbody>
</table>

Table 22: Recoveries of nitro-PAH (percent) obtained by a 3rd PUF plug extraction, and in total after 3 extractions

<table>
<thead>
<tr>
<th>Nitro PAH</th>
<th>Recoveries in % of first 2 extractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plug 1</td>
</tr>
<tr>
<td>2-Nitronaphthalene</td>
<td>83.9</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>84.3</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>68.9</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>84.0</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>85.6</td>
</tr>
<tr>
<td>3-Nitrofluoranthen</td>
<td>75.5</td>
</tr>
<tr>
<td>7-Nitrobenz[a]anthracene</td>
<td>86.3</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>92.6</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>97.9</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>84.3</td>
</tr>
<tr>
<td><strong>Std. dev.</strong></td>
<td>8.5</td>
</tr>
<tr>
<td>(Std. dev.) x (100) %</td>
<td>10.1</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>68.9</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>97.9</td>
</tr>
</tbody>
</table>

Table 23: Recoveries of nitro-PAH (percent) after two PUF plug extractions
Even though the recoveries of the individual nitro-PAH of each plug showed considerable variation (68.9% - 97.9%), the average recovery of each of the four plugs stayed rather constant: 82% - 85%. The third extraction yielded on the average 4.3% (PUF 2) or 5.5% (PUF 3), giving a total recovery of 82.2% (PUF 2) and 90.6% (PUF 3).

The data of the methylene chloride measurements indicate that a substantial amount of the 300 ml of methylene chloride was not recovered after the first extraction. One third of the solvent added remained in the plug or evaporated. After the second extraction, a significant amount of the original methylene chloride could not be recovered - about one eighth of the 300 ml added originally. After the third extraction, however, 94.4% of the original volume of the solvent could be recovered. The plugs kept a volume of about 100 ml that was not extractable even though the plugs were pressed during decanting.

It should be kept in mind that nitro-PAH and methylene chloride have very different molecular weights and volatilities. Some of the losses observed for methylene chloride are due to evaporation during extraction at room temperature. It is not likely that the nitro-PAH under study showed any measurable evaporation during this experiment. Therefore the nitro-PAH were probably concentrated to a certain extent during extraction.

Nitro-PAH are very soluble in methylene chloride, but due to their aromatic nature are also readily adsorbed on a matrix of similar
aromatic composition such as polyurethane foam. During this extraction the nitro-PAH were partly dissolved from the foam into the solvent. It was hypothesized that the distribution of the nitro-PAH between solid and liquid phase would be shifted to the solvent side because

- constant pressing of the PUF plugs generated a relative high solvent flow within the plugs;
- a relative large amount of solvent compared to the rather small amount of foam material in the plug was used.

<table>
<thead>
<tr>
<th>Average recovery</th>
<th>Nitro-PAH</th>
<th>Methylene chloride, original 300 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PUF 2</td>
<td>PUF 3</td>
</tr>
<tr>
<td>after 2nd extraction</td>
<td>83.9</td>
<td>85.0</td>
</tr>
<tr>
<td>after 3rd extraction</td>
<td>88.2</td>
<td>90.6</td>
</tr>
</tbody>
</table>

Table 24: Average recoveries (percent) of nitro-PAH and methylene chloride after 2nd and 3rd PUF plug extraction

The data obtained from these experiments support this hypothesis. The measured nitro-PAH recovery shows a close correlation with the expected recovery as calculated with methylene chloride (see Table 24). Therefore it can be concluded that almost the total mass of nitro-PAH was dissolved during extraction.

Nevertheless, it was of concern that the final nitro-PAH solution used for analysis contained a substantial amount of methylene
chloride. This solvent has a boiling point of only 40 °C, which makes it a very "risky" solvent for storage if one has to rely on the volume stability of the sample solution. In order to avoid this concern, the sample preparation procedure was changed during subsequent experiments. Instead of having a mixture of methylene chloride and methanol in the final solution, the methylene chloride was blown down to dryness and a known amount of methanol was added. Methanol has a boiling point of 64.5 °C, and is already present in the HPLC eluent, thus providing better storage stability and compatibility with the HPLC system.

10.2.5.4 Conclusions

The results indicate that the three-fold extraction procedure can recover nitro-PAH absorbed on PUF plugs with recoveries better than 83.9%. This procedure was therefore used for all further experiments. Nitro-PAH recoveries can be estimated by calculating the recovery of the initial volume of methylene chloride used for the first extraction.
10.2.6 Recovery and Determination of Contamination of Methylene Chloride in the Rotary Evaporator for Recycling Purposes

The extraction of one sample (filter and 2 PUF plugs) required two liters of methylene chloride. Since this is a rather expensive solvent and contributes to the depletion of the ozone layer of our atmosphere, the recycling of methylene chloride (example: for cleaning glass ware) was investigated.

Methylene chloride was collected in the receiving flask of the rotary evaporator during concentration. The collected methylene chloride was stored in one of the solvent bottles of the manufacturer until four liters were accumulated. 300 ml of this solution were concentrated to about 2 ml using a gentle stream of nitrogen and a water bath of approximately 30 °C. The liquid was transferred to a vial where the solvent was then evaporated to dryness for a very short time using a gentle stream of nitrogen. Without delay, 0.25 ml of methanol were added and the sample analyzed on the analytical HPLC.

Among the nine nitro-PAHs of this study, only 2-nitronaphthalene was present in a substantial concentration. The concentrations of the other eight nitro-PAHs were below the detection limit.
From this experiment, two conclusions can be drawn:

1) Assuming that the concentration by a stream of nitrogen did not cause losses of nitro-PAHs by itself, rotary evaporation of nitro-PAHs as described in Chapter 10.2.3 does not significantly cause losses of these compounds except for 2-nitronaphthalene.

2) The collected methylene chloride can be recycled and used for cleaning glass ware and other laboratory items in all cases in which the presence of small quantities of 2-nitronaphthalene does not cause a contamination problem during subsequent analysis.
11. Sample Preparation Procedure after Extraction

The procedures for extraction of the collector media (filter and PUF plugs) have already been described in Chapter 9.4 and 10.2.3. Here, the total procedure will be presented in a flow chart in Figure 7 in order to give an overview. The steps after extraction will be described in detail.

A common feature in the extraction procedure both for filter and plugs was that the solvent was collected after extraction in a round bottom flask. The volume was reduced in a rotary evaporator to about 5 - 10 ml, transferred into sample vials and further concentrated using a stream of nitrogen and a water bath of about 30 0C.

When the sample volume was reduced to about 0.5 ml it was transferred for filtration into a precleaned pasteur pipette with a small glass fiber plug. The original vial and the filter pipette were washed with methylene chloride; sample and washing solution combined added up to about 4 ml. This solution was again concentrated to about 0.25 ml by a gentle stream of nitrogen using a water bath of 37 0C. The vial was then stored at -60 0C until fractionation.

At the day of fractionation the volume was further reduced using a stream of nitrogen and a water bath until a volume of about 0.1 ml
was reached. This volume was drawn up into a precleaned 0.25 ml syringe, the vial was washed 2 - 3 times with a little methylene chloride which was drawn up into the syringe, too, until the total volume in the syringe was approximately 0.25 ml. The syringe volume was then injected into the fractionation HPLC.

The collected Nitro-PAH fraction consisted of a solvent mixture of methylene chloride and n-hexane. It was concentrated to about 1.5 ml using a gentle stream of nitrogen and a water bath of about 37 degrees C. The sample was transferred to a vial. The collection tube was washed three times with methylene chloride, and the washing solution was combined with the sample. The solution was concentrated again to about 0.25 ml using nitrogen and a water bath as described above. The sample was stored at -60 °C until analysis by HPLC.

On the day of analysis, the solvent was evaporated to dryness using a stream of nitrogen but no water bath. Thus the speed of evaporation was slower and the process could be better controlled. When the solvent was "just" completely evaporated, the sample was immediately removed from the nitrogen stream and 0.25 ml of methanol were added. The vial was capped, the walls washed by the methanol by turning the vial and the sample stored in a refrigerator until injection.

Each sample was injected 3 - 4 times to compensate for injection variations and to detect also components that either coelute (9-
nitrofluorene and 9-nitroanthracene) or elute relatively short after each other (1-nitropyrene and 3-nitrofluoranthenone). 2-Nitrofluorene and 9-nitroanthracene could be differentiated using their specific excitation and emission spectra for detection.
Filter

PUF 1
+ Methylene chloride 4 times 100 rpm

Extraction

PUF 2
+ Methylene chloride

Rotary Evaporator
Water bath at 57°C no vacuum

Concentration

5-10 ml
+ Methylene chloride for washing

Concentration

N₂

Storage

Filter sample, Storage at -60°C

PUF 1, Storage at -60°C

PUF 2 Storage at -60°C

Filtration

+ Methylene chloride for washing
Filter → PUF 1 → PUF 2

**Preparation for injection**

No water bath, ~100 μl final volume

+ Little methylene chloride for washing

**Fractionation in HPLC**

83 min per run

Nitro-PAH fraction of PUF 1 in MeCl₂ / Hex.

Nitro-PAH fraction of PUF 2 in MeCl₂ / Hex.

Concentration

Methylene chloride for washing

Storage

Filter, storage at -60 °C

PUF 1, storage at -60 °C

PUF 2, storage at -60 °C
Figure 7: Flow chart of sample preparation procedure after extraction

1. PUF 1
2. PUF 2
3. Concentration “just” to dryness
4. Addition of 250 μL methanol
5. Storage in refrigerator at +4°C until injection in analytic HPLC
6. Analysis by HPLC
   17 min per run
12. Determination of Recoveries after Simulated Sampling with the Selected Collector Design

12.1 Introduction

The purpose of these experiments was to simulate a real sampling situation to find out whether the selected procedures for sampling, fractionation and analysis showed an adequate performance under these conditions.

The approach was to volatilize standard nitro-PAH added onto the filter of a sampling train (consisting of a filter and two PUF plugs downstream) by operating the sampling pump.

It was hypothesized that the obtained recoveries from the sampling media would serve as an estimation for the usefulness of this sampling method for nitro-PAH in real field sampling situations.
12.2 Experimental Methods

12.2.1 Standards - Preparation and Use

A mixture of nitro-PAH in methanol was prepared using undiluted NBS standard reference material 1587 in methanol as well as laboratory standards of 2-nitronaphthalene and 2-nitroanthracene in methanol. Two aliquots were taken from it: 0.35 ml were added onto the Teflon coated filter in each of the 3 runs. In run 3 the glass fiber filter upstream of the Teflon coated filter cleaned the incoming air and was not analyzed. It was assumed that the glass fiber filter did not significantly catalyze chemical reactions in this run because the nitro-PAH were adsorbed throughout the Teflon coated filter material, not just on its outer surface. The second aliquot (0.15 ml) was diluted 1:1 with methanol to give 0.3 ml of solution, stored at -60 degrees Celsius and was used as a reference for subsequent quantification of recoveries.

The total mass of each compound added on a Teflon coated filter is shown in Table 25. The average mass of the nine nitro-PAH added on each filter is 92.8 ng. A total air volume of approximately 440 m$^3$ would therefore result in a simulated airborne concentration of about 0.2 ng/m$^3$ which is not much higher than the maximum concentrations of 1-nitropyrene found in ambient air (see Table 3, Chapter 3.2).
Table 25: Masses of nitro-PAH added to each filter of sampler used in simulated sampling experiments

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>112</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>106.96</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>55.44</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>ca. 100</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>98.98</td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>102.2</td>
</tr>
<tr>
<td>7-Nitrobenzo[a]anthracene</td>
<td>102.48</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>89.88</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>67.2</td>
</tr>
</tbody>
</table>

12.2.2 Sampling Train and Experimental Conditions

The sampling train consisted of a high volume sampling pump, a filter holder, and two metal cartridges each containing a PUF plug. The sampling train with its dimensions is shown in Figure 8. The flow rate was determined using a calibration curve obtained by measuring pressure drop at pump outlet against a calibrated orifice. The primary standard was a pitot tube. The effective diameter of filters exposed to the air stream was 12.2 cm, the internal diameter of the cartridges was 10 cm, each plug was about 5.5 cm thick and 11 cm in diameter if outside the cartridge. Each plug was blank extracted prior to use and checked for contaminations. PUF plugs were assigned a number to keep a record of their last use, their blank values and storage time since last
extraction. Blanks of PUF plugs used in the runs were analyzed prior to the experiment. An experiment was only conducted if the blanks were clean or showed only contaminations that would affect the peak heights of nitro-PAH less than 1 % (100 % = expected peak heights caused by total mass added onto the filter). Filters were extracted four times with methylene chloride according to the procedure described in Chapter 9.4 prior to an experiment in order to eliminate contaminations. The experimental conditions of the three runs are given in Table 26.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of air</td>
<td>none</td>
<td>PUF plug</td>
<td>PUF plug + glass fiber filter</td>
</tr>
<tr>
<td>precleaning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>1.5</td>
<td>23</td>
<td>12 - 17</td>
</tr>
<tr>
<td>Run time</td>
<td>6 hs 47 min</td>
<td>5 hs 13 min</td>
<td>6 hs 34</td>
</tr>
<tr>
<td>Flow rate, l/s</td>
<td>21.9</td>
<td>21.9</td>
<td>16.05</td>
</tr>
<tr>
<td>Total volume, m³</td>
<td>536</td>
<td>412</td>
<td>379</td>
</tr>
<tr>
<td>Filter face velocity, m/s</td>
<td>1.87</td>
<td>1.87</td>
<td>1.37</td>
</tr>
<tr>
<td>Approx. air residence time in each plug in seconds</td>
<td>0.02</td>
<td>0.02</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Table 26: Sampling parameters for simulated sampling experiments
Figure 8: Sampling train used for simulated sampling experiments
12.2.3 Prefiltration of Air Entering the Sampling Train

In the first experiment no air prefiltration was conducted. However, in order to eliminate the concern of contamination due to ambient nitro-PAH, precautions were taken in run 2 and 3 to eliminate traces of nitro-PAH by prefiltration of the air entering the sampling train. In run 2, a single PUF plug was used that covered the total filter head plus the collar. The pump caused sufficient suction to hold the plug in place and to ensure good fitting.

In run 3, the set up was changed in two ways:
1) A PUF plug was placed upstream of the filter in a plastic tube cleaned previously with soap solution. The tube had the same internal diameter as the internal circle of the filter head collar. The tube was kept in place by some strips of duct tape. There was no direct contact between the duct tape and the filter surface.
2) A precleaned glass fiber filter was placed on top of the Teflon coated glass fiber filter to prevent potential interferences during subsequent analysis due to particle adsorbed compounds. This measure was taken because it was observed in run 2 that very small particles can penetrate even an eight cm thick PUF plug.

None of the prefiltration plugs were precleaned. The inner surface of the sampling train was cleaned by rinsing with methylene chloride prior to use.
12.3 Results and Discussion

12.3.1 Evaluation of Sample Fractionation Chromatograms

The UV detector chromatogram during fractionation might be a first indication on how much of UV absorbing material was adsorbed on the filter or PUF plug. Significant contaminations of blank extracts — which would require reextraction — could be detected already at this step, thus saving the time otherwise spent for further sample handling and analysis.

Figure 9 shows a chromatogram obtained by fractionating the extract of the top PUF plug of run 3. It should be noted that the standard shown in Figure 6 in Chapter 7.3 was detected with a 4-times greater sensitivity than the PUF plug sample.

It can be seen that the PUF plug contained a number of other compounds, mostly compounds of low polarity such as unsubstituted PAH (first big peak). However, within the nitro-PAH fraction there were also several huge peaks. They were most likely caused by compounds other than the nitro-PAH under study because during subsequent analysis the nine nitro-PAH studied in this project were detected at concentrations substantially lower than those indicated by the UV chromatogram. In general, the UV detector response is much less sensitive than that of a fluorescence detector. The
masses of the nine nitro-PAH used in that experiment were far too small to cause significant peaks in the UV-chromatogram during fractionation. The expected peak heights in Figure 9 would be about 1/50th of the peak heights seen in Figure 6. However, it was possible to quantify the nine compounds free from interferences and with peak heights that could reasonably be expected by fluorescence detection after separation by the analytical HPLC.

As an example, the chromatograms of the top PUF plug in run 3 before (blank, Figure 10) and after (sample, Figure 9) are shown. Note that the detector condition in the PUF plug chromatogram before and after run 3 are the same. Even though some peaks can be seen in the nitro-PAH fraction, no significant interference during subsequent analysis was detected.
Figure 9: UV-Chromatogram of the upstream PUF plug of run 3 of the simulated sampling experiments, extracted after the run.
Figure 10: UV-Chromatogram of the upstream PUF plug of run 3 of the simulated sampling experiments, extracted before the run (blank)
The large peak within the non-polar fraction found on the PUF plug of run 3 was the main difference between the two chromatograms of the plug extracts before and after the experiment. All the additional peaks must have been caused by airborne substances that were capable of penetrating the PUF plug placed upstream the filter for prefiltration purposes as well as the two filters (the glass fiber pre-filter and the Teflon coated glass fiber filter). Therefore it can be assumed that the extracted compounds are probably of rather low molecular weight and high volatility.

The literature suggests that PUF plugs have a better trapping efficiency for more polar than for less polar compounds (49) and that they do not trap two-ring PAH very well (4). One would therefore expect that the PUF plug placed upstream of the collector would not trap the less polar substances with low molecular weight as efficiently as the more polar ones of high molecular weight. Indeed, the hypothesis is supported by the findings of these experiments, since all chromatograms of crude PUF plug extracts in runs 1-3 showed big peaks of early eluting compounds.

12.3.2 Recoveries of Nitro-PAH

The recoveries of nitro-PAH in percent (100 % = peak height of total mass of nitro-PAH added onto the filter of each run) are given in Tables 27 - 30.
Most of the masses of 2-nitronaphthalene, 2-nitrofluorene and 9-nitroanthracene were recovered from the first PUF plug (58 % on the average), only about 12 % stayed on the filter. Contrary to these three compounds the other six nitro-PAH were largely not blown off and thus found in the filter sample (72 % on the average). Only roughly 9 % of these six nitro-PAH were collected by the first PUF plug. The second plug retained only little mass, the average recovery of all nine nitro-PAH was only little more than 2 %. The observation of unequal volatization rated was expected because Table 1 in Chapter 3.1 shows there are relatively large ranges of molecular weights (173.17 - 297.3) and melting points (79 - 251 °C) among the nine nitro-PAH studied. Low molecular weight and low melting point are closely related to a high vapor pressure.

Total recoveries ranged from about 57.2 - 93.8 %. If one defines the total recoveries of each nitro-PAH in run 1 (without 2-nitrofluorene), run 2 and run 3 (without 2-nitronaphthalene and 2-nitrofluorene) as one set of data, the average recovery is 80.8 % with a standard deviation of 8 %. This appears to be a reasonable performance given the long procedure of sample preparation with its 7 concentration steps. Compared to literature data the average recovery obtained here is in the same range as the one described for some pesticides which were collected and recovered from filters and PUF plugs with in total about 75 % efficiency (49).
The results of these three runs will also be discussed regarding the influence of several sampling parameters.

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>10.4</td>
<td>-</td>
<td>3.2</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>15.3</td>
<td>-</td>
<td>14.3</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>15.1</td>
<td>-</td>
<td>15.0</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>-</td>
<td>-</td>
<td>38.3</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>69.7</td>
<td>64.5</td>
<td>74.9</td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>73.7</td>
<td>61.1</td>
<td>85.8</td>
</tr>
<tr>
<td>7-Nitrobenzo[a]anthracene</td>
<td>69.4</td>
<td>63.7</td>
<td>93.0</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>76.6</td>
<td>69.3</td>
<td>79.1</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>76.3</td>
<td>76.6</td>
<td>82.7</td>
</tr>
</tbody>
</table>

Table 27: Recoveries of nitro-PAH from filter samples (percent) of simulated sampling experiments

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>65.4</td>
<td>-</td>
<td>62.1</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>(3.6)</td>
<td>-</td>
<td>63.6</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>56.3</td>
<td>-</td>
<td>41.7</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>-</td>
<td>-</td>
<td>34.1</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>6.5</td>
<td>15.7</td>
<td>5.6</td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>4.0</td>
<td>20.4</td>
<td>5.6</td>
</tr>
<tr>
<td>7-Nitrobenzo[a]anthracene</td>
<td>8.2</td>
<td>21.2</td>
<td>0.75</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>5.2</td>
<td>10.9</td>
<td>0.65</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>0.7</td>
<td>3.0</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 28: Recoveries of nitro-PAH from PUF 1 samples (percent) of simulated sampling experiments
### Table 29: Recoveries of nitro-PAH from PUF 2 samples (percent) of simulated sampling experiments

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>9.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>9.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>2.2</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>-</td>
<td>-</td>
<td>0.0</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>0.7</td>
<td>0.9</td>
<td>0.43</td>
</tr>
<tr>
<td>3-Nitrofluoranthenone</td>
<td>0.5</td>
<td>0.8</td>
<td>0.22</td>
</tr>
<tr>
<td>7-Nitrobenzo[a]anthracene</td>
<td>8.5</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>7.3</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>1.1</td>
<td>0.0</td>
<td>0.26</td>
</tr>
</tbody>
</table>

### Table 30: Total recoveries of nitro-PAH from filters and PUF plugs (percent) of simulated sampling experiments

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitronaphthalene</td>
<td>85.2</td>
<td>-</td>
<td>&gt;65.3</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>(28.1)</td>
<td>-</td>
<td>&gt;77.9</td>
</tr>
<tr>
<td>9-Nitroanthracene</td>
<td>73.6</td>
<td>-</td>
<td>&gt;57.2</td>
</tr>
<tr>
<td>2-Nitroanthracene</td>
<td>-</td>
<td>-</td>
<td>72.4</td>
</tr>
<tr>
<td>1-Nitropyrene</td>
<td>76.9</td>
<td>81.1</td>
<td>80.9</td>
</tr>
<tr>
<td>3-Nitrofluoranthenone</td>
<td>78.2</td>
<td>82.3</td>
<td>91.6</td>
</tr>
<tr>
<td>7-Nitrobenzo[a]anthracene</td>
<td>86.1</td>
<td>85.1</td>
<td>93.8</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>89.1</td>
<td>81.2</td>
<td>79.7</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>78.1</td>
<td>79.6</td>
<td>83.1</td>
</tr>
</tbody>
</table>

Table 29: Recoveries of nitro-PAH from PUF 2 samples (percent) of simulated sampling experiments

Table 30: Total recoveries of nitro-PAH from filters and PUF plugs (percent) of simulated sampling experiments
As can be seen from Table 26 the temperature of the three experiments varied considerably. As pointed out earlier, there is an exponential relationship between temperature and vapor pressure of a compound. Therefore it was expected that a temperature change from 1.5 - 23 degrees Celsius would cause a significant difference in the observed mass distribution of the nitro-PAH on the three sampling matrices. However, the results did not reveal such strong differences. The less volatile compounds (the last five compounds in Tables 27 - 30) were recovered from filter and PUF 1 samples with similar efficiencies in all three runs. Unfortunately, the more volatile compounds could not be completely quantified in run 2 (the warmest run) and run 3. Therefore interpretations regarding temperature effects can only be made with great uncertainty. Further studies on temperature effects are described in Chapters 13.1 - 13.4 with respect to the relationships between the parameters temperature, nitro-PAH concentrations both in the vapor and in the particulate phase, and total suspended particulated matter.

The surprising observation was made that recoveries of 2-nitronaphthalene, the most volatile of the nine nitro-PAH, were approximately the same in filter and PUF 1 samples of run 1 and 3, even though the temperature difference was about 12.7 degrees Celsius. However, the filter data of this compound as well as for
2-nitrofluorene even showed behaviors opposite to that expected: at higher temperature more mass was recovered from the filter than at lower temperatures. Two explanations for this phenomenon appear to be possible:

1) Possible differences in the sample preparation or analysis might have occurred; during the concentration steps of the procedure there is a great potential for losses.

2) Considerable contamination due to 2-nitronaphthalene and possibly other nitro-PAH in the ambient air occurred during run 1 when no air precleaning was conducted.

At this point it is impossible to determine the actual reason for this contradiction.

12.3.4 Effects of Potential Contamination by Ambient Nitro-PAH

Since these experiments relied on nitro-PAH free air being sucked through the sampling train the possibility of contamination by ambient nitro-PAH needed to be further evaluated using literature data of nitro-PAH concentrations found in ambient air. These data were used as estimates with respect to nitro-PAH concentrations found in the experiments described here.
In a rural area in Denmark the particulate bound 1-nitropyrene concentration (using a 24 hour sample) was found to be about 0.009 ng/m$^3$ (61). Assuming the same concentration during run 1 and no volatilization from the filter during sampling, the additional mass of 1-nitropyrene in run 1 would be 4.8 ng compared to the 98.08 ng added, this would result in an error of 4.8 %.

6-Nitrochrysene is not known to occur as such in nature (2). It was not found in methylene chloride extracts of airborne particulate matter collected in Prague, Czechoslovakia, using an analytical technique with a detection limit of 100 ng (34).

Experiments were performed with loaded filters spiked with 9-nitroanthracene in order to get information on the possible transformation products of mono-nitro-PAH (61). After 24 hours of sampling, one filter was spiked with a standard solution of 9-nitroanthracene, another filter only with the corresponding solvent. Sampling was continued for 24 hours using the same filters. During subsequent analysis two reaction products of 9-nitroanthracene were identified by means of their retention time: 9,10 dinitroanthracene and 10-nitroanthrone. In addition, 4 unknown products were observed. 9.6 % of the spiked mass was recovered as 9-nitroanthracene, 90 % was blown off the filter, and 0.4 % were recovered as reaction products of the sampling procedure (61).
The difference between 90% blow off (of 9-nitroanthracene) in (61) and 85% in the experiments of this project (see Table 27) might be due to:

- different sampling volumes (2000 m$^3$ in (61) versus 538 m$^3$ here)
- contamination by ambient 9-nitroanthracene. In (61) a concentration of 0.03 ng/m$^3$ was determined, which would give a total mass of 16.08 ng in run 1, corresponding to 29% of the mass added onto the filter.

However, contamination by ambient 9-nitroanthracene seemed to have caused an error much less than 29% because:

1) The lower temperature of run 1 would favor less blow off than in run 3 with its higher temperature.
2) No air precleaning in run 1 would favor higher concentrations of 9-nitroanthracene for run 1 than in run 3 with good precleaning.

Both arguments would support the hypothesis of higher concentrations in run 1 than in run 3. If there had been significant airborne concentrations of 9-nitroanthracene, they would have caused much higher concentrations both on the filter and the foam plugs during run 1 compared to run 3. However, it was actually found that the filter data were approximately equal, and that the data for PUF plug 1 differ only by 14.6%. These rather small differences might be due to experimental variations as well as due to little contamination caused by airborne 9-nitroanthracene.
For most of the nitro-PAH used in this project, concentrations in ambient air have not yet been published. From estimations described above one might hypothesize that these concentrations in the air of the three runs were low and did not interfere significantly with the recovery experiments of this project. But since no data were available to confirm this hypothesis it was felt that prefiltration during the run was important.

12.3.5 Effects of Different Flow Rates

The flow rate of the three runs varied only moderately: 21.9 versus 16.05 liters per second. The lower flow rate of run 3 was most likely caused by the additional glass fiber filter.

From literature data it can be concluded that flow rate is not a very important factor for the collection efficiency of PUF-plugs. For example, in a field study it has been found that the collection efficiency of PCB vapors on PUF plugs was not flow dependent within the high volume sampling equipment (80). Therefore no efforts were undertaken to study such an dependency.
12.3.6 Effects of Different Total Air Volume

The total air volume showed some variability but did not change as much as did temperature. The collection efficiency of a PUF plug depends strongly on sample volatility and on the total volume of air that passes through the collector (80). Temperature strongly affects the volatility of a compound: The vapor pressures of naphthalene and pyrene increase by a factor of about 1.65 and 1.8, respectively, per increase in temperature of 5 degrees Celsius (72). Since both variables (temperature and total air volume) changed between the runs, it was difficult to determine their effect on the results.

12.3.7 Problems during Analysis

There were several samples where the concentration of some nitro-PAH could not be determined. Contamination during sample preparation was probably the reason for difficulties in determining the concentrations of the nitro-PAH in run 2 that eluted early in the analytical chromatogram. The later eluting nitro-PAH did not seem to be affected. 2-Nitronaphthalene and 2-nitrofluorene of PUF 2 in run 3 were masked by two very big peaks before and after their elution in the analytical HPLC, thus making quantification
impossible. The origin and nature of these confounding peaks is not known.

The recovery of 2-nitrofluorene on PUF 1 in run 1 is probably incorrect and should be ignored, since the number is based on only a single injection. 2-Nitroanthracene was not included in the standard solution added onto the filter in run 1.

12.3.8 Breakthrough during Sampling

A sampling cartridge filled with a PUF plug behaves as a frontal gas-chromatographic system, in which sample is continuously introduced to the column and subsequently moved through the column by the air flow. The breakthrough volume has been defined as the amount of air necessary to move the sample front through the adsorber (80). Breakthrough means the period during sampling when the analyte is no longer completely adsorbed on the sampling matrix but is lost in the exhaust air. Breakthrough makes the quantification of the analyte impossible and therefore needs to be prevented. Breakthrough of an analyte on an adsorbent is a function of the ambient temperature, the sampling volume, the adsorbent volume, the affinity of the analyte to the adsorbent, and the dimensions of the adsorbent trap (50). The strongest influence is caused by temperature.
Even though a large air volume of approximately $500 \text{ m}^3$ was drawn through the sampling apparatus no breakthrough occurred during these experiments. The small amounts of ca. $0.0 - 0.5 \%$ measured on the second PUF plug compared to a relative large recovery ($>65.3 - 93.8 \%$) of the total system indicated that breakthrough would occur only at much larger air volumes than $500 \text{ m}^3$.

12.4 Conclusions

Polyurethane foam, when used as described in this project, traps the major part of a number of nitro-PAH in air. The foam permits a high sampling rate and therefore a short sampling time. The procedure for sample preparation and analysis is time consuming but reasonably accurate. For the sampling conditions used no breakthrough of nitro-PAH was observed.
Evaluation of Possibilities to Simplify the Sampling of Volatile Nitro-PAH by Application of an Empirical Equation

13.1 Introduction

The procedure necessary to generate one data point (ng of nitro-PAH/m³) is described in Chapters 9.4, 10.2.3, 11, and required about 4 - 6 days of laboratory work under the conditions of this project. The long sample handling procedure is the major disadvantage of this procedure and poses some limitations on its usefulness for a large sampling program. Any reduction in time necessary to process a sample is therefore to be highly desirable.

A possible way for such a time reduction would be to measure only particulate adsorbed nitro-PAHs and to calculate the concentrations expected in the vapor phase by a mathematical formula.
One possibility would be the application of Junge’s equation (46):

\[ R = \frac{C \times D}{P + C \times D} \]

with

- \( R \) = ratio of the amount of that organic compound in both gaseous and particulate-associated phases
- \( C \) = constant, depends on the molecular weight and heat of condensation of compound of interest;
- \( D \) = surface area of the particulate matter per unit volume of air
- \( P \) = saturation vapor pressure at given temperature.

There are three major disadvantages associated with this formula for the purpose of estimation of concentrations expected on vapor traps during sampling:

1. The parameters \( C \) and \( P \) for nitro-PAH are not available in the literature, and parameter \( D \) requires an analysis of the particle size distribution and several assumptions regarding composition and shape of particles.

2. Junge’s equation does not take into account the effect of temperature on the phase distribution of semivolatile organic compounds in the atmosphere.
3. During sampling, the equilibrium between particulate and vapor phase is different from the one in the free atmosphere. Junge’s equation does not describe the phase distribution during sampling, but can only be used in this case as an approximation.

In another study by Yamasaki (90) a different formula was developed that avoids the above disadvantages

number 1 (the empirical approach used does not require the determination of the parameters C, D and P. of Junge’s equation), and

number 2 (sampling temperature is taken into account).

Yamasaki used a high volume sampler equipped with a filter and two PUF plugs in series downstream the filter to measure total suspended particulate matter (TSP) and PAH concentrations in air. For the PAH he differentiated between the mass found on particles ((PAHₚ), collected on the filter) and the mass in the vapor phase (PAHᵥ, collected on PUF plugs). The temperature (T) during sampling was monitored. He then used the Langmuir adsorption concept to develop the following equation that empirically describes the relationship between the parameters mentioned above.

\[
\log \left\{ \frac{PAH_v}{PAH_p} \times TSP \right\} = -A \times \frac{1}{T} + B
\]
A and B are constants if the following 5 parameters remain constant as well:

- compound measured;
- sampling apparatus used;
- sample handling procedure applied;
- type of particles;
- climate of sampling sites.

It should be noted that - if ambient air samples are to be taken - TSP concentrations as well as PAH$_V$, PAH$_p$, and T change from day to day and therefore need to be included as a variable in the empirical model. However, they need to remain constant during the sampling period.

Once these two constants A and B are determined (for a given set of these five parameters), one only needs to measure T, TSP and PAH$_p$ in order to calculate PAH$_V$, the expected concentration of the analyte on the PUF plugs. In this way, the efforts to generate one data point would be drastically reduced, which is the major advantage of this approach. This method does not attempt to mathematically describe the complex relationships between those 5 parameters mentioned above, but accounts for them on an empirical basis. Therefore this approach is very simple.
However there are also two disadvantages associated with this equation. One is its inflexibility in cases of changes or fluctuations

- of one or more of the 5 parameters mentioned from one sampling period to another, or
- of TSP, PAH_p, PAH_v and T during any sampling period.

For example, the two constants A and B would probably be different in cases of a sampling site in the spring with many pollens in the air and another sampling site close to a large building construction area in the fall when particles would mostly be of mineral nature. In these cases, particle surface properties would be different as well as particle size and mass loading (TSP) which would affect extraction efficiency, potential for chemical reactions and particle surface area available for adsorption of organics (33).

The other disadvantage is that Yamasaki’s equation still does not distinguish between equilibrium distributions in the free atmosphere and those that occur due to volatilization from the filter during sampling. Post-collection volatilization would bias the sample towards the gas phase.

One should keep in mind that a collector system consisting of a filter and a cartridge-type vapor trap can principally not measure the true phase distribution of an analyte in the free atmosphere. The filtration process causes a considerable disruption of the particle/vapor equilibrium of semivolatile organic compounds.
present in the free atmosphere and permits post-collection volatilisation from the filter. Up to now, only a denuder sampler is capable of measuring the true phase distribution in the atmosphere (35). The filter/PUF plug cartridge system needs to be calibrated for each set of environmental and analytical conditions in order to give accurate results that represent the true phase distribution of an analyte in the free atmosphere.

In order to calibrate the filter/PUF plug cartridge system it is necessary to compare both sampling methods (denuder and filter/PUF plug cartridge system) to obtain correction factors for each compound. In this case the denuder would serve as a standard reference. Also, the constants A and B in Yamasaki’s equation could be calculated for the filter part of the filter/PUF plug cartridge system for a given set of the five parameters mentioned; these constants could then be modified so that the predicted vapor phase concentration of an analyte corresponds to the result obtained by the denuder. Equilibrium vapor phase concentrations could then be

- predicted by Yamasaki’s equation (with A and B reflecting the filter/PUF plug cartridge system; the calculated results would then have to be multiplied with the corresponding correction factors;

- measured by the filter/PUF plug cartridge system and then multiplied with the corresponding correction factors.

Since the analytical procedure described in this report is very time consuming, the potentially time saving alternative described above was explored.
13.2 Description and Discussion of Approach

The approach was to determine the constants A and B and to evaluate how well Yamasaki's equation fits the data of the three runs.

First, the recoveries of the filter and PUF plugs of the runs were normalized to the total air volume of run one. This normalization can be justified because a linear relationship has been described between

- total air volume and
- the blow off from a filter or the penetration depth of a chromatographic front of a compound in a PUF plug (14).

The normalization was performed in the following manner for each nitro-PAH that was completely quantified in all three runs.
\[
\{ (\%{PUF \ 1}) + (\%{PUF \ 2}) \} \times \frac{Q_1}{Q_X} = N-PAH_{\nu} \\
(Total\%) - (N-PAH_{\nu}) = N-PAH_{\rho}
\]

with

Total\% : Total recovery of a nitro-PAH from filter and PUF plugs in percent;
\%{PUF \ 1} : Recovery of a nitro-PAH from PUF plug 1 in percent;
\%{PUF \ 2} : Recovery of a nitro-PAH from PUF plug 2 in percent;
\(Q_1\) : Total sample air volume of run 1;
\(Q_X\) : Total sample air volume of run to be modified with run 1 being the standard;
\(N-PAH_{\nu}\) : Recovery of a nitro-PAH from PUF 1 and 2 of a run, normalized to the total air sample volume of run 1;
\(N-PAH_{\rho}\) : Recovery of a nitro-PAH from the filter of a run, normalized to the total air sample volume of run 1;

Values for \(N-PAH_{\nu}\) correspond to the parameter \(PAH_{\nu}\) in Yamasaki’s equation; similarly, values for \(N-PAH_{\rho}\) correspond to \(PAH_{\rho}\). Since TSP was not measured, a value was assumed: 96.1 ug/m\(^3\) which has been the annual average concentration of Berlin airborne particles that were smaller than 10.2 micrometer in diameter (73).

Next, a linear regression was performed between

\[
\frac{N-PAH_{\nu}}{(\ log \frac{\ --- \times TSP \ )}{N-PAH_{\rho}} \ and \ ( \ --- \ )} = 1
\]
with T in °K in order to determine the slope A of the regression line, the point of intersection B with the log-axis, and the correlation coefficient r.

A conceptual difficulty is that actually no particles were allowed to reach the filters in runs 2 and 3, and that the surface properties of a Teflon coated glass fiber filter are different from those of airborne particulate matter. Also, the nitro-PAH were all adsorbed on a filter when an experiment was started, none were already in the gaseous phase as in Yamasaki's experiments where an equilibrium between gaseous and particulate phase PAH concentrations had been reached in the air prior to entering the sampler.

In addressing these difficulties one needs to consider that the "natural" equilibrium present in ambient air between gas and particulate phase is disturbed during sampling in any case. The equilibrium-disrupting conditions are identical in both studies for compounds adsorbed on solid matter: the compounds are blown off from the solid matter of/on the filter due to unsaturated air passing through. However, a difference is present in terms of the more volatile compounds with a significant mass fraction in the gaseous phase: in Yamasaki's work the vapor fraction of a certain PAH easily passed through the filter and was adsorbed on the plugs; in this project there was no such vapor fraction, the entire mass of a compound first needed to be vaporized from the filter before it was adsorbed on the plugs. Here the equilibrium-disrupting
conditions were identical only downstream of the filter in the PUF plugs. This difference will ultimately result in different values for the constants A and B in Yamasaki’s equation if one compares typical sampling situations with the experiments of run 1 - 3. This means that the values for A and B calculated from the three runs in Chapter 12.4.3 can not be generalized for ambient air sampling without making an assumption, even if the same sampler and analytical procedure were to be used. The necessary assumption is that the constants A and B will not differ much if one compares real and simulated sampling situations.

The differences in
- presence/absence of particulates on the filter, and
- surface properties between filter and particulate matter all refer to the parameter "TSP" in Yamasaki’s equation. Again, it is helpful to remember that Yamasaki’s equation reflects those differences on an empirical basis. In order to apply the equation with respect to the parameter "TSP" it only is necessary that the characteristics of the TSP-material stay constant and that the total "TSP"-surface is much greater than the surface fraction of it covered by the nitro-PAH. "TSP" does not have to consist of particles but can be of any shape and material in a given experimental system. Consequently, it is not important whether airborne particles or any other material (such as filter material) is present as TSP in a particular situation. The differences regarding TSP between simulated and real sampling experiments
certainly change the values of constants A and B but do not question their existence.

In the three runs described here, the filter material (TSP) did not change but became a function of the apparatus used. Any number can therefore be used for TSP in this case. It was arbitrarily decided to choose a value that reflects typical TSP concentrations in ambient air. In the experiments described therefore only three variables remained: PAH\textsubscript{v}, PAH\textsubscript{p}, and T.

### 13.3 Results and Discussion

Among the nine nitro-PAH studied only the five less volatile compounds could be completely quantified in all three runs and were used for the calculations described. Table 31 shows the values for the constants A and B in Yamasaki's equation for the nitro-PAH investigated as well as the correlation coefficient \( r \).

<table>
<thead>
<tr>
<th>Nitro-PAH</th>
<th>( r )</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Nitropyrene</td>
<td>-0.841</td>
<td>1891</td>
<td>7.5</td>
</tr>
<tr>
<td>3-Nitrofluoranthene</td>
<td>-0.902</td>
<td>3246</td>
<td>12.5</td>
</tr>
<tr>
<td>7-Nitrobenzo[a]anthracene</td>
<td>-0.040</td>
<td>252</td>
<td>1.9</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>-0.033</td>
<td>186</td>
<td>19.7</td>
</tr>
<tr>
<td>6-Nitrobenzo[a]pyrene</td>
<td>-0.264</td>
<td>845</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Table 31: Correlation coefficients and constants for selected nitro-PAH in Yamasaki's empirical equation
Only for 1-nitropyrene and 3-nitrofluoranthene could acceptable correlation coefficients be obtained that were close to Yamasaki's average correlation coefficient of -0.886. For these two compounds values for A and B were similar to those that Yamasaki calculated for several common unsubstituted PAH in ambient air.

Figure 11 and 12 show the data for 1-nitropyrene and 3-nitrofluoranthene, respectively.
Figure 11: Plot of $\log(\frac{N-PAH_v \times TSP}{N-PAH_p})$ against $[1/T]$ and the corresponding regression line for 1-nitropyrene
Figure 12: Plot of $\log(N-PAH_v \times TSP / N-PAH_p)$ against $[1/T]$ and the corresponding regression line for 3-nitrofluoranthene.
Unfortunately, for 6-nitrobenzo[a]pyrene only a weak correlation could be found \((r = -0.264)\), and for 7-nitrobenzo[a]anthracene and 6-nitrochrysene there was no correlation at all between the log-expression and the inverse of the sampling temperature.

The variability can be explained mainly by the very small set of data available (only three data points determined A and B). Also, the observed variations might be due to

- experimental variation; Yamasaki had available several micrograms of each compound, whereas the samples analyzed for this Master's project were all in the ng-range (although a more sensitive detector was used here than the GC-FID in Yamasaki's work);

- differences in filter surface between run 1 (particulates were present on filter) and runs 2 and 3 (no particles on filters),

- the temperature of run 3 ranged from 12 - 17 °C. For calculation purposes the temperature was averaged to 14.5 °C. However, a temperature change of 5 °C during sampling (as observed in run 3) might have about the same effect as almost doubling the total air volume \((11, 33, 41, 72)\). Yamasaki explicitly points out the importants of a stable temperature during sampling for the application of his formula \((90)\).
Conclusions

Given the many causes for variations and a set of only three data points per compound it appears to be likely that the accuracy of the determination of A and B will be negatively affected. More runs would be necessary to compensate for these variations. Due to these limitations an evaluation of the usefulness of Yamasaki's equation is difficult. At this point it is impossible to determine whether the assumption of similar values for A and B in real and simulated sampling experiments is valid or not. Further experiments are necessary to determine the accuracy of the equation's predictions of nitro-PAH concentrations found on the PUF plugs. Values for A and B obtained by simulated sampling experiments can not be generalized for ambient air sampling experiments without making the assumption that the values in both types of experiments will be very similar. The validity of this assumption still needs to be demonstrated.
A very sensitive and selective analytical technique is necessary to detect nitro-PAH in extracts of ambient air samples. Fluorescence detection appears to be the most suitable technique: it offers the lowest detection limits for nitro-PAH analysis described in the literature and discriminates against all non-fluorescent compounds. Of the two fluorescence methods compared in this project the procedure described by Tejada (83) was the only suitable one. Detection limits (peak heights three times the noise width of the baseline, 10 microliters injection volume, in methanol) as low as 0.5 pg per injection, were obtained. However, since HPLC does not provide as good a resolution capacity as gas chromatography, fractionation of the crude extract is essential in order to avoid interferences during subsequent analysis. In this project a fractionation scheme developed by Rich Kamens was modified for shorter run time. Recoveries of about 93% and a relatively small nitro-PAH fraction were obtained, suggesting this new program was efficient for fractionating complex mixtures of ambient organics for nitro-PAH analysis.

PAH can chemically react with other substances in air or adsorbed on particles. Since literature findings suggest that glass fiber filters catalyze chemical reactions of PAH, this type of filter did not seem to be appropriate. Teflon coated filters decrease the amount of chemical decomposition and were therefore selected for
this project. The filter extraction method used is fast (less than an hour), and easy to use.

The molecular weight and other physical and chemical characteristics of nitro-PAH do not allow an accurate measurement of ambient air concentrations by filter sampling alone, since a significant proportion of the total mass present is either in the vapor phase or volatilized during sampling. Therefore an adsorbent capable of trapping vaporized nitro-PAH during sampling is necessary. Of the two collector materials tested for vapor phase sampling in this project -- XAD-2 and polyurethane foam plugs -- the foam technique showed superior performance in terms of recovery of adsorbed nitro aromatics. Approximately 88% of the total amount of nitro-PAH injected into PUF-plugs could be recovered after a three-fold extraction, which can be considered adequate for sampling purposes.

Organic extracts of ambient air samples are usually rather complex mixtures of many compounds, thus posing the problem of confirming the identity between analyzed sample and standard compounds. The technique suggested in this project for identity confirmation of nitro-PAH peaks appears to be a fast, sensitive, easy and elegant way to solve this problem.

With the selected procedure for sampling semivolatile nitro-PAH in air it was possible to collect and recover nine selected nitro aromatics added in solution on a filter surface and volatilized
during simulated sampling. The recoveries obtained ranged between 72.4 and 91.6% of the mass initially added on the filter. These values are adequate for industrial hygiene surveys and other applications.

The sample preparation procedure used is rather tedious and time consuming. It requires a certain routine in order to give consistent results. The total procedure — taking a sample until obtaining the calculated concentrations as ng/m³ required about 4-6 days, which limits its use for routine measurements such as in industrial hygiene surveys. Improvements of the analytical procedure such as the introduction of an internal standard, the use of an integrator for the HPLC detector, an programmable fluorescence detector, and the application of Yamasaki’s empirical equation are therefore highly desireable. Among these changes the introduction of an internal standard would probably be the most effective time saver. Even without application of Yamasaki’s equation a reduction to about 3 days of total time needed per sample appears to be within reach. These improvements would make the remaining procedure suitable for industrial hygiene surveys with a relatively large number of samples. But even without these improvements one can conclude that -- if a high sensitivity and selectivity is needed -- this method appears to be a very good alternative to existing methods that use less sensitive detection systems such as gas chromatography with FID detection.
Nitro-PAH are highly mutagenic and carcinogenic compounds. Their presence in a variety of source emissions such as diesel exhaust or stack gases from power plants has been documented. Occupational exposure to nitro-PAH has hardly been investigated; but it appears reasonable to assume that significant concentrations can be found in industrial operations that have the following characteristics: heated coal tar pitch, incomplete combustion, exposure to ultraviolet light, nitric acid or ozone. Further research such as an industrial hygiene sampling survey is necessary to determine whether or not nitro-PAH pose an occupational health hazard.

The results obtained in this project suggest that the developed method for sampling, fractionation and analysis of nitro-PAH in air should be applied to industrial hygiene surveys and related measurements.
15. Recommendations for Future Work

The sample preparation procedure used requires a significant amount of time to be implemented to guarantee accuracy of analysis. Also, it is very time consuming in itself. In this work, the procedure has been tested only with clean Teflon coated filters spiked with nitro-PAH but not with large amounts of ambient airborne particulate matter on the filter. Given these limitations, it is recommended that future work focuses on three goals:

1) Reduction of the time required for sample handling;
2) Further characterization of the sampling procedure by experiments that include the chemical and structural complexity of ambient particulate matter;
3) Application of the modified procedure in industry or other suitable sampling locations.

The laboratory time requirement per sample could be reduced by improvements of the analytical procedure such as the introduction of an internal standard, the use of an integrator for the HPLC detector, a programmable fluorescence detector, and the application of Yamasaki’s empirical equation. Among these changes the introduction of an internal standard would probably be the most effective time saver. Even without application of Yamasaki’s equation a reduction to about 3 days of total time needed per sample appears to be within reach.
To further characterize the entire procedure additional (simulated) sampling experiments are desirable that include airborne particulate matter as sorbent for nitro-PAH. One option for such an experiment is outlined below.

Ambient airborne particulate matter is collected at the same sampling location and time by two samplers similar to the ones used in this work. After approximately 4 mg of particulate mass have been collected the samplers are stopped. One of the two filters is then spiked with a nitro-PAH standard solution, the other one is spiked with clean solvent only. A simulated sampling experiment similar to run 3 of this Master’s project follows during which both samplers run in parallel. Filters and PUF plugs are analysed and the nitro-PAH recoveries are determined. The results would show how selective and sensitive the HPLC fractionation and analysis procedures can determine nitro-PAH in sample extracts that contain many other environmental compounds. These results would therefore indicate whether or not further modifications of the analytical procedure are necessary.

Finally, an application of the (modified) procedure in industry is recommended in order to demonstrate its usefulness for industrial hygiene surveys that include large numbers of samples.
16. Acknowledgements

This project was completed because a number of people helped me with their experience, their resources, and their moral support.

Outstanding in this group is my adviser Professor Richard M. Kamens who served as the chair of my thesis committee and contributed especially his enormous experience as a research scientist with specialty in atmospheric chemistry and kinetics of PAH reactions in air. He was also so kind to let me use his laboratory facilities including a lot of chemicals, especially the nitro-PAH standards. But besides his experience, resources and the valuable discussions with him I could also enjoy the productive spirit and support of his research group consisting of Jim Fulcher, Douglas Bell, Guo Zhishi, and Hani Karam. All of them helped me with the many small difficulties that came up during my lab work. The discussions with both Professor Richard M. Kamens and his group members were a big learning experience for me and definitely supported this project, and I owe them thanks.

My project, however, did not consist only of laboratory work; the contacts with several industries regarding permission to take samples at some potential nitro-PAH sources at their facilities would not have been possible without Professor David A. Fraser’s efforts and help. As a member of my thesis committee he shared with me much of his "real world" experience in the field of occupational
safety and health in industry and greatly influenced this work in those aspects.

I am also very thankful to Professor Steven L. Simon who was kind enough to spend a lot of time in discussions with me. He certainly influenced this project with his public health point of view.

Besides Prof. Richard M. Kamens several other sources contributed material support for this project and are hereby appreciated: Professor Frederic K. Pfaender and the Program of Industrial Hygiene in the Department of Environmental Science and Engineering need to be honored for their support: Professor Frederic K. Pfaender, because he allowed me to use his Waters HPLC equipment including a fluorescence spectrophotometer, and the Program of Industrial Hygiene, because it supported the purchase of one HPLC analytical column and some solvents with a grand of $300. Another thank you I would like to say to Dr. Franc Sasser, Senior Chemist of Olympic Products Company, Greensboro, NC, for the generous supply of the polyurethane foam used in this project.

Especially during the last part of this work a number of very good friends encouraged me and gave me moral support: first of all, I would like to mention here Jennifer Gold who helped me finding a way out of many difficulties and also proof read the final draft of the report. My "Chapel Hill family" Lillian Stanienda and Yasushi Yoshimoto both helped me a lot especially with the many "last minute details" for the oral defense of this work.
To all of the persons mentioned I owe my deep thanks and the experience that even something fairly difficult and comprehensive can become manageable and be successfully completed if human and material support is available. Their help will not be forgotten.
17. References

1. Anonymous
   Verein Deutscher Ingenieure
   "Maximale Immissions-Konzentrationen (MIK), Organische
   Verbindungen", Duesseldorf, 1966

2. Anonymous
   International Agency for Research on Cancer,
   "IARC Monograph on the Evaluation of the Carcinogenic
   Risk of Chemicals to Humans"
   Part 2, volume 33, pages 171 - 222, 1984

3. Alfheim, I., Wirkstroem, L.
   "Air Pollution from Aluminum Smelting Plants I
   The Emissions of Polycyclic Aromatic Hydrocarbons and
   of Mutagens from an Aluminum Smelting Plant using the
   Soederberg Process"
   Toxicological and Environmental Chemistry
   Volume 8, pages 55 - 72, 1984

4. Alfheim, I., Lindskog, A.
   "A Comparisment Between Different High Volume Sampling System
   for Collecting Ambient Airborne Particles for Mutagenicity
   Testing and for Analysis of Organic Compounds"
   The Science of the Total Environment, volume 34, pages
   203 - 222, 1984

5. Alfheim, I., Moeller, M.
   "Mutagenic Activity in Photocopies"
   Science, volume 209, pages 1037 - 1039, 1980

6. Andersson, K.
   "Sampling and Analysis of Particulate and Gasous
   Polycyclic Aromatic Hydrocarbons from Coal Tar Sources in the
   Working Environment"
   Chemosphere, volume 12, number 2, pages 197 - 207, 1983

7. Andersson, K.
   "Sampling of Polychlorinated Aromatics in Workroom Air
   Using Amberlite XAD-2 Resin"
   Chemosphere, volume 10, number 2, pages 137 - 142, 1981

8. Becker, G., Bjorseth, A.
   "A Novel Method for the Determination of Occupational Exposure
   to Polycyclic Aromatic Hydrocarbons by Analysis of Body
   Fluids"
   in: Cooke, M., Dennis, A. J.
<table>
<thead>
<tr>
<th>No.</th>
<th>Author(s)</th>
<th>Title</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Bell, D. A.</td>
<td>&quot;Photoreaction of Wood Smoke Particles: Destruction and Creation of Mutagens in Sunlight&quot;</td>
<td>79th Annual Meeting of the Air Pollution Control Association, June 22, 1986, Minneapolis, Minnesota</td>
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<td>10</td>
<td>Berry, D. L.</td>
<td>&quot;Mutagenicity of Nitrofluoranthenes, 3-Aminofluoranthen and 1-Nitropyrene in Chinese Hamster V79 Cells&quot;</td>
<td>Carcinogenesis, volume 6, number 10, pages 1403 - 1407, 1985</td>
</tr>
</tbody>
</table>
18 Erickson, M. D.
"Development of Methods for Sampling and Analysis of Polychlorinated Naphthalenes in Ambient Air"
Environmental Science and Technology, volume 12, number 8, Pages 927 - 931, 1978

19 Fellin, P.
"A Critical Review of Nitro- and Oxy-PAH Compounds in the Atmosphere"
Prepared for Atmospheric Environment Service, Concord Scientific Corporation, 2 Tippet Rd., Downsview, Ontario M3H 2V2, Canada, March 1985

20 Flotard, R. D., Stetter, J. R.
"Workplace Air Sampling at Coal Conversion Facilities"
in: "Coal Conversion and the Environment: Chemical, Biological and Ecological Considerations"

21 Galasyn, J. F.
"The Loss of PAH from Quartz Fiber High Volume Filters"
Journal of the Air Pollution Control Association, volume 34, Number 1, pages 57 - 59, 1984

22 Gibson, T. L.
"Sources of Direct Acting Nitroarene Mutagens in Airborne Particulate Matter"
Mutation Research, volume 122, pages 115 - 121, 1983

23 Gibson, T. L.
"Nitro Derivatives of Polynuclear Aromatic Hydrocarbons in Airborne and Source Particulate Matter"
Atmospheric Environment, volume 16, number 8, pages 2037 - 2040, 1982

24 Gibson, T. L.
"Measurement of Polynuclear Aromatic Hydrocarbons, their Derivatives, and their Reactivity in Diesel Automobile Exhaust"
in: Cooke, M., Dennis, A. J. (Eds.)
"Polynuclear Aromatic Hydrocarbons", 1981

25 Gorse, R. A.
"1-Nitropyrene Concentrations and Bacterial Mutagenicity in On- Road Vehicle Particulate Emissions"
Environmental Science & Technology, volume 17, number 4, pages 198 - 202, 1983
26 Greensberg, A.
"Development of an Improved HPLC Analysis for Nitrated Polycyclic Aromatic Hydrocarbons and Quinones from Airborne Inhalable Particulate Matter"

27 Greensberg, A.
"Analysis of Nitrated Polycyclic Aromatic Hydrocarbons, PAH-Quinones and Related Compounds in Ambient Air", 1986, send by: Arthur Greensberg, Department of Chemical Engineering and Chemistry, New Jersey Institute of Technology, Newark, NJ 07102

28 Griest, W. H., Caton, J. E.
"Extraction of Polycyclic Aromatic Hydrocarbons for Quantitative Analysis",

29 Grosjean, D.
"Polycyclic Aromatic Hydrocarbons in Los Angeles Air from Samples Collected on Teflon, Glass and Quarz Filters"Atmospheric Environment, volume 17, number 12, pages 2565 - 2573, 1983

30 Grosjean, D.
"The Effect of Nitrogen Dioxide and Ozone on the Decomposition of Particle - Associated Polycyclic Aromatic Hydrocarbons During Sampling from the Atmosphere"Atmospheric Environment, volume 17, number 10, pages 2112 - 2114, 1983

31 Grosjean, D.
"Interactions of Polycyclic Aromatic Hydrocarbons with Atmospheric Pollutants"Environmental Science & Technology, volume 17, pages 673 - 679, 1983

32 Hanson, R. L.

33 Hunt, G. T.
34 Jaeger, J.
"Detection and Characterization of Nitro Derivatives of some Polycyclic Aromatic Hydrocarbons by Fluorescence Quenching after Thin - Layer Chromatography: Application to Air Pollution Analysis"

35 Johnson, N. D., Lane, D. A., Schroeder, W. H.
"Evaluation of a Denuder - Based Gas/Particle Sampler for Chlorinated Organic Compounds"

36 Jones, P. W.
"Efficient Collection of Polycyclic Aromatic Hydrocarbons from Combustion Effluents"
Environmental Science & Technology, volume 10, number 8, pages 806 - 810, 1976

37 Kamens, R. M.
"Effects of Temperature on Wood Soot PAH Decay in Atmospheres with Sunlight and Low NOx"
Atmospheric Environment, volume 20, number 8, pages 1579 - 1587, 1986

38 Kamens, R. M.
"The Influence of Temperature on the Daytime PAH Decay on Atmospheric Soot Particles"
79th Annual Meeting of the Air Pollution Control Association, June 22 - 27, 1986, Minneapolis, Minnesota

39 Kamens, R. M.
"Mutagenic Transformations of Dilute Wood Smoke Systems in the Presence of Ozone and Nitrogen Dioxide - Analysis of Selected High Pressure Liquid Chromatography Fractions from Wood Smoke Particulate Extracts"
Environmental Science & Technology, volume 19, number 1, pages 63 - 69, 1985

40 Kamens, R. M.
"Factors which Influence Polycyclic Aromatic Hydrocarbon Decomposition on Wood Smoke Particles"

41 Keller, C. D.
"Collection of Polycyclic Aromatic Hydrocarbons and other organics with a Glass fiber filter - Polyurethane Foam System"
Atmospheric Environment, volume 18, number 4, pages 837 - 845, 1984

42 King, L. C.
"Binding of 1-Nitropyrene to DNA and Protein in Cultured Lung Macrophages and Respiratory Tissue"
Cancer Letters, volume 19, pages 241 - 246, 1983
43. Korfmacher, W. A.
"Oxidative Transformation of Polycyclic Aromatic Hydrocarbons Adsorbed on Coal Fly Ash"
Science, volume 207, pages 763 - 765, 1980

44. Larsson, B.
"Polycyclic Aromatic Hydrocarbons in Lettuce - Influence of a Highway and an Aluminum Smelter"
in: Cooke, M., Dennis, A. J.
"Polynuclear Aromatic Hydrocarbons", pages 417 - 426, 1982

45. Lee, F. S. - C.
"The Problem of PAH Degregation During Filter Collection of Airborne Particulates - An Evaluation of Several commonly used Filter Media"
in: Bjorseth, A., Dennis, A. J. (Eds.)

46. Lewis, R. G.
"Problems Associated with Sampling for Semivolatile Organic Chemicals in Air."

47. Lewis, R. G.
"Portable Sampler for Pesticides and Semivolatile Industrial Organic Chemicals in Air"
Analytical Chemistry, volume 54, pages 310 - 315, 1982

48. Lewis, R. G.
"Modification and Evaluation of a High-Volume Air Sampler for Pesticides and Semivolatile Industrial Organic Chemicals"
Analytical Chemistry, volume 54, number 3, pages 592 - 594, 1982

49. Lewis, R. G.
"Evaluation of Polyurethane Foam for Sampling of Pesticides, Polychlorinated Biphenyls and Polychlorinated Naphthalenes in Ambient Air"
Analytical Chemistry, volume 49, number 12, pages 1168 - 1672, 1977

50. Ligocki, M. P.
"Assessment of Adsorption/Solvent Extraction with Polyurethane Foam and Adsorption/Thermal Desorption with Tenax-GC for the Collection and Analysis of Ambient Organic Vapors"
Analytical Chemistry, volume 57, pages 1138 - 1144, 1985
51 Lindgren, J. L.
"A Comparison of Two Techniques for the Collection and Analysis of Polynuclear Aromatic Compounds in Ambient Air"
Journal of the Air Pollution Control Association, Volume 30, number 2, pages 166 - 168, 1980

52 Lindner, W.
"Analysis of Nitro-PAH in Diesel Exhaust Particulate Extracts with Multicolumn HPLC"
Chromatographia, volume 20, number 4, pages 213 - 218, 1985

53 Lindskog, A.
"Transformation of Polycyclic Aromatic Hydrocarbons During Sampling"
Environmental Health Perspectives, volume 47, pages 81 - 84, 1983

54 Lioy, P. J.
"Air Sampling Instruments for Evaluation of Atmospheric Contaminants"
Chapter D: "Air Monitoring for Organic Constituents"
6th ed., 1983, ACGIH, Cincinatty, Ohio

55 MacCrehan, W. A., May, W. E.
"Oxygen Removal in Liquid Chromatography with a Zinc Oxigen-Scrubber Column"
Analytical Chemistry, volume 56, pages 625 - 628, 1984

56 MacCrehan, W. A., May, W. E.
"Determination of Nitro-Polynuclear Aromatic Hydrocarbons in Diesel Sood by Liquid Chromatography with Fluorescence and Electrochemical Detection"
Organic Analytical Research Division, National Bureau of Standards, Washington, DC 20234

57 Maeda, T.
"Induction of Squamous Cell Carcinoma in the Rat Lung by 1,6-Dinitropyrene"
Journal of the National Cancer Institute, volume 76, number 4, Pages 693 - 701, 1986

58 March, J.
Advanced Organic Chemistry
2nd edition, 1977

59 Morita, K., Fukamachi, K., Tokiwa, H.
"Studies on Aromatic Nitro Compounds in Air; II. Determination of Aromatic Nitro compounds in Airborne Particulates by Gas Chromatography" (in Japanese), Bunseki Kagaku, volume 31, pages 255 - 260, (citated according to reference 2)
60 Mosberg, A.
"Properties of 3H-Labeled 1-Nitropyrene deposited onto Fly Ash",
in: Cooke, M., Dennis, A. J.
"Polynuclear Aromatic Hydrocarbons", pages 551 - 566, 1982

61 Nielsen, T.
"The Presence of Nitro-PAH in Samples of Airborne Particulate Matter"
in: "Polynuclear Aromatic Hydrocarbons", 7th International Symposium, Columbus, OH., Battelle Press, pages 963 - 970, 1983

62 Nielsen, T.
"Isolation of Polycyclic Aromatic Hydrocarbons and Nitro Derivatives in Complex Mixtures by Liquid Chromatography"
Analytical Chemistry, volume 55, pages 286 - 290, 1983

63 Oehme, M.
"Quantitative Determination of Ultra-Traces of Chlorinated Compounds in High - Volume Samples from the Arctic Using Polyurethane Foam as Collection Medium"
Fresenius Zeitschrift fuer Analytische Chemie, volume 311, Pages 665 - 673, 1982

64 Oehme, M.
"Determination of Nitrated Polycyclic Hydrocarbons in Aerosols Using Capillary Gas Chromatography Combined with Different Electron Capture Detection Methods"

65 Ohgaki, H.
"Carcinogenicity in Rats of the Mutagenic Compound 1-Nitropyrene and 3-Nitrofluoranthene"
Cancer Letters, volume 15, pages 1 - 7, 1982

66 Pitts, J. N.
"The Gas-Phase Reaction of Naphthalene with N2O5 to Form Nitronaphthalenes"
Atmospheric Environment, volume 19, number 5, pages 701 - 705, 1985

67 Pitts, J. N.
"Reaction of Adsorbed Pyrene and Perylene with Gaseous N2O5 under Simulated Atmospheric Conditions"
Atmospheric Environment, volume 19, number 6, pages 911 - 915, 1985

68 Pitts, J. N.
"Atmospheric Reactions of Polycyclic Aromatic Hydrocarbons : Facile Formation of Mutagenic Nitro Derivatives"
Science, volume 202, pages 515 - 519, 1979
69 Ramdahl, T.
"Nitration of Polycyclic Aromatic Hydrocarbons Adsorbed to Different Carriers in a Fluidized Bed Reactor"
Chemosphere, volume 13, number 4, pages 527 - 534, 1984

70 Rappaport, S.
"High Performance Liquid Chromatography with Reduction-Electrochemical Detection of Mutagenic Nitro-Substituted Polynuclear Aromatic Hydrocarbons in Diesel Extracts"

71 Rickert, D.
"Toxicity of Nitroaromatic Compounds"

72 Robbat, A.
"Gas Chromatographic Chemiluminescent Detection and Evaluation of Predictive Models for Identifying Nitro-PAH in a Diesel Fuel Particulate Extract"

73 Rohlfs, W.
"Determination of Concentration and Mutagenicity of Eleven Organic Fractions adsorbed on both Lung-Penetratable and Non-Lung-Penetratable Berlin Airborne Particles during One Year of Sampling"; original title in German:
"Konzentrationsbestimmungen und Mutagenitaet von Elf Organischen Fraktionen der Lungengaengigen und Nicht-Lungengaengigen Partikel des Berliner Stadtaerosols wahrend eines Jahres"
Main Study Project for the degree "Diplom-Ingenieur" (transl.:Master's degree) in environmental engineering, 1984, Dipl.-Ing. Werner Rohlfs, Technische Universitaet Berlin, Fachbereich Umwelttechnik, Fachgebiet Hygiene, Amrumer Strasse, D-1000 Berlin 65, West Germany

74 Rosenkranz, H. S.
"The Genotoxicity, Metabolism and Carcinogenicity of Nitrated Polycyclic Aromatic Hydrocarbons"

75 Rosenkranz, H. S.
"Mutagenicity and Genotoxicity of Nitroarenès - All Nitro-Containing Chemicals were not Created Equal"
Mutation Research, volume 114, pages 217 - 267, 1983

76 Rosenkranz, H. S.
"Nitropyrenes: Isolation, Identification, and Reduction of Mutagenic Impurities in Carbon Black and Toners"
Science, volume 209, pages 1039 - 1043, 1980
77 Schuetzle, D.
"Sampling of Vehicle Emissions for Chemical Analysis and Biological Testing"
Environmental Health Perspectives, volume 47, pages 65 - 80, 1983

78 Schuetzle, D.
"Factors influencing the Emission of Nitrated Polynuclear Aromatic Hydrocarbons (Nitro-PAH) from Diesel Engines"
Journal of the Air Pollution Control Association, volume 33, Number 8, pages 751 - 755, 1983

79 Siak, J.
"Contribution to Bacterial Mutagenicity from Nitro-PAH Combustions in Ambient Aerosols"
Atmospheric Environment, volume 19, number 2, pages 369 - 376, 1985

80 Simon, C. G.
"Sampling Airborne Polychlorinated Biphenyls with Polyurethane Foam - Chromatographic Approach to Determining Retention Efficiencies"
Analytical Chemistry, volume 51, number 8, pages 1110 - 1113, 1979

81 Stray, H.
"Determination of Substituted Polycyclic Aromatic Hydrocarbons in Urban Air Particulate Matter - Method Development and Quantitative Results"
Norwegian Institute of Air Research, Report No. NILU OR 5/84, 1984

82 Stray, H.
"Selective Determination of Substituted PAH in Aerosols Using Liquid CO2-Extraction, HPLC Prefractionation on Chemically Activated Silica, and HRGC Combined with Negative Ion Mass Spectrometry"

83 Tejada, S. B.
"Analysis of Nitroaromatics in Diesel and Gasoline Car Emissions"

84 Thrane, K. E.
"High-Volume Sampling of Airborne Polycyclic Aromatic Hydrocarbons Using Filters and Polyurethane Foam"
Atmospheric Environment, volume 15, number 6, pages 909 - 918, 1981
Tokiwa, H.  
"Mutagenic Assay of Aromatic Nitro Compounds with Salmonella typhimurium"  
Mutation Research, volume 91, pages 321 - 325, 1981

Wang, Y. Y.  
"Direct - Acting Mutagens in Automobile Exhaust"  

Weast, R. C. (Edit.)  
"Handbook of Chemistry and Physics"  

White, C. M.  
"Nitrated Polycyclic Aromatic Hydrocarbons"  
Dr. Alfred Huethig Verlag, Heidelberg, 1985  
ISBN 3-7785-1029-0

Xu, X. B.  
"Isolation and Identification of Mutagenic Nitro-PAH in Diesel - Exhaust Particulates"  
Analytica Chimica Acta, volume 136, pages 163 - 174, 1982

Yamasaki, H.  
"Effects of Ambient Temperature on Aspects of Airborne Polycyclic Aromatic Hydrocarbons"  
Environmental Science & Technology, volume 16, number 4, pages 189 - 194, 1982

Yokley, R. A.  
"The Effect of Nitrogen Dioxide on the Photochemical and Nonphotochemical Degradation of Pyrene and Benzo[a]pyrene Adsorbed on Coal Fly Ash"  
Chemosphere, volume 14, pages 1771 - 1778, 1985

You, F.  
"Influence of Volatility on the Collection of Polycyclic Aromatic Hydrocarbon Vapors with Polyurethane Foam"  
Environmental Science & Technology, volume 18, pages 330 - 333, 1984

Zimmerman, N. J.  
"An Initial Investigation to Determine the Effectiveness of Polyurethane Foam as a Collecting Medium for Atmospheric Pesticide Residues"  
A report (thesis) submitted to the faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering in the Department of Environmental Science and Engineering, Chapel Hill, NC, 1976
Appendix: Proposal for Sampling of Nitro-PAH in Industry

The developed method was attempted in industry. In order to find suitable sampling sites the proposal presented below was send to a number of industries that were suspected to have nitro-PAH emitting operations.

Unfortunately, it became very difficult to get access to such sampling sites so that the initial goal of a practical application of this method in industry could not be reached. However, it is believed that the performance of the method developed in this project is capable of measuring nitro-PAH in air given the good results obtained under controlled conditions.
GENERAL BACKGROUND AND PURPOSE:

This proposal should be seen in context as a Master's project which will help to determine the extent to which nitrated polynuclear aromatic hydrocarbons (Nitro-PAH) exist in the indoor environment of various industries and ultimately the potential occupational exposure of workers in these environments.

This project includes the following aspects:
1. development of a procedure to sample and analyse Nitro-PAH in air,
2. evaluate its analytical parameters (recovery efficiency, variability of data, etc.). The analytical evaluation is currently under way and will be completed by mid September.
3. sampling for Nitro-PAH in an occupational environment. This will be done in areas where the potential for PAH emissions exists.

For the last part of this project I will need your help. Primarily I will need to take a few samples at an industrial operation that potentially includes considerable PAH emissions.

**FACULTY ADVISORS OF THIS MASTER'S PROJECT:**

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HEALTH HAZARDS OF NITRO-PAH:

Many Nitro-PAH have been proven to be very strong mutagens in bacterial test systems (1) and some have recently been identified to be carcinogenic in animal test systems (2). Their potential health hazard for humans is still not known.

POTENTIAL SOURCES OF NITRO-PAH EXPOSURE IN INDUSTRY:

Presently there is almost no information in the technical literature which addresses the presence or absence of Nitro-PAH in the industrial environment. The one qualitative study on Nitro-PAH in the indoor environment of an aluminum smelter found some of these compounds (3).

Nitro-PAH are possibly emitted or generated during operations that include heating or baking of coal or pitch or their products to temperatures typically used in industry. The emitted vapours may contain traces of Nitro-PAH.

Moreover, Nitro-PAH can be formed in the atmosphere by photochemical reactions between nitrogen oxides or nitric acid with non-nitrated PAH. Laboratory studies (4, 5, 6, 7) demonstrate reaction pathways which may also be present in the indoor working environment. These pathways can be summarized as follows:
OH radicals, NO₂, light, probably catalytic

\[ \text{Pyrene} \rightarrow \text{2-Nitropyrene} \]

**SAMPLING PROGRAM:**

**Time of sampling:** One or two sampling visits within the period of mid September - mid October, sampling time per sample: 2 - 8 hrs, depending on estimated concentration of organics in the air.

**Number of samples:** 2 - 6 per visit.

**Kind of sampling:** High vol sampling. Teflon-coated glass fiber filters for particle bound Nitro-PAH, polyurethane foam plugs and XAD-2 resin for volatile Nitro-PAH. At present two high vol samplers and their aluminum sampling houses are ready for use.

**Principle of analysis:** Fractionation of extract by high performance liquid chromatography (HPLC), separation of Nitro-PAH compounds in the corresponding fraction by reversed phase HPLC, followed by on-line reduction using a Pt/Rh catalyst into amines for fluorescence detection. These techniques have already been implemented in the Department of Environmental Science and Engineering.
HELP REQUESTED FROM YOUR COMPANY:

1. The identification of an industrial operation or process in your company that involves expected or known emissions of PAH into air. If such an operation is in use during this fall I would ask for an introduction into this process, preferably by an industrial hygienist or a safety engineer, as well as for information about the types of exposure involved with this operation. Data about the concentrations of PAH measured previously at the operation would be extremely useful.

2. The permission to take samples as outlined above (including the permission to use your company's power supply system for the samplers). A contact person would be necessary. If a visit of your company were to be inappropriate or inconvenient I would ask you to take the samples yourself by an industrial hygienist or another person of your company trained in taking area samples. In this case I would send to you by mail the sampling equipment as well as information about how to use it. It would be already calibrated and ready for use. After sampling is finished, I would ask you to send it back to me without opening the device or otherwise touching the sampling media. Moreover, I would need to know several sampling parameters (sampling time, temperature, description of sampling location, size of room/hall where the operation is located, etc).

3. The Master's report will not include any information that would help to identify your company. I only ask for the permission to use the data obtained as well as descriptive information about the
particular process in my Master's report. However, the scientific interpretation of the data must be mine. You would receive a draft copy of the report prior to submission for your review.

Please let me or one of my advisors know about your decision about this proposal by September 30, 1986.

Thank you very much for your interest!

Yours respectfully

Werner Rohlfs
REFERENCES (of appendix):

1. Tokiwa H. et al.  

2. Rosenkranz H. S., Mermelstein R.  


4. Pitts J. N. et al.  

5. Bell D. A., Kamens R. M., Claxton L. D., Lewtas J.  
"Photoreaction of Wood Smoke Particles: Destruction and Creation of Mutagens in Sunlight" Paper presented at the 79th Annual Meeting of the Air Pollution Control Association, APCA 86-77.4, June 22, 1986, Minneapolis, Minnesota

6. Jager J., Hanus V.  

7. White C. M., Volume editor  
"Nitrated Polycyclic Aromatic Hydrocarbons" Dr. Alfred Huethig Verlag, Heidelberg (West Germany), 1985 ISBN 3-7785-1029-0