INVESTIGATIONS INTO THE AQUATIC TOXICITY OF PHENANTHRENE

(cover-report for reproduction tests with the waterflea *Daphnia magna* and an Early Life Stage (ELS) test with the zebra fish *Brachydanio rerio*

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Authors : Drs R.N. Hooftman
Ms A. Evers-de Ruiter

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Sponsor : RIZA and Ministry of Housing, Physical Planning and the Environment
c/o RIZA
Maerlant 4-6
Postbus 17
LELYSTAD

CONTENTS

SUMMARY

In the present report the results of studies into the aquatic toxicity of phenanthrene and preliminary experiments for these studies are summarized.

Phenanthrene was the first Polycyclic Aromatic Hydrocarbon (PAH) studied in a project on the aquatic toxicity of PAHs. Specific problems, related to the solubility and stability of phenanthrene in the dilution water used for the tests, were met. Dosing of phenanthrene directly into the dilution water or dosing from stock solutions in an organic solvent did not result in the expected concentrations. It was therefore decided to prepare saturated phenanthrene solutions by column technique.

Dilution water was purged through a stainless steel column, packed with small inert particles, coated with phenanthrene. The generated solution was analyzed and used to prepare dilutions for the aquatic toxicity tests. Although concentrations were reached, which could be expected on basis of the aqueous solubility, these solutions appeared not to be stable. From some tests on the stability of phenanthrene solutions it was concluded that its stabilty in semi-static tests was at least influenced by:

- The presence of the test animals and food.
- Size of the test vessel.
- Aeration of the test vessels (to a lesser extent).

Because it appeared not to be possible to keep phenanthrene concentrations stable in semi-static tests and only peak exposures could be reached, it was concluded that the use of a flow through system was the only approach to keep phenanthrene concentrations more or less stable during aquatic toxicity tests. In the flow through system phenanthrene concentrations could be maintained on a relatively constant level; actual concentrations were between 78% and 142% (average percentage present during the test: 118%) of the nominal concentrations.

Ultimately three tests with phenanthrene were carried out:

- a semi- static test with the zebra fish *Brachydanio rerio*
- a semi-static test with the water flea *Daphnia magna*
- a flow through test with the water flea *Daphnia magna*.

The results of these tests were in nominal and actual concentrations and in mg/l:

Semi-static test with B.rerio

	nominal conc.	actual conc.
NOEC (growth):	0.032	peak of 0.028 decreasing to below the detection level
LOEC (growth):	0.056	peak of 0.049 decreasing to below the detection level

Semi-static test with D. magna

	nominal conc.	actual conc.
EC50 (reprod):	>0.1 and < 0.18	peak >0.08 and <0.15 decreasing to below the detection level
NOEC (reproduction):	0.18	peak of 0.15 decreasing to below the detection level
LOEC (reproduction):	0.32	peak of 0.27 decreasing to below the detection level

Flow through test with D. magna

	nominal conc.	actual conc.
EC50 (reprod):	0.042	0.050
NOEC (reprod):	0.018	0.021
LOEC (reprod):	0.032	0.037

LC50, NOEC and LOEC values for *D. magna* are 3 - 10 times lower in the flow through test compared with those of the semi-static test (in which only peak exposures could be reached). The lowest NOEC value quantitatively determined was 0.018 mg/l.

The ELS test with the zebra fish *Brachydanio rerio* was only carried out semi-statically. It can be expected that exposure to more stable solutions will also result in toxicity values of at least three times lower than those in the semi-static test.

1. INTRODUCTION

Safety levels for Polycyclic Aromatic Hydrocarbons (PAHs) are now mainly based on QSARS. At the request of RIZA and the Dutch Ministry of Housing, Physical Planning and the Environment aquatic toxicity tests according the OECD Guidelines have been carried out to provide a better basis for the establishment of these levels.
Initially semi static tests (reproduction test with *Daphnia magna* and an early life stage test with the zebra fish *Brachydanio rerio*) with several PAHs, including phenanthrene, were planned. Due to problems in the stability of the test substances in aqeuous solution it was necessary to change the program.
Semi static tests with *D. magna* and *B. rerio* and (intermittent) flow through tests with *D. magna* have been carried out with phenanthrene. The results of this work are reported in the following reports:

REPORT IMW-TNO R 91/060
THE INFLUENCE OF PHENANTHRENE ON THE REPRODUCTION OF *DAPHNIA MAGNA* (semi-static test, OECD GUIDELINE NO. 202)

REPORT IMW-TNO R 91/058
THE INFLUENCE OF PHENANTHRENE ON THE REPRODUCTION OF *DAPHNIA MAGNA* (intermittent flow through test, OECD GUIDELINE NO. 202)

REPORT IMW-TNO R 91/059
THE INFLUENCE OF PHENANTHRENE ON THE EARLY LIFE STAGES OF *BRACHYDANIO RERIO* (semi-static test, OECD DRAFT GUIDELINE)

The present report is a "Cover Report" in which the the work carried out with phenanthrene is summarized. Particular attention is given to:

- Preparation of test solutions by column technique
- Maintainance of test concentrations during the test
- Comparison of the results in the tests carried out

2. STABILITY OF PHENANTHRENE IN THE TEST SOLUTIONS

2.1 Preliminary experiments

In preliminary experiments an attempt was made to prepare solutions of phenanthrene by dosing it from a stock solution in an organic solvent (tertiary butyl alcohol = TBA). After 30 minutes of ultrasonic vibration a quantity of 100 mg of phenanthrene appeared to be dissolved (visually assessed) in 10 ml TBA. Furthermore the stability of the test substance was investigated in test solutions without test animals.
Dosing of the test substance from stock solutions in TBA resulted in actual concentrations, which were far less than the nominal concentrations (between 3% and 63% of nominal (just after dosing) and between the detection limit and 4% after 48 hours
Shaking of 1 mg phenanthrene with one litre of dilution water resulted in a solution of only 0.063 mg per litre.

2.2 Stability of test solutions during the testing

Chemical analysis showed that concentrations of phenanthrene decreased in the semi-static tests with *D. magna* and *B. rerio* from average values of respectively 87 % and 83 % to values just above or below the detection limit immediately before replacement of the test solutions. The instability of phenanthrene solutions can be attributed to various factors such as adorbtion to the walls of the test beakers, absorbtion by food, absorbtion by the test animals, volatilization and degradation of the compound.

2.3 Test for stability

Some tests for stability were carried out with phenanthrene solutions with a nominal concentration of 0.18 mg/l. Phenanthrene solutions were prepared by column technique as described in section 3. Thereafter they were analyzed and diluted to 0.18 mg/l.
400 ml of this phenanthrene solution was placed in test beakers of 800 ml and 50 ml was placed in both a glass beaker and a plasic beaker of 100 ml. These test vessels were treated as follows.

400 ml test vessels without aeration; no additions
400 ml test vessels with aereation; no additions
400 ml test vessels with aereation + *C. pyrenoidosa*
400 ml test vessels with aereation + *C. pyrenoidosa* + *D. magna*
400 ml test vessels with aereation + yeast
400 ml test animals with aereation + yeast + *D. magna*
50 ml glastic vessels without aereation; no additions
50 ml plastic vessel without aereation; no additions

Daphnias of one week old and a quantity of yeast and *Chlorella pyrenoidosa*, required at that age were added to each test vessel.

Samples were taken from the newly prepared solutions and after 24 hours and 48 hours from the "spent" solutions.

The results of these stability tests are given in Table 1.

Table 1 *Test for the stability of phenanthrene. Phenanthrene concentrations in solutions kept under various conditions are measured at the start of the test, after 24 h and after 48 h.*

Time	400 ml solution in glass vessel						50 ml solution	
(h)	a	n.a.	a + Cp	a + Cp + Dm	a + y	a + y + Dm	glass vessel	poly-ethylene vessel
0	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
24	0.12	0.10	0.086	0.038	0.020	0.029	0.040	0.031
48	0.020	0.051	0.034	< 0.010	0.012	< 0.010	< 0.010	< 0.010

a = aerated
n.a. = not aerated
Cp = *Chlorella pyrenoidosa* added
y = yeast added
Dm = *Daphnia magna* (ten animals, one week old) added

From the stability test the following can be concluded:

- There is some influence of aeration on the test substance concentration. However, this remains small

- By addition of the test animals and/or food (in particular yeast) the test substance concentration decreases.
- The material of which the test vessels are made has no influence on the test substance concentration.
- The stability of phenanthrene is influenced by the size of the test vessel.
- It is not possible to maintain the test substance concentrations in a semi-static test.

Because the desired concentrations could not be reached it was decided to determine the solubility of phenanthrene in DSWL (the dilution water used) by preparing saturated solutions by column technique and to use these solutions with several dilutions of it for the toxicity tests. The method used is described in section 3 and Annex A.

3. PREPARATION OF SATURATED SOLUTIONS BY COLUMN GENERATION

3.1 Principle of column generation

Methods for the determination of the aqueous solubility of PAHs by column generation have been developed in the past. The same technique can also be used to prepare saturated solutions in dilution water for aquatic toxicity tests (Billington et al, 1988; ref. 1 and May et al, 1978; ref. 2). The method is based on pumping water through a column packed with small inert particles, which are coated with the substance being tested. As a result of the high surface area and a sufficient contact time between water and particles, water saturated solutions can be produced in a reproducable way.

3.2 Preparation of the column

Saturated phenanthrene solutions were prepared by column technique. A solution of 0.1 % (w/v) phenanthrene in acetone was prepared. Chromosorb Q, 60/80 mesh, Chrompack, was used as high surface area packing material and coated with the phenanthrene solution. The acetone was stripped by rotation evaporation. The coated material was stored at room temperature in a brown glass bottle in the dark. Due to the photosensitivity of PAHs all handling was carried out under a darkened hood.
Stainless steel columns of 28 cm and a diameter of 0.6 cm were filled with about 1.0 gram of the coated material. DSWL water was purged through the column with an HPLC pump. The flow rate varied between 1 ml and 3 ml per minute. The column was warmed in a water basin to 25 degrees celsius. The flow was received in a brown glass bottle.

Preliminary experiments resulted in measured concentrations of 0.88 - 1.5 mg/l. This is in conformity with the aqueous solubility (in distilled water ?) as reported for phenanthrene in the Integrated Criteria Document PAHs (ref. 3). The highest concentrations were measured when analyses were carried out immediately after release of the solution from the column. From later experience it is clear that the differences in concentrations are probably caused by the instability of the the compound.

The concentrations measured in the solutions used in the ecotoxicity tests varied between 0.42 and 1.6 mg/l (see Tables 1 in R 91/059 en R 91/060). Rinsing the bottles with phenanthrene solutions did not solve the problem of the variation in concentration. Therefore the generated solutions were analyzed and on basis of these analyses appropriate dilutions were then made to prepare the desired test solutions.

The method used for the preparation of the saturated solutions of phenanthrene is described in a Standard Operating Procedure, which is given in Annex A (drafted by Drs A.J.M. Blom).

4. THE TOXICITY OF PHENANTHRENE TO AQUATIC ORGANISMS

Three tests with phenanthrene and aquatic organisms were carried out in this project. A semi-static and a flow-through reproduction test with *Daphnia magna* and a semi-static early life stage test with the zebra fish *Brachydanio rerio*. These studies are reported in the IMW-TNO reports R91/060, R 91/058 and R 91/059 and the summaries of these reports are given here below.

Semi-static early life stage test with *Brachydanio rerio* (Rapport IMW R91/059)

SUMMARY

This report is a part of a study for the aquatic toxicity of phenanthrene.
The influence of phenanthrene on hatching, mortality and presence of malformations of eggs and larvae of the fresh water fish species *Brachydanio rerio* was determined, as laid down in the Draft OECD Guideline, with an exposure period of 28 days.
The test was carried out as a semi static test, with a replacement of the test solutions three times per week. Saturated solutions were prepared by column technique. All concentrations tested were prepared by dilution of these saturated solutions.
The nominal concentrations of phenanthrene tested were 0.018, 0.032, 0.056, 0.10, 0.18, 0.32 and 0.56 $mg.l^{-1}$. The concentration of 0.56 $mg.l^{-1}$ was tested in a second (additional) test together with again the 0.32 $mg.l^{-1}$ concentration (this concentration was thus tested twice).
The results of the second (additional) test corresponded with those of the first test.

Hatching of eggs of *Brachydanio rerio* was not affected by phenanthrene at any of the concentrations tested.
Mortality was not significantly different from the control animals at any of the concentrations tested.
At a concentration of 0.056 $mg.l^{-1}$ and at the higher concentrations tested the length of the test animals was significantly less than that of the control animals ($p = 0.05$ and $p = 0.01$ respectively).
At a concentration of 0.10 $mg.l^{-1}$ and higher concentrations tested the wet weight of the test animals was significantly less ($p = 0.01$) than that of the control animals.
The lowest NOEC for the criteria quantitatively investigated was 0.032 $mg.l^{-1}$ (nominal value)

The detailed results of the tests were (in nominal concentrations):

<u>With respect to mortality</u>
28 days LOEC: > 0.56 $mg.l^{-1}$ 28 days NOEC: ≥ 0.56 $mg.l^{-1}$

<u>With respect to hatching of eggs</u>
28 days LOEC: > 0.56 $mg.l^{-1}$ 28 days NOEC: ≥ 0.56 $mg.l^{-1}$

With respect to growth

28 days LOEC (length) : 0.056 $mg.l^{-1}$ 28 days NOEC: 0.032 $mg.l^{-1}$

28 days LOEC (weight) : 0.10 $mg.l^{-1}$ 28 days NOEC: 0.056 $mg.l^{-1}$

Chemical analysis showed that concentrations of phenanthrene decreased from an average value of 87% of the nominal concentration (just after preparation of the solutions) to values just above or below the detection limit just before replacement of the test solutions.

The results of this test thus counts for "peak" exposures to phenanthrene. In a semi static test it appeared to be not possible to maintain concentrations. The problems on the stability of phenanthrene in aquatic toxicity tests are further discussed in the "cover" report R 91/057.

Semi-static reproduction test with *Daphnia magna* (Rapport IMW R91/060)

SUMMARY AND CONCLUSIONS

This report is part of a study for the aquatic toxicity of phenanthrene.
Phenanthrene was tested for inhibition of reproduction of *Daphnia magna* according to the OECD Guideline no. 202 (ref. 1). Two tests were carried out because the control mortality in the first test exceeded the allowed 20%.
4 x 10 daphnias were used for each test concentration (quadruplicates for each concentration). The exposure duration was 19 days (first test) and 21 days (second test).
The test was carried out as a semi-static test with replacement of the test solutions three times per week.
The nominal concentrations tested were 0.018, 0.032, 0.056, 0.10, 0.18 and 0.32 $mg.l^{-1}$ (first test) and 0.032, 0.056, 010, 0.18 and 0.32 $mg.l^{-1}$ (second test). Saturated solutions were generated by column technique. All concentrations tested were prepared by dilution of these saturated solutions.

The detailed results of the reproduction test were in nominal concentrations:

First test:

with respect to reproduction			with respect to mortality		
mean number of offspring in the control: 65.					
19d EC50	:	> 0.10 and < 0.18 $mg.l^{-1}$	19d LC50	:	0.28 $mg.l^{-1}$
19d NOEC[1)]	:	≥ 0.10 $mg.l^{-1}$	19d NOEC[1)]	:	0.18 $mg.l^{-1}$
19d LOEC[2)]	:	> 0.18 $mg.l^{-1}$	19d LOEC[2)]	:	0.32 $mg.l^{-1}$
			The control mortality was 25% at t = 19 days.		

with respect to size and condition (swimming behaviour, colour or any other visual observable morphological or behavioural criterion) as visually assessed
NOEC or LOEC values could not be determined because differences in condition were observed in the replicates of test concentrations and the lack of a dose effect relationship.

1) No observed effect concentration according to OECD Guideline no. 202.
2) Lowest observed effect concentration according to OECD Guideline no. 202.

Second test:

with respect to reproduction			with respect to mortality
mean number of offspring in the control: 84.			
21d EC50	:	about 0.32 $mg.l^{-1}$	Could not be determined because of differences in mortality were observed in the replicates of test concentrations and the lack of a dose effect relationship.
21d NOEC[1)]	:	0.18 $mg.l^{-1}$	
21d LOEC[2)]	:	0.32 $mg.l^{-1}$	

with respect to condition (visually assessed)

Could not be determined because of differences in condition were observed in the replicates of test concentrations and the lack of a dose effect relationship.

Chemical analyses showed that the concentrations of phenanthrene decreased in 48 h from an average value of 83% of the nominal concentration (just after preparation of the solutions) to values just above or below the detection limit (just before replacement of the test solutions). At t = one day the level of phenanthrene was maintained at 57% of the dosed value during, however, a 24 h period. The problems on the stability of phenanthrene are discussed in the "cover" report R 91/057.

The results obtained for mortality and reproduction varied in both tests within replicate test concentrations and there were no clear dose effect relationships. This may indicate that a semi static test method is not suitable for a reproduction test with *D. magna* and phenanthrene. The problems on stability of the test substance and the irregular results obtained were solved by using an intermittent flow through system as reported in subreport R 91/059.

1) No observed effect concentration according to OECD Guideline no. 202.
2) Lowest observed effect concentration according to OECD Guideline no. 202.

Flow through reproduction test with *Daphnia magna* (Rapport IMW R91/058)

SUMMARY

This report is part of a study for the toxicity of phenanthrene. Phenanthrene was tested for inhibition of reproduction of *Daphnia magna* according to the OECD Guideline no. 202 (ref. 1).

The test was carried out with 4 x 10 daphnias in an intermittent flow through system (quadruplicates for each concentration). The exposure duration was 21 days.

The nominal concentrations tested were 0.018, 0.032, 0.056, 0.10, 0.18 and 0.32 $mg.l^{-1}$.

The test substance was dosed from concentrated solutions in tertiary butyl alcohol and appeared to be completely dissolved at all concentrations tested (visually assessed).

The actual concentrations of phenanthrene were determined with HPLC. Measured concentrations of phenanthrene in the test solutions were between 78% and 142% of the nominal concentrations (average 118%).

The lowest NOEC for the criteria quantitatively investigated was 0.018 $mg.l^{-1}$ (nominal and measured)

The detailed results of the reproduction test were in nominal concentrations:

<u>with respect to reproduction</u>	<u>with respect to mortality</u>
mean number of offspring in the control: 161	
21d EC50 : 0.042 $mg.l^{-1}$	21d LC50 : 0.11 $mg.l^{-1}$
21d NOEC[1] : 0.018 $mg.l^{-1}$	21d NOEC[1] : 0.056 $mg.l^{-1}$
21d LOEC[2] : 0.032 $mg.l^{-1}$	21d LOEC[2] : 0.10 $mg.l^{-1}$

<u>with respect to size and condition</u> (swimming behaviour, colour or any other visual observable morphological or behavioural criterion)

21d NOEC[1] : 0.032 $mg.l^{-1}$

21d LOEC[2] : 0.056 $mg.l^{-1}$

1) No observed effect concentration according to OECD Guideline no. 202.
2) Lowest observed effect concentration according to OECD Guideline no. 202.

Comparison of both reproduction tests with *Daphnia magna* shows that the flow through test (in which phenanthrene concentrations could be maintained at a relatively constant level) resulted in effect-, NOEC- and LOEC-values being three to ten times lower than those in the semi-static test (in which peak exposure appeared to occur). The lowest NOEC value for the criteria quantitatively examined was 0.018 mg per litre.
The ELS test with the zebra fish *Brachydanio rerio* was only carried out semi-statically. The lowest NOEC found in this test was 0.032 mg per litre. It can be expected that in a flow through system effects would also occur at a lower concentration level of at least three times lower than in the semi-static test. For the ELS test with the zebra fish a level of 0.010 mg per litre or lower may be expected, beeing comparable with those of 0.018 mg per litre found for *Daphnia magna* in the flow through test.

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ANNEX A

1. INLEIDING

Het maken van een waterverzadigde oplossing van een stof gebeurt meestal door een overmaat van de stof toe te voegen aan een volume water en het geheel gedurende geruime tijd bij de gewenste temperatuur te mengen. Bij stoffen die slecht in water oplosbaar zijn wordt op deze manier niet altijd de verzadigingsconcentratie gehaald verder zijn de behaalde concentraties niet altijd reproduceerbaar. De oorzaak hiervan kan zijn dat er onvoldoende contact is tussen de stof en het water. Ook kan de stof nog als emulsie of deeltjes geresuspendeerd zijn in het water en is scheiding door filtratie of centrifugatie niet goed mogelijk.
Is de op te lossen stof bovendien nog vluchtig of lichtgevoelig dan geeft dit nog extra problemen. De hier beschreven kolomtechniek lost een aantal van de hierboven beschreven problemen op. Door te zorgen voor een groot contactoppervlak en voldoende contacttijd en door te voorkomen dat de stof gedispergeerd is in het water, kunnen op reproduceerbare wijze waterverzadigde oplossingen gemaakt worden. De stof wordt hiervoor gecoat op een kleine deeltjes inert drager materiaal en hiermee wordt een kolom gevuld. Door het variëren van de stroomsnelheid van het water door de kolom kan deze aangepast worden aan oplosbaarheid van de stof (zie voor een uitgebreidere uitleg Billington et al. 1988).

2. COATING DRAGERMATERIAAL

- Een 0,05-0,2% w/v oplossing maken van de stof in een organisch oplosmiddel.
- Dragermateriaal toevoegen zodat de verhouding drager/oplosmiddel is 1:10.
- Oplosmiddel verdampen in rotatieverdamper bij een lage temperatuur.
- Het verkregen dragermateriaal gecoat met 0,5-2% w/w van de stof in een bruine fles/pot doen en droog en in het donker bewaren.

3. KOLOM GIETEN

- Lege kolom voorzien van glaswolpluggen en inclusief sluitingen wegen.
- Sluitingen aan één kant vastdraaien, van de andere kant de sluiting en de glaswolplug verwijderen en de kolom verticaal vastklemmen.

- De kolom tot ca. 3 mm onder de rand vullen met gecoat dragermateriaal met behulp van een glazen trechter. Een goede homogene pakking wordt verkregen als tijdens het vullen tegen de kolom getikt wordt, of deze met behulp van een vibrator in trilling wordt gebracht.
  Vullen in de zuurkast en opletten voor stuivend dragermateriaal.

4. POMPAANSLUITINGEN

- Water vlak voor gebruik ontgassen door filtratie over filters met behulp van vacuüm of door doorgassing met helium gedurende 15 min.
- Voorraadvat aansluiten op de HPLC-pomp door de teflon aanvoerleiding met het inlaatfilter in het vat te plaatsen.
  Opletten dat het inlaatfilter niet verstopt zit, deze moet regelmatig (na elke 10-15 l) goed doorgespoeld worden in de andere richting, eventueel schoonmaken met hoogfrequente trillingen (sonic-bad). Als dit niet meer helpt vervangen.
- Pompkoppen "purgen" tot alle lucht uit de aanvoerleiding en koppen verwijderd is.
  "Purgen" Gynkotek HPLC-pomp:
  1. Purge-klep-schroef halve slag openen (tegen de klok in) en bekertje onder de opening houden.
  2. "Flow" schakelaar in de "AAN" positie gedrukt houden tot er een regelmatige stroom uit de pruge-uitlaat komt.
  3. "Flow" schakelaar loslaten geeft een continue stroom met de ingestelde snelheid. Uitzetten van de pomp door de "flow" schakelaar kort in de "OFF" positie te drukken.
  4. Purge-klep-schroef dicht draaien.
- Gepakte kolom aansluiten op de toevoerleiding (RVS, OD 1/16", ID 0,01") en de afvoerleiding (RVS, OD 1/16", ID 0,01") met behulp van fittings (Fingertights of metalen met ferrules).
- Kolom "purgen" tot alle lucht verwijderd is. De achterkant van de kolom omhooghouden versneld het verwijderen van de lucht.
- Opvangvat onder de afvoerleiding plaatsen.
- Stroomsnelheid instellen (1-5 ml/min).
- Pomp aanschakelen met de "Flow" schakelaar of met de klok en tussen schakelaar.

5 OPMERKINGEN, TIPS EN TRUCS

Algemeen

- De optimale stroomsnelheid door de kolom moet voor elke verbinding apart uitgezocht worden. Indien grote hoeveelheden waterige oplossing nodig zijn en de stof een erg lage oplosbaarheid heeft moet rekening gehouden worden met de snelheid waarmee de stof aan de glaswand hecht en met de tijd die nodig is om een voldoende hoeveelheid te maken.

- Indien de kolom nog niet uitgeput is en opgeslagen moet worden moeten de openingen na het verwijderen van de toe- en afvoerleidingen afgesloten worden met schroefjes om te voorkomen dat hij deels uitdroogt.

- Het gebruik van "fingertights" fittingen vergemakkelijkt het verwisselen van de kolommen, wel dient na elke verwisseling gecontroleerd te worden of de koppelingen niet lekken.

- Temperatuurverschillen tijdens het maken van een verzadigde oplossing geeft fluctuaties in de concentratie (May and Wasik, 1978). Om dergelijke concentratie verschillen te voorkomen wordt de kolom met een waterbad of kolomoven tot dezelfde temperatuur verwarmd als waarbij de oplossing nodig is.

- In verband met de instabiliteit van PAK's onder invloed van UV wordt zowel het droogdampen van het oplosmiddel als het maken van de waterige oplossing in een verduisterde zuurkast uitgevoerd.
  Tevens moeten het gecoate dragermateriaal en de waterige oplossingen zoveel mogelijk in het donker worden bewaard.

- Waterige oplossingen van PAK's gaan snel in concentratie achteruit zodat deze oplossingen alleen vlak voor gebruik gemaakt kunnen worden.

- Bij waterige oplossingen van PAK's hechten de PAK-verbindingen zich zeer snel en makkelijk aan oppervlakken van bijvoorbeeld leidingen en glaswerk. Voorspoelen van deze oppervlakken maakte bij fenantreen weinig uit, integendeel hoe vaker de

opvangflessen voor fenantreenoplossingen gebruikt waren des te sneller ging de concentratie van fenantreen in oplossing achteruit.

- Aceton is een geschikt organisch oplosmiddel voor PAK's.

6. MATERIALEN EN APPARATUUR

- Rotatie verdamper, met verwarmingsbad.

- Vacuümopstelling (waterstraalpomp met terugslagvat voldoet uitstekend).

- HPLC-pomp die een niet of zeer weinig pulserende vloeistofstroom kan geven met een instelbare stroomsnelheid tussen de 0,1 en 5 ml/min.

- Ontgassingsinstallatie
  a. Filterhouder voor teflon-, nylon- of nitrocellulosefilters, poriëngrootte 0,45-0,8 µm met een afzuigkolf aan te sluiten op de vacuüm opstelling. Filter/degasser Unit van Supelco voldoet uitstekend omdat hierbij geen speciale afzuigkolf nodig is.
  b. Aansluiting op helium fles/leiding via teflon slang en een RVS bruissteentje (b.v. deeltjes filter zoals aan de aanvoerleiding).

- Lege kolommen, RVS, lengte 25 cm, binnen diameter 10 of 4,6 mm. Hiervoor kunnen oude analytische en preparatieve kolommen gebruikt worden waaruit het oorspronkelijke dragermateriaal en de fritjes zijn verwijderd. Deze kolommen moeten goed schoongemaakt worden met aceton en methanol om eventuele resten van chemicaliën te verwijderen.
  Nieuwe lege kolommen zijn te verkrijgen bij Supelco; "Blanc columns" cat. no. 5-9101 (ID 4,6 mm) en 5-98217 (ID 10 mm).

- RVS HPLC-leidingen OD 1/16", ID 0,01".

- Teflon aanvoerleidingen met RVS-deeltjesfilter, poriëngrootte 0,2 µm.

- Fittingen voor de verbindingen. "Fingerthights" van plastic of RVS-fittingen met ferrules, voor 1/16" leidingen.

- Dragermateriaal Chromosorb Q, 60-80 mesh van Chrompack.

- DMCS (dimethylchloorsilaan) gecoat glaswol.

- Gereedschap voor het vast/losdraaien van de kolomsluitingen en -verbindingen.

7. LITERATUUR

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