Methods for Determination of Environmentally Important Physical-Chemical Properties of Polar Polycyclic Organic Material

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Methods for Determination of Environmentally Important Physical-Chemical Properties of Polar Polycyclic Organic Material

Ph.D. Thesis

by

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Preface

This dissertation constitutes my Ph.D. thesis in Environmental Chemistry. The work presented in the thesis has been carried out at Risø National Laboratory in Roskilde, Denmark, as a part of the Strategical Environmental Research Program. The supervisors have been Professor Poul Erik Hansen, University of Roskilde, Department of Life Sciences and Chemistry and Torben Nielsen, Risø National Laboratory, Department of Plant biology and Bio-Geochemistry.

The majority of the work presented here has been published in three peer reviewed articles in international journals and books (Helweg et al 1997 a, Helweg et al 1997 b and Helweg et al 1997 c). Extra reference data has been aquired since these publications and correlations presented in this thesis may therefore deviate slightly from those presented in the articles. Conclusions however are the same in all cases.

This thesis, with the subject of methods for determination of environmentally important physical-chemical properties of polar polycyclic organic material, has been divided into 10 chapters. After the introduction, three background chapters follow describing the properties and environmental occurrence of the polar polycyclic aromatic compounds studied, the definition and nature of environmentally important physical chemical parameters and available methods for determination of the physical-chemical parameters. These chapters give basic information that has been applied in the choice and development of methods as well as the evaluation of results as are described in chapters 5-9. Chapter 5, 6 and 7 concerns determination of the octanol water partition coefficients by direct experimental, indirect experimental and theoretical methods and chapter 8 and 9 concerns determination of organic matter-water partition coefficients and soil sorption coefficients respectively. After each chapter a short summary and discussion is given. Chapter 10 contains a final summary and a conclusion.

I wish to thank my supervisors Torben Nielsen and Poul Erik Hansen for their dedicated involvement in the project as well as for their invaluable advice. I have surely learned from their great experience. I gratefully acknowledge the economic support of the Center for Ecotoxicological Research under the Danish Environmental Research Program which has made the project possible. Anders Feilberg is acknowledged for his help with the GC-MS analyses and I also wish to thank the laboratory personnel that has assisted me with the experimental part of the project: Lene Engelbrecht Lind, Christian B. Søndergaard, Tina Sonne Sonberg, Elsebeth Kendix, Charlotte Grønlund Christiansen and Kristina Maria Goldberg who all were a great help and good company. Finally I wish to thank the regular staff at the Chemical Reactivity Section at RISØ for their helpfullness as well as the RISØ library who effectively provided whatever litterature I was interested in.

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1. Introduction

The contamination of soil with creosote and coal tar has become a world wide problem. There are about 1000 sites in Germany and the UK (Wild and Jones 1995) and more than 300 coal tar polluted sites have been registered in Denmark (ROKA 1995, Dyreborg and Arvin 1994). Groundwater pollution arising from coal tar and creosote polluted sites has likewise become an international problem (Pereira et al. 1983) and in Denmark several groundwater wells have been closed due to the decreasing quality of the groundwater caused by leaching coal-tar pollutions. The risk evaluation at such sites is therefore more important than ever before. The components of tar pollutions that have traditionally been the greatest cause of concern are the polycyclic aromatic hydrocarbons (PAHs) as these compounds are hazardous to human health and are present in large amounts in tar. The PAHs are nonpolar compounds that when released to soil partition strongly into the organic matter of the soil which largely reduces the spreading to groundwater. When the extent of a tar pollution is to be established, groundwater is often examined for the presence of naphthalene as this is the PAH that is expected to have travelled the farthest. But beside the PAHs, another group of compounds, the polar endocyclic nitrogen containing azaarenes, are also found in tar pollutions. They are similar to the PAH except that they contain a nitrogen incoorporated in the ring system. The azaarenes are present in smaller amounts than the PAHs but due to their polar structure they are less susceptible to partitioning into soil organic matter and will consequently be transported further and faster than equivalent PAHs. As the azaarenes at the same time have carcinogenic properties comparable to those of the PAHs, they may be at least just as problematic. Another group of polar compounds, the polar substituted PAHs, may for the same reasons as for the azaarenes be problematic for example in relation to drinking water quality. Just how large the problem may be depends on the physical-chemical properties of the compounds. Therefore, the objective of this study has been to develop methods for the determination of environmentally important physical chemical properties of polar polycyclic aromatic compounds and to determine important physical chemical properties of polar polycyclic aromatic compounds, i.e., azaarenes and polar substituted PAH.

2. The polar polycyclic aromatic compounds

2.1 Introduction

The polar polycyclic aromatic compounds are, like the polycyclic aromatic hydrocarbons (PAHs), a sub class of polycylic aromatic compounds (PACs). They have a polycyclic aromatic structure which means that they have at least two aromatic rings. For the compounds to be polar, atoms with a different electronegativity than C and H, typically N, O, Br or Cl atoms, must appear and there is two ways in which this can be the case. Either the electronegative atoms are incorporated into the ring structure (endocyclic), as in the so called N,S and O heterocycles or they are present in substituents (exocyclic). The existence of an electronegative atom in a molecule however, does not necessarily make the molecule polar.

Atom	H	C	N	S	O	Cl	Br
electronegativity	2.2	2.5	3.0	2.5	3.5	3.0	2.8

Table 2.1. *Pauling scale, (Swarzenbach et al. 1993).*

A detailed view of the classifications employed in this thesis is given in fig 2.1. As this study is mainly concerned with unsubstituted N-heterocycles or azaarenes and polar substituted PAH, this chapter is focused on these groups.

The term "NSO compounds" has been popular, but wrongly indicates that these types of heterocyclic PACs to a large extent share physical properties. In contrast, the S and O heterocycles has more in common with the PAH than with the heterocylic N-PACs due to the electronic structure (see below). The different properties of heterocyclic N-PACs and heterocyclic S,O- PACs is noted in extraction procedures, where the S and O heterocycles end up together with the PAHs while the N-heterocycles end up in a different fraction (Wright et al. 1985). Also, in the chromatographing on silica columns, as used in gas chromatography, the S,O-heterocycles elute together with the PAH indicating similar nonpolarity.

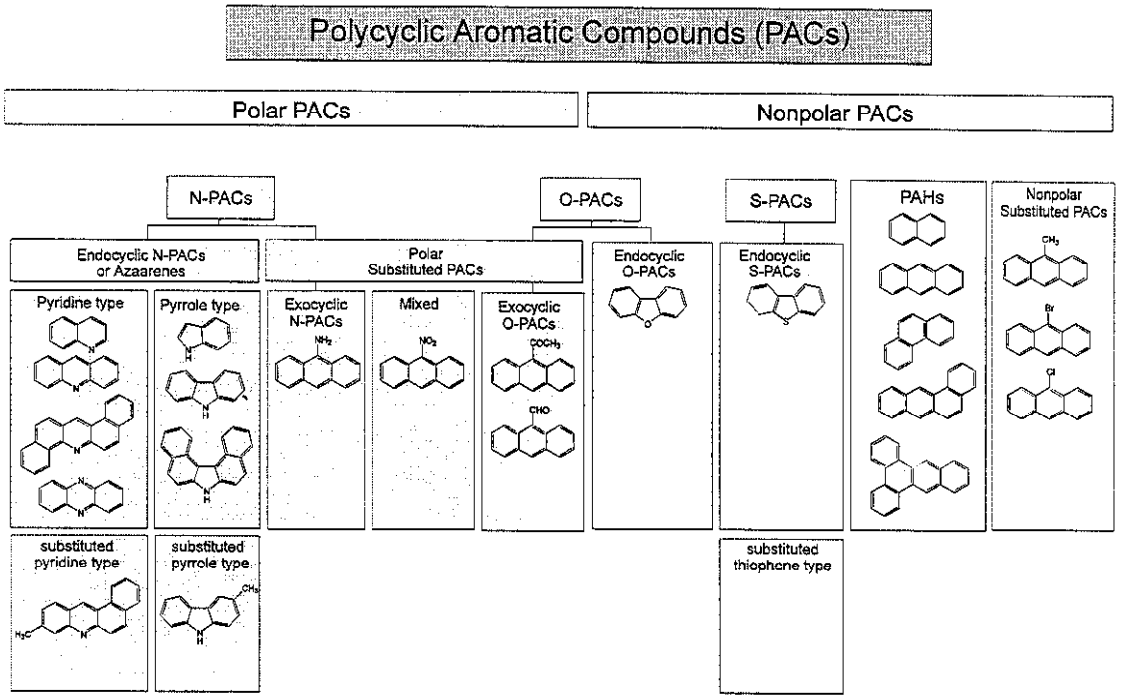


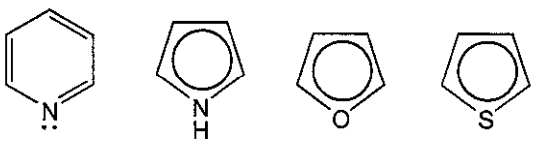
Fig 2.1. *The grouping of compounds according to chemical structure as empolyed in this thesis*

2.2 The azaarenes

The azaarenes are endocyclic nitrogen containing PACs (N-PACs) and there are two types of azaarenes, the pyrrole type and the pyridine type.

2.2.1 Pyridine type azaarenes

The pyridine type azaarenes contain a pyridine ring which is a six membered aromatic ring system of which one member is a N atom (see Fig 2.2). The pyridine azaarenes have a highly aromatic structure with one nitrogen electron participating in the π system of the ring. The pyridine N-atom has an unshared electron pair (free lonepair) in a sp^2 hybrid orbital in the same plane as the ring. This lonepair is available for sharing and therefore has a very large influence on the physical properties of the pyridine type azaarenes. The free lonepair makes the pyridine type azaarenes weak bases and strong hydrogen bond acceptors. The N-atom in the pyridine type azaarenes has a surplus negative charge which make the molecules polar. Due to the possibilities for placement of the nitrogen atom in the ring systems many isomeric pyridine type azaarenes exist. Examples of pyridine type azaarenes are quinoline, acridine and benz[a]acridine.



Pyridine Pyrrole Furan Thiophene

Fig 2.2. *The structure of different heterocycles.*

2.2.2 The pyrrole type azaarenes

The pyrrole type azaarenes contain a pyrrole ring which is a five membered planar aromatic ring system of which one member is a N atom. The carbons and nitrogen are held together by σ bonds with sp^2 hybridization while a single electron from each carbon and two from nitrogen reside in the p orbitals and form the sextet required for aromaticity. In pyrrole type azaarenes the N-atom does not have any free lonepairs for sharing. (see Fig 2.2). Due to the strong electronegativity of the N-atom compared to that of the H-atom, the electrons in the N-H bond is drawn away from the hydrogen leaving it electron poor and therefore a good hydrogen bond donor. This also makes the pyrrole type azaarenes less basic than water. In the pyrrole type azaarenes there are a surplus negative charge on the ring system, but the dipolarity of pyrrole is smaller than that of pyridine (Later 1985). Examples of pyrrole type azaarenes are carbazole and indole.

2.2.3 Other N-heterocycles

Apart from the pyridine and pyrrole type heterocyclic N-PACs, diazaarenes or triazaarenes, substituted azaarenes and thioazaarenes also exist. Wakeham (1979) found a rather large number of diazaarenes in lake sediments, oils, automotive exhaust and street dust. He also found that in lake sediments and street dust, the largest part of the azaarenes and PAH were unsubstituted whereas it was opposite in fossil material. Wright et al. (1985) found many substituted, mainly methylated, azaarenes in creosote samples, but this work has been limited to the monoazaarenes.

2.2.4 Physical-chemical properties of azaarenes

The pyridine type azaarenes are all weak bases due to the free lonepair of nitrogen and they can therefore occur in a protonated form which has drastically different physico-chemical properties than the neutral form. In Table 2.2 pKa values are given for some azaarenes and the distribution between neutral and protonated form at different pH are given in Fig 2.3.

N-PAC	pKa	Baseform at pH 7	Reference
quinoline	4.87, 4.92	99.21 %	Sangster 1989, Later 1985
isoquinoline	5.39	97.60 %	Sangster 1989, Later 1985
benzo[f]quinoline	5.11	98.73 %	Later 1985
benzo[h]quinoline	4.21	99.84 %	Later 1985
phenanthridine	4.61	99.59 %	Sangster 1989
benz[a]acridine	3.95	99.91 %	Later 1985
benz[c]acridine	4.70	99.50 %	Later 1985
acridine	5.58	96.34 %	Later 1985
carbazole	-1.9	100.00 %	Later 1985
dibenz[a,h]acridine	3.6	99.96 %	Southworth and Keller 1984

Table 2.2. *The pKa values for some N-PACs.*

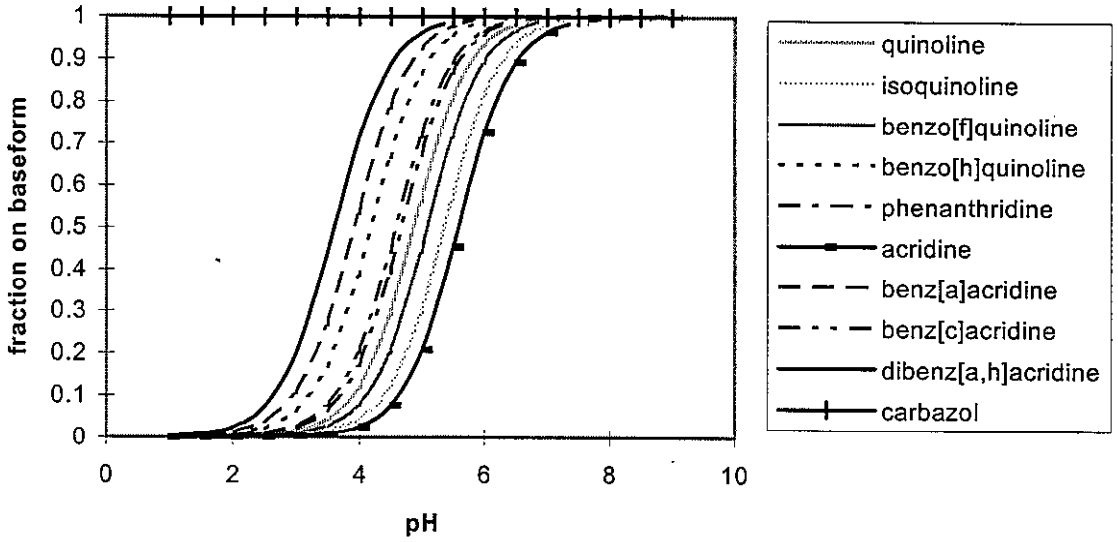


Fig 2.3. *The speciation of azaarenes as a function of pH.*

The octanol water partition coefficient (K_{ow}) and water solubility has been measured for some azaarenes and the values are given in Table A2 and A3 in appendix. However, as the physical-chemical properties are the subject of this thesis they will be discussed in detail in the following chapters.

2.2.5 Biological activity of the azaarenes

N-PACs are one of the compound groups in coal products that has been identified as genotoxics and they may be equally important carcinogenic pollutants as their homocyclic analogs (PAHs) although they have been less studied (Warshawsky 1992). Often the original compounds do not possess carcinogenic or mutagenic properties but when the compounds are metabolized in organisms or otherwise environmentally transformed, derived compounds may submerge that are highly carcinogenic or mutagenic. Most of the mammal metabolism pathways studied with azaarenes seem to lead to epoxides and dihydrodiols and to a lesser degree hydroxy and N-oxides (Warshawsky 1992).

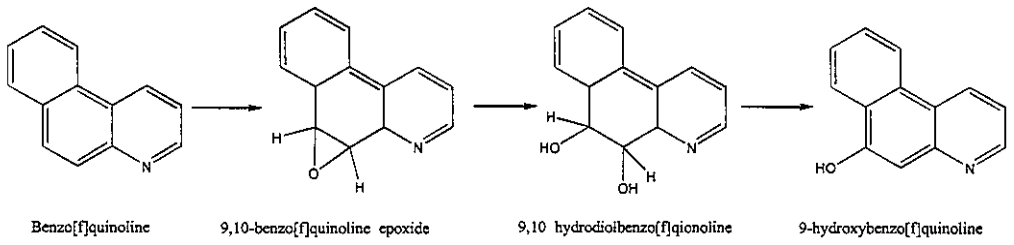


Fig. 2.4. *Proposed metabolism pathway of benzo[f]quinoline, (Warshawsky 1992).*

When the PAHs and the N-PACs are compared it can be noted that N-PACs starting from two rings and up are mutagenic and carcinogenic while PAHs with less than four rings are relatively inactive mutagenics/carcinogenics (Later 1985, Nielsen et al. 1996).

This is important as the small N-PACs/azaarenes are the compounds that most readily partition into water and therefore are compounds for which there is a high risk of human exposure.

Tests for mutagenicity and carcinogenicity, methodology

As the availability of human subjects for carcinogenicity experiments is low, the tests referred here have mostly used mice instead. The test compounds have been administered to the mice on the skin surface, under the skin, in the lungs, and through the diet. Results from testing on live animal cells like liver cells or lung preparations, have also been reported.

Mutagenicity is normally tested with mutated cells that in their mutated form cannot live without histimine. These cells are often the TA100 or TA98 strains. When these cells are exposed to the test compound and/or metabolites they may mutate back and gain the ability to live without histimine, which can be registered in the test. Metabolites relevant to humans can be generated during the test by adding rat liver cells S9, to the medium.

Biological activity of the two ringed azaarenes (Quinolines)

The biological activity of quinolines seems not to have been investigated in detail, but in a study done in connection with this project, mutagenicity was tested with TA100 and TA98, S9 rat metabolism activated. The tests showed that quinoline was a rather strong mutagenic compared to f.ex. acridine. This has also been the results of Weyand et al. (1993) who furthermore found that quinoline and 4-methyl quinoline was strong tumor initiators (liver) in newborn mice.

Compound	Mutagenicity
Quinoline	2558 ± 108
8-hydroxyquinoline	2674 ± 152
Benzo[a]pyrene	12188 ± 730

Table 2.3. *The mutagenicity of azaarenes as tested on TA100, S9 metabolism activated. The numbers refer to the slope of the linear part of dose response curves. (Nielsen et al. 1996). Benzo[a]pyrene has been included for comparison.*

Biological activity of the three ringed azaarenes

Acridine is carcinogenic according to (IARC 1983), but only weakly mutagenic according to Nielsen et al. (1996) (see Table 2.4).

In a study done in connection with this project, mutagenicity was tested with TA100 and TA98, S9 rat metabolism activated, and the results are given in Table 2.4.

Compound	Mutagenicity
acridine	574 ± 95
phenantridine	1048 ± 68
Benzo[a]pyrene	12188 ± 730

Table 2.4. *The mutagenicity of azaarenes as tested on TA100, S9 metabolism activated. The numbers refer to the slope of the linear part of dose response curves. (Nielsen et al. 1996)*

For the benzoquinolines the placement of the nitrogen has a marked effect on both the mutagenic activities of the diol epoxides and the metabolic activation of the parent compounds (Warshawsky 1992).

	Carcinogenic activity Liver tumors when given in diet	Carcinogenic activity Mouse skin tumors	Mutagenicity in Rat aroclor1254 induced in S-9 Salmonella typhimurium TA100
Benzo[f]quinoline	inactive/active	inactive	Yes
Benzo[h]quinoline	inactive	inactive	Yes
phenanthridine	inactive	inactive	Yes
4-methylquinoline	active		

Table 2.5. *Carcinogenic and mutagenic activity of azaarenes. Extracted from (Warshawsky 1992 and Later 1985)*

Biological activity of the four ringed azaarenes (benzacridines)

the carcinogenicity of benzacridines are varied. Benz[a]acridine is not carcinogenic and benz[c]acridine is only mildly carcinogenic, but some of the substituted benzacridines are highly active carcinogenics while, again, others are not. Warshawsky (1992) states in a summary that: the benz[a]acridines are at least weakly active and the benz[c]acridines are weakly to moderately active but the activity of these compounds depend on the functional groups attached to specific sites. In the tests that have been performed, the methylated benzacridines have been found to be mutagenics. (Warshawsky 1992)

Some examples of carcinogenic properties of benzacridines are given in Table 2.6 below.

Compound	Relative carcinogenic potency
benz[a]acridine	-
9-methylbenz[a]acridine	-
8,10,12-trimethylbenz[a]acridine	++
8,12-dimethyl-9-chlorobenz[a]acridine	++
benz[c]acridine	-
7-methylbenz[c]acridine	+++
9-methylbenz[c]acridine	-
7-cyanobenz[c]acridine	+

Table 2.6. *Relative carcinogenic potencies for benzacridines. Extracted from (Warshawsky 1992) Application to mouse skin, - not active, + weakly active, ++ moderately active, +++ highly active*

Biological activity of the dibenzacridines and azabenzo[a]pyrenes

In the literature as compiled by Warshawsky (1992) the dibenzacridines have been found to be weakly to moderately mutagenic and, weakly to moderately active carcinogenics. Tests done under this project found that dibenz[c,h]acridine was a rather strong mutagenic, see Table 2.8. From the data in Table 2.7, it can be seen that differences in molecular structure are causing the dibenzacridines to have different carcinogenic potencies.

Compound	Relative carcinogenic potency
dibenz[a,j]acridine	+
14-methyldibenz[a,j]acridine	++
dibenz[a,h]acridine	+
14-methyldibenz[a,h]acridine	++
10-ethyldibenz[a,h]acridine	-
dibenz[c,h]acridine	++
7-methyldibenzacridine	++
dibenzo[a,h]phenazine	-

Table 2.7. *Relative carcinogenic potencies for dibenzacridines. Extracted from (Warshawsky 1992)·Application to mouse skin, - not active, + weakly active, ++ moderately active, +++ highly active.*

Compound	Mutagenicity
10-azabenzo[a]pyrene	6029 ± 530
dibenz[c,h]acridine	7310 ± 717
benzo[a]pyrene	12188 ± 730

Table 2.8. *The mutagenicity of azaarenes as tested on TA100, S9 metabolism activated. The numbers refers to the slope of the linear part of dose response curves. (Nielsen et al. 1996)*

Other studies have also shown that the mutagenic activity of 10-azabenzo[a]pyrene is as strong as that of benzo[a]pyrene on the TA100 cells and furthermore that 10-azabenzo[a]pyrene is a moderately active carcinogenic when applied to mouse skin (Warshawsky 1992).

Biological activity of the carbazoles

The pyrrole or carbazole type N-PACs have mainly been studied in the form of dibenzocarbazoles and of these, many are potent carcinogenics(tested on mouse) and mutagenics. While carbazole is not mutagenic towards strains of S. typhimurium 9-ethyl- and 9-methyl- carbazole are. Neither carbazole, 9-ethyl or 9-methyl-carbazole was tumorgenic in newborn mice in total doses of 1.75 µmol. (Weyand et al. 1993).

For the pyrrole type N-PACs, the following results was gathered by Later (1985)

Compound	Mutagenicity	Carcinogenicity
indole	-	+
carbazole	-	-
benzo[a]carbazole	+	++
dibenzo[c,g]carbazole	-	+++

Table 2.9. *Mutagenic and carcinogenic potencies of pyrrole type N-PACs. - not active, + weakly active, ++ moderately active, +++ highly active*

Toxicity of azaarenes

The toxicity of azaarenes is a relatively untouched area of research, but Wayne T Shultz and co workers have published some work on the subject. Tetrahymena pyriformis, a zoo plankton, has been used as test material by Schultz et al. (1983). Tetrahymena is a representative of microfauna in aquatic ecosystems and important in turnover of organic matter. In nature, Tetrahymena is an indicator of a healthy aquatic environment. Schultz used a sensitive test where population growth impairment is quantified by IGC50 which is the inhibitor growth concentration for 50 % of control populations (Schultz 1983). The results are given in Table 2.10 below.

Compound	60-hr. IGC50 mmol/L
quinoline	0.97 (0.67-1.41)
isoquinoline	0.97 (80-1.17)
indole	0.62 (0.44-0.87)
carbazole	0.04 (0.01-0.07)
phenanthridine	0.04 (0.03-0.05)
acridine	0.04 (0.03-0.06)

Table 2.10. *Growth inhibitor concentration (IGC50) for Tetrahymena pyriformis for different azaarenes (Schultz 1983).*

The results show that the three ringed compounds are much more toxic than the two ringed compounds. In another study acridine was found to be toxic towards Tetrahymena pyriformis with 24h-LC100 = 35 mg/L and 24h-LC50 = 30 mg/L, and caused cytolysis (Schultz et al. 1981).

Experiments with the green algae *scenedesmus acuminatus* showed that the two ringed azaarenes indole, quinoline, isoquinoline and the three ringed carbazole did not inhibit growth rate in concentrations up to 10 mg/L. For three ringed benzoquinolines there was a large difference between isomers when chlorophyll-a or growth rate was used as an indicator of toxicity. The differences in toxicity could be correlated well with ionization potential and Homo-Lumo gap (van Vlaardingen et al. 1996).

compound	Growth rate EC50 mg/L
acridine	0.32
benzo[f]quinoline	1.55
phenanthridine	5.24
benzo[h]quinoline	6.65

Table 2.11. *Growth rate effect concentrations with scenedesmus acuminatus for various azaarenes (van Vlaardingen et al. 1996).*

Experiments in connection with this study have shown that acridine was toxic to plants in that growth of Italian rye-grass was inhibited at soil concentrations of 1 ppm. At soil concentrations of 100 ppm nawev seeds did not germinate or sprouts did not survive (Gissel-Nielsen and Nielsen 1996).

Degradability

Biological

In a large experiment performed by Catallo (1996), degradation rates were established for two and three ringed azaarenes in different eustarine sediments under different redox conditions. In stirred open microcosms, the degradation of the three ringed azaarenes in sand was faster than in clay and faster in oxidized sediment than in reduced sediment. There was a reduction from 45 ppm to zero in about 28 days for most three ringed compounds such as acridine and benzo[f]quinoline. In sealed microcosms not stirred the degradation rates were considerably lower. The results showed that the compounds were slowly and incompletely degraded in sediments under conditions found in eustarine, wetland and marine environment. It is suggested that the azaarenes form complexes with Fe(III) found in oxidized sediments which can hamper degradation. The fastest degradation rate for sealed microcosms was found in methanogenic fine particle sediments.

Compound	Oxidized sand	Reduced sand	Oxidized clay	Reduced clay
quinoline	nt	87	nt	nt
dibenzofuran	174	174	nt	nt
4-azaflourene	174	260	nt	nt
benzo[f]quinoline	130	208	nt	69
acridine	174	208	nt	347
phenanthridine	149	260	nt	65
carbazole	149	347	nt	260

Table 2.12. *Half lives ($t_{½}$ days) for selected azaarenes in eustarine sediments. nt- no significant transformation vs killed controls (Catallo 1996).*

Two ringed azaarenes

Many examples have shown that the two ringed azaarenes, quinoline and isoquinoline are degradable by bacteria present in nature under both anaerobic and aerobic conditions.

Four strains of bacteria, which were able to degrade isoquinoline, was isolated from an oil contaminated site. The bacteria were able to use isoquinoline as the sole carbon source. The initial degradation product was 1-hydroxyquinoline (Aislabie et al. 1994). Others have isolated bacteria from garden soil that were able to use quinoline as sole carbon and nitrogen source. 2-hydroxyquinoline was an initial product.

Pereira (1987) found that quinoline and isoquinoline was transformed in an aquifer contaminated with wood treatment chemicals.

Three ringed azaarenes

Acridine has been shown to be degraded by bacteria from groundwater aquifers in laboratory under denitrifying, sulfate reducing and methanogenic conditions (Kaiser et al. 1996). However, Pereira (1987) found that acridine and benzoquinoline were not degraded in an aquifer contaminated by wood treatment chemicals.

Larger azaarenes.

Unfortunately no studies seems to have been made for the larger azaarenes so far.

Non biological

Abiotic degradation of azaarenes can be physical or chemical, i.e. photolysis, hydrolysis or other chemical transformation, but information in literature on these subjects are scarce for azaarenes. However, Kochany and Maguire (1994) have reported that quinoline was photolyzed in lake water by sunlight (Half life =14 days in summer, 123 days in winter). The occurrence of $NaNO_3$ or dissolved organic material increased the degradation rate as did low pH (4.5 as opposed to 7).

2.2.6 Environmental sources of N-PACs

N-PACs are present in original fossil material like coal and oil and can be released to the environment through combustion, pyrolysis or processing of the fossil materials but N-PACs can also be formed during the conversion of fossil fuels such as nitrogen rich coal and oil shale. (Santodonato and Howard 1981, Pereira 1987) see Fig 2.5.

Ways for azaarenes to enter the environment

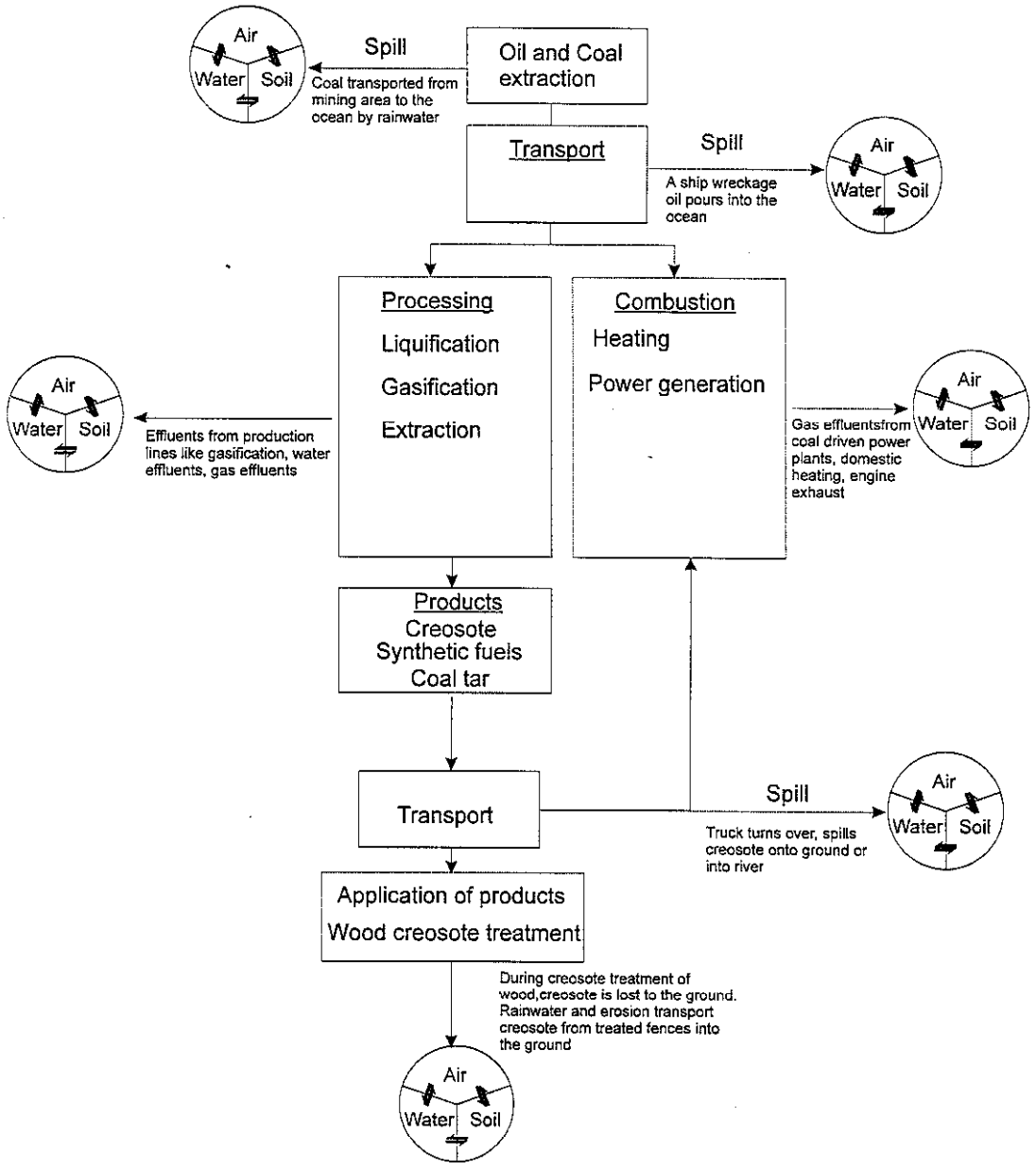


Fig 2.5. *Ways for the azaarenes to enter the environment.*

N-PACs in unprocessed fossil materials

N-PACs are indigenous to fossil materials and pyridine and pyrrole type N-PACs have been extracted directly from coal (Later 1985). As can be seen in Table 2.13 natural oils likewise contain azaarenes in different amounts. Of individual compounds, benzo[f]quinoline, benzo[h]quinoline and phenanthridine have been identified in crude oil. (Scmitter et al. 1982).

The content of different oils have been investigated by Wakeham (1979), and a large variation in the amount of indigenous azaarenes was found.

Oil	mg azaarene/g oil
Serpiano shale	0.04
Kuwait crude	1.06
South Louisiana crude	0.4

Table 2.13. *Azaarene content in oils (Wakeham 1979).*

N-PACs in processed fossil materials

Processing of coal includes coking, gasification, solvent refining, and liquefaction, and most of the products or by-products contain significant levels of N-PAC ranging from 5 to 40 % w/w of the total sample composition (Later 1985). Some of the products of coal conversion processes are heavy distillate coal-tar and creosote which contain high amounts of PAH and N-PAC. A National Bureau of Standards (NBS) coal tar standard and a solvent refined coal heavy distillate material contained aza aromatic fractions of 31% and 18 % w/w respectively (Wakeham 1979) while creosote contained 11% w/w. (Wright et al. 1985) Also in other coal and oil shale products like synthetic fuels, azaarenes (carbazole, acridine, benzo[h]qinoline, benzo[f]quinoline and phenanthridine) have been identified (Later 1981). A commercial scale gasifier can generate 5*105 kg condensate water per hour (Mohr and King 1985) and the condensate will contain large amounts of N-PACs. In table 2.14 below, examples of concentrations of N-PACs in some coal and oil products are given.

	Jet fuel JP-8	Diesel fuel marine	hydro treated Sohio crude
Hydrocarbon	93.1	93.1	-
Pyridine fraction	1.70	2.05	2.84
Pyrrole fraction	1.49	1.85	5.90

Table 2.14. *Composition of fuel products derived from Paraho shale oil. Fractions are weight % of samples (Ford et al. 1981).*

Examples of N-PACs in coal tars and creosote are given in Table 2.15 to 2.17.

Heavy Coal Tar distillate	Conc. mg/g distillate
benzo[h]quinoline	1.68
benzo[f]quinoline	3.31
acridine	0.97
carbazole	15.3
3-methylcarbazole	5.8
1 or 4-azapyrene	2.36
benzo[a]carbazole	1.42
benzo[b]carbazole	0.72
benzo[c]carbazole	1.21
dibenzocarbazoles	0.08

Table 2.15. *Concentration of selected compounds in heavy coal tar distillate (Warshawsky 1992).*

NBS coal tar	ppt
benzo[h]quinoline	1.81
acridine	2.85
phenanthridine	1.65
carbazole	11.5
2-azapyrene	0.42
benz[a]acridine	1.8
benzo[a]carbazole	4.18
dibenz[a,i]carbazole and dibenz[a,j]acridine	0.72
dibenzo[c,g or a,g]carbazole	0.56

Table 2.16. *Concentration of selected compounds in NBS coal tar (Warshawsky 1992).*

Coal gasification tar	µg/g (approximate concentrations)
quinoline	147
isoquinoline	55
4-azaflourene	40
benzo[h]quinoline	36
acridine	35
phenathridine	134
2-azapyrene	4
benz[a]acridine	29
azabenzopyrenes	18

Table 2.17. *Concentration of selected compounds in coal gasification tar (Later 1985)*

Creosote oils are produced as a by-product of conventional coal coking and have been used as wood preservatives whereas hydrogenated coal tar distillates are used as industrial solvents (Wright et al. 1985). A commercial creosote had a composition as seen in Table 2.18.

	Commercial creosote	Hydrogenated creosote
Compound class	weight %	weight %
aromatic hydrocarbons	7	18
PAH	69	70
N-PAC	11	8
Hydroxy-PAH	11	8

Table 2.18. *Commercial creosote compositions (Wright et al. 1985).*

Compound	mg/g commercial creosote
quinoline	20.3 ± 1.2
isoquinoline	6.77 ± 0.36
benzo[h]quinoline	11.6 ±1.0
acridine	22.2 ± 2.4
phenanthridine	27.8 ± 2.1
carbazole	38.9 ± 1.7
methylacridine	8.93 ± 0.55

Table 2.19. *Concentration of selected compounds in commercial creosote (Wright et al. 1985)*

The high concentration of N-PACs in coal tar produced during combustion or pyrolysis of coal is not entirely unwanted. In Japan there has been research to determine processing parameters of coal pyrolysers that gave a maximum of N-PACs in the coal tar, as this would reduce the effluent of NOx, a severe air quality problem (Chen et al. 1995).

The environmental distribution/occurrence

After the azaarenes have been released from a process or material they will be found in the environmental compartments sediment, soil, water and air besides possibly in plants and animals. As the compounds are hydrophobic they will tend to end up in the organic material of soil and sediments and if they are not degraded they will accumulate there. An illustration of how the azaarenes enters the environment is given in Fig 2.5.

Soil and sediment

The azaarenes can reach soil and sediment from either contaminated water or air or through direct spill in connection with production or transport of products containing azaarenes. In lake Zurich sediments concentrations of ND to 0.006 mg azaarene/g sediment was found and this contamination was suggested to originate from coal combustion where particles were formed that later fell out into the lake (Wakeham 1979). Blumer (1977) reported that a mix of azaarenes was found in coastal surface sediments and some of the larger and very carcinogenic azaarenes have been found in the sediment of the black river Ohio USA. If one considers the azaarene concentrations in the water of Dohkai bay found by Shinohara et al. (1983) the concentrations in the sediment could be substantial.

	ng/g dry sediment
benzo[a]acridine	870
benzo[b]carbazole	1100
dibenzacridines	550
dibenzocarbazoles	1000

Table 2.20. *Azaarene concentrations in contaminated sediments from the Black river Ohio USA, (Warshawsky 1992).*

Compound	µg/kg sediment
acridine	43.7
methylacridine	6.0
azapyrene	12.0
carbazole	463
methylcarbazole	61.5

Table 2.21. *Azaarene concentrations in pudget sound sediments, Eagle Harbor (Brumley et al 1991).*

In soil azaarenes have been found mainly in connection with coal tar or creosote contaminated sites (Lauer et al. 1988, Pereira et al. 1987, Brumley et al. 1991).

Compound	µg/g soil
quinoline	0.62
isoquinoline	0.70
benzo[h]quinoline	20.5
azaflourene	6.84
acridine	30.8
phenanthridine	1.94
carbazole	34.8
benzocarbazole	0.117
dibenzothiazole	2.01

Table 2.22. *Azaarenes in a creosote contaminated soil from spotlylvania VA (L.A. Clark site) soxhlet extracted (Brumley et al. 1991).*

Water

Azaarenes can reach water from either spills in connection with transport or production, from production watery effluents or indirect from air or soil. Bark et al. (1972) mentions that the river board of Avon Bristol set a limit on organic bases of 1 mg/L in the effluents entering the main stream. The activated sludge of sewage water treatment plants does often not remove the organic bases from carbonization process effluents why these may enter rivers and streams in large amounts (Bark et al. 1972). In tar plant drainage water, concentrations of 0.5-1 mg/L isoquinoline and 0.8-2.9 mg/L quinoline were measured while higher concentrations were found in coke oven liquors (Bark et al. 1972)

Azaarenes have been found in groundwater in the vicinity of coal tar distillation and wood preserving facilities (Pereira 1983, 1987, Warshawsky 1992,), but also in surface water like the sea water from Dohkai bay Japan (Shinohara et al. 1983) and must be present in the fresh water of Lake Zürich as azaarenes are found in the sediment (Wakeham 1979).

	ng/L
quinoline	22
isoquinoline	13
acridine	9.5
dibenz[c,h]acridine	0.66
dibenz[a,h]acridine	3.1
dibenz[a,j]acridine	4.1

Table 2.23. *Azaarenes in contaminated sea water from Dohkai bay, Japan (Shinohara et al. 1983).*

In sewage sludges from upper silesia Poland, quinoline/isoquinoline, 4-azaflourene, methylacridiine, benzoquinolines and benzoisoquinolines, acridine, benzoacridine, azaflouranthene and/or azapyrene were identified by GC-MS. (Bodzek et al. 1996)

Air

The largest release of azaarenes to the environment is most likely happening through the air but as the pollution is spread over a large volume concentrations are low. Azaarenes have been found in ambient air and appears to originate mainly from coal driven power plant effluents and mobile exhaust, where azaarenes have been measured. The azaarenes in air are found either as vapor phase or associated with particles.

Most studies of azaarenes in air have focused on particles, but a study has shown that ambient air vapor phase concentrations of two and three ringed azaarenes are just as important (Adams et al. 1982).

Compound	Vapor phase ng/m3
Isoquinoine	24
methylquinolines	5.5
quinoline	2.23
C_2-quinolines/isoquinolines	0.58
benzoquinolines	0.22

Table 2.24. *Vapor phase azaarenes sampled with sorption techniques at College station, Texas, C2 = ethyl or dimethyl (Adams et al. 1982).*

In automotive exhaust concentrations of 2.3 mg azaarene /m3 exhaust was found, mainly quinolines and alkylquinolines (Wakeham 1979). Benzo[h]quinoline 0.3 µg/g benzene soluble, benz[c]acridine 0.6 µg/g, dibenzacridines <0.3 µg/g, indenoquinolines 1.6 µg/g have also been found. (Warshawsky 1992). In street dust, 0.01-0.03 mg azaarene /g dust has been found (Wakeham 1979). Further examples of azaarenes in air are given in Table 2.25 to 2.29.

Compound in air	µg/g benzene soluble
Benzo[h]quinoline in air	
Urban air	30
coal tar pitch polluted air	2300
coal burning effluent	300
incinerator effluents	2900
Dibenz[a,h]acridine in air	
Coal burning effluents	160
Incinerator effluents	4.0
Dibenz[a,j]acridine in air	
Coal burning and incinerator effluents	20-13

Table 2.25. *Azaarenes in air (Warshawsky 1992).*

Average American urban air	µg/g airborne particle	µg/1000 m^3 air
benz[a]acridine	2	0.2
benz[c]acridine	4	0.6
dibenz[a,h]acridine	0.6	0.08
dibenz[a,j]acridine	0.3	0.04

Table 2.26. *Azaarenes in an average American urban air as per g particle or pr. m^3 air. (Warshawsky 1992)*

New York City USA	ng/1000 m^3 from urban particles
4-azapyrene	21
1-azaflouranthene	5
acridine	41
benzo[h]quinoline	10
phenanthridine	22

Table 2.27. *Azaarenes in New York city air (Warshawsky 1992).*

Tokyo Japan	µg/g particle
4-azaflourene	0.03-3.5
acridine	0.03-3.5
benz[a]acridine	0.03-3.5
dibenz[a,j]acridine	0.03-3.5
dibenz[a,h]acridine	0.03-3.5
dibenz[a,c]acridine	0.03-3.5
10-aza-benzo[a]pyrene	0.03-3.5

Table 2.28. *Azaarenes in urban air from Tokyo, Japan (Warshawsky 1992).*

Coal heating in residential coal furnaces creates high concentrations of azaarenes as can be seen from Table 2.29.

Compound	mg/1000 m^3 gas
Acridine	111
Benzo[f]quinoline	57
Benzo[h]quinoline	38
Phenantridine	32
Benz[a]acridine	26
Benz[c]acridine	15
Dibenz[a,h]acridine	17
Dibenz[a,j]acridine	2
Benz[a]pyrene	500

Table 2.29. *Residential coal furnace gas concentrations (Sawicki et al. 1965).*

Also coal pitch tar fumes can have high concentrations of azaarenes as can be seen from Table 2.30. This may have implications for clean up procedures at contaminated sites and possibly for the choice of soil remediation methods.

Compound	mg/1000 m^3 air
Acridine	0.870
Benzo[f]quinoline	0.420
Benzo[h]quinoline	0.260
Phenantridine	0.020
Benz[a]acridine	0.200
Benz[c]acridine	0.120
Dibenz[a,h]acridine	0.010
Dibenz[a,j]acridine	0.001
Benzo[a]pyrene	0.400

Table 2.30. *Concentration of azaarenes in coal tar pitch fumes (Sawicki et al. 1965).*

Tobacco smoke
A large number of smaller nitrogen containing compounds was found in dark air cured tobacco and Virginia tobacco cigarette smoke but also the azaarenes quinoline, 2-methylquinoline and isoquinoline were identified (Saint-Jalm and Moree-Testa 1980)

2.3 The sulfur and oxygen containing PACs (S and O PACs)

The S and O heterocyclics contain S and O atoms incorporated in 5 membered rings of the thiophene and furan type respectively. In furan and thiophene type compounds, S and O atoms have two lonepairs of which one, like in pyrrole, is taking part in the extended π system of the aromatic structure. In thiophene and furan the ring has a high electron density, or surplus of negative charge, and the second lonepair are kept so close that it loses the ability to interact with other molecules. Furan and thiophene are nonpolar molecules and the benzologs are similarly nonpolar.

The S-PACs are found in air particulate matter, carbon blacks, coal liquids, shale oil, coal tar, lubricating oils, aerosols, automobile exhausts, asphalt residues and fish (Warshawsky 1992).
Some of these are carcinogenics like 4,9-dimethyl-1,2,7,8-dibenzothiophanthrene while others like dibenzo[b,d]thiophene are neither mutagenics nor carcinogenics (Warshawsky 1992).

2.4 Substituted PACs

An infinite number of substituted PACs can be imagined as a large number of PACs exists, a large number of substituents exists and as there is a large number of positions where the substituents can be attached to the PAC. In this study mainly polar substituted PAHs, where the substituents, besides C and H, contain either O ,N , Br or Cl, have been investigated.

2.4.1 Environmental occurrence

Like the azaarenes, the polar substituted PACs do exist in the environment, but as it is such a diverse group of compounds only examples of environmental occurrence are given here. Pristine oil may contain different alkyl substituted components formed during the petrogenesis(Youngblood and Blumer 1975) but substituted PACs can also be formed by physical, chemical or biological interactions during the spreading of oil- or coal-derived materials in the environment. For example, different PAH oxidation products may be present in tar (Zander 1990, Schultz et al. 1972), microbial transformation may transform unsubstituted PAH to their hydroxy derivatives (Pothuluri et al. 1995) and photolytic reactions appear to be a possible source for chlorinated PAH in the marine environment and on the soil surface (Sugiyama 1996). Furthermore, combustion processes and photochemical reactions in the atmosphere may produce a number of different PAC derivatives, e.g., nitro (Nielsen et al. 1984, Finlayson-Pitts and Pitts 1986, Nielsen et al. 1983, Paputa-Peck et al. 1983, Schuetzle 1983, Sera et al. 1994 and Fan et al. 1996) cyano (Krisknan et al. 1979), chloro, bromo (Nilsson and Colmsjö 1993) methoxy (Choudhury and Bush 1981) and some oxygenated derivatives like phenols, quinones, ketones, aldehydes and carboxylic acid derivatives (Finlayson-Pitts and Pitts 1986, Nielsen et al. 1983, Kamens et al. 1989, David and Boule 1993, Kirso et al. 1993 and Kochany and Maguire 1994)

2.4.2 Physical chemical properties of substituted PACs

The behavior of the polar substituted PACs in the environment is largely unknown so data, like the octanol water partition coefficient K_{ow} is greatly needed. The polarity of the substituted PACs make them less hydrophobic than non substituted PACs and consequently a greater threat to the drinking water supply.

2.5 Summary

From this chapter it is clear that azaarenes are potentially problematic compounds as they occur in all compartments of the environment in some quantities and as they are hazardous to human health as well as the health of other living organisms including plants. Also polar substituted polycyclic aromatic hydrocarbons are present in the environment, and they may, like the azaarenes, be problematic. As the diverse group they constitute they have received rather little attention but it can be suspected that they, like their parent PACs, will have carcinogenic and toxic properties and the polar substituents do increase water solubility whereby the risk of exposure to living organisms also increases. For both azaarenes and polar substituted PACs the risk is strongly dependent on the physical properties which is the subject of the following chapters.

3. Environmentally important physical-chemical properties

In all calculations of environmental distribution of a compound, two things play a major role, the physical nature of the compound and the physical nature of the environmental compartments. These things however have been combined in the definition of partition coefficients which are physical properties related to certain systems. A partition coefficient expresses the sum of molecular interactions between a compound and two phases, and knowledge about the molecular interactions as well as the thermodynamic theory can help to understand the nature of partition coefficients.

3.1 Thermodynamic background

3.1.1 Chemical potential

Chemical potential of a compound is defined as the increase in Gibbs free energy of a system when a certain amount of the compound is added to the system. There is no way of measuring chemical potential directly, but in an ideal gas, when additional gas is added to the system, the increase in chemical potential is proportional to the increase in pressure. This is expressed in a differential equation.

$$(d\mu_i)_T = \frac{V}{n_i} dP_i \qquad \text{eq 3.1}$$

where μ_i is the chemical potential of compound i, V is volume, n_i is mole of i and P_i is partial pressure of compound i. And for an ideal gas ($P_iV_i = n_iRT$) (T is temperature)

$$(d\mu_i)_T = \frac{RT}{P_i} dP_i \qquad \text{eq 3.2}$$

By integration of this differential equation the chemical potential of a gas can now be expressed relative to a reference state μ_i^0.

$$\int_{\mu_0}^{\mu_i} (d\mu_i)_T = RT \int_{P_i^0}^{P_i} \frac{1}{P_i} dP_i \qquad \text{eq 3.3}$$

which has the solution

$$\mu_i = \mu_i^0 + RT \ln\left[\frac{P_i}{P_i^0}\right] \qquad \text{eq 3.4}$$

This was for an ideal gas, but can be extended to ideal solutions, in that liquids also excerts a pressure, the vapor pressure $P_i(l)$ and pure liquid can be used as the reference state.

$$\mu_i = \mu^0_{i,pure\ liquid} + RT \ln \left[\frac{P_i(l)}{P^0_{i,pure\ liquid}(l)} \right] \qquad \text{eq 3.5}$$

For a real solution the vapor pressure alone is not sufficient to describe the chemical potential as the chemical potential is also influenced by non ideal interactions (se below). Therefore the vapor pressures are exchanged with fugacities *f*.

$$\mu_i = \mu^0_{i\,pure liquid} + RT \ln \frac{f_i}{f_{i\,pure liquid}} \qquad \text{eq 3.6}$$

The fugacity f_i is equal to the vapor pressure times an activity coefficient γ_i accounting for any non ideal behavior and using Raoults law $P_i(l) = x_i P^0_{i\,pure liquid}(l)$, where x_i is the molar fraction, one gets

$$f_i = \gamma_i x_i P^0_{i\,pure liquid}(l) \qquad \text{eq 3.7}$$

And the fugacity of the pure liquid $f_{i\,pure\ liquid}$ is equal to the vapor pressure of the pure liquid, when the pure liquid is used as the reference state. Therefore the equation can be transformed one last time to yield

$$\mu_i = \mu^0_{i\,pure liquid} + RT \ln \gamma_i x_i \qquad \text{eq 3.8}$$

This is an important equation in that it describes that the chemical potential of a compound in a solution is dependent of the concentration of the chemical and the activity coefficient of the chemical in that solution.

3.1.2 Partitioning

In a two phase system a solute *i* will have a chemical potential in each of the phases, 1 and 2.

$$\mu_{i,1} = \mu^0_{i,pure liquid} + RT \ln \gamma_{i,1} x_{i,1} \qquad \text{eq 3.9}$$

and

$$\mu_{i,2} = \mu^0_{i,pure liquid} + RT \ln \gamma_{i,2} x_{i,2} \qquad \text{eq 3.10}$$

At equilibrium the potentials are equal $\mu_{i,1} = \mu_{i,2}$ which gives

$$RT \ln \gamma_{i,1} x_{i,1} = RT \ln \gamma_{i,2} x_{i,2} \qquad \text{eq 3.11}$$

and

$$RT \ln \frac{x_{i,1}}{x_{,2}} = -(RT \ln \gamma_{i,1} - RT \ln \gamma_{i,2}) \qquad \text{eq 3.12}$$

Equation 3.12 is named the Boltzmann equation and it clearly shows that the activity coefficients are the key to partitioning of a solute between two phases.

As stated before, activity coefficients accounts for non ideality and it can be said that they are a measure of the additional free energy that a compound is carrying in a non ideal mixture as compared with the reference state considered to be the ideal state (pure liquid compound in the case of liquids).

An ideal mixture versus a non ideal mixture

An ideal mixture or solution is a mixture in which the molecular interactions between unlike molecules (A with B) are identical in strength and type to interactions between like molecules (A with A and B with B) Methanol-water is an example of a nearly ideal mixture. As all interactions are equal, any change in proportion between the components in the system would leave the system energy unchanged. This is not the case for non ideal behavior. Here A with B interactions could be very different from A with A interactions. As for example if a nonpolar molecule like naphthalene in water is considered, there will be no hydrogen bonds or dipole-dipole interactions between naphthalene and the water molecules while these interactions will exist between water molecules. One could say that it is non ideality that determine the partitioning.

The fact that activity coefficients are a measure of the additional free energy that a compound is carrying in a non ideal solution is expressed in the term: partial molar excess free energy, which for a compound *i* is defined as :

$$g_i^e = RT \ln \gamma_i \qquad \text{eq 3.13}$$

With this, boltzmanns equation can now be reformulated to

$$RT \ln \frac{x_{i,1}}{x_{i,2}} = -(g_{i,1}^e - g_{i,2}^e) \qquad \text{eq 3.14}$$

and finally

$$K_{i,12}^{'} = e^{\frac{\Delta g_{i,12}^e}{RT}} \qquad \text{eq 3.15}$$

So partitioning can be considered a question of excess energy, caused by non ideality and, as free energy can be divided into enthalpy end entropy

$$\Delta g_{12}^e = \Delta h_{12}^e - T\Delta s_{12}^e \qquad \text{eq 3.16}$$

the mechanisms responsible for partitioning are the mechanisms deciding entropy and enthalpy of the solute and solvent in the two phases.

Dissolution in water

To outline the mechanisms responsible for entropy and enthalpy changes, the dissolution in water of a organic compound from a pure liquid phase can be considered. The following 'happens': The organic molecule leaves the pure liquid phase without leaving a cavity and forms a cavity in the water phase. To leave the pure liquid phase

organic intermolecular bonds is broken which cost energy ΔH_1(corresponds to the heat of vaporization) and to enter the aqueous phase a cavity is formed. To form the cavity water molecule bonds are broken and water removed. The removed water form the bonds again elsewhere, but the water molecules surrounding the organic molecule do not form as many water intermolecular bonds as before. This costs part of the water heat of vaporization ΔH_2 and is of course very dependent on the size of the organic molecule since larger organic molecules needs bigger cavities with more water molecules lining the cavity. When the organic molecule enters the cavity some of the energy spent is returned ΔH_3 from forming new water organic molecule interactions. In ideal solutions the result is 0. The forces responsible for these energies are van der waals, induced dipole interactions and if the organic molecule contains polar functional groups dipole-dipole and in relevant cases hydrogen bonding effects (see chapter 3.1.3). Iceberg formation is also of importance. The molecules around the cavity freezes to form a crystalline structure which releases some energy ΔH_4. Most important, all these enthalpy effects depend on the size or surface area of the solute!. (Schwarzenbach et al. 1993, Grant and Higuchi 1990)

Besides enthalpy, entropy also changes during dissolution.
Entropy is related to cavity formation, ice formation and the enhanced mixing of dissimilar molecules. Unlike enthalpy, entropy of cavity formation and ice formation can not easily be related to molecular structure but the total entropy seems to get larger for larger molecules, if for instance alcohols are considered (Schwarzenbach et al. 1993).

The total excess free energy of dissolution of a compound from its liquid phase to the water phase can be written:

$$\Delta g_s^e = \Delta h_s^e - T\Delta s_s^e = (\Delta h_{cav} - \Delta h_{ice}) - T(\Delta s_{cav} + \Delta s_{ice} + \Delta s_{mix}^e) = RT \ln \gamma_w \quad \text{eq 3.17}$$

The entropy term dominates for the smaller molecules like the alcohols and the smaller PAH, but for larger molecules like phenanthrene and up, enthalpy becomes more important than entropy terms (Schwarzenbach et al. 1993).

Of course, enthalpy and entropy likewise changes if a solute enters an organic phase like octanol from a pure liquid state even though the changes for a nonpolar compound will be much smaller. The relationship between the sum of enthalpy and entropy effects in the two phases of question will ultimately determine the partitioning.

3.1.3 The mechanisms responsible for partitioning.

As mentioned above, partitioning can be narrowed down to energies of enthalpy and entropy for which intermolecular forces are responsible. These intermolecular forces are as follows.

A. Coulumbic interactions which are simple interactions between positively and negatively charged compounds. (Interionic).

B. Van der Waals forces which includes the following three attractive forces:

1. Dipole-dipole interactions (Keesom forces). Molecules that are polar, having a permanent dipole moment, will interact in that positive will attract negative. In solubility phenomena, the dipole moment of bonds or groups are of greater significance than the dipole moment of the full molecule because the solvating molecules usually are much smaller than the solute molecule (Grant and Higuchi 1990).

2. Dipole- induced dipole interactions (Debye forces). Molecules that are normally not polar, but polarizable, can be made polar by an electric field. The field can be created by a nearby permanent dipole or by an ion, and dipole-dipole forces between the polarizable molecule and the permanent dipole will occur (Grant and Higuchi 1990).

3. Induced dipole-induced dipole interactions(London forces). All molecules, polar as nonpolar interact by induced dipole induced dipole interactions also called dispersion forces or London forces

C. Hydrogen bonding. Hydrogen bonding occurs between electronegative atoms (A) and hydrogen bound to electronegative atoms (B). Hydrogen bonding will occur only if atoms A and B are strongly electronegative and small and in practice A and B must Fluorine, Oxygen or Nitrogen. Sulphur can form weak hydrogen bonds. Hydrogen bonding is strongly directional because orbital overlap must be present and it is therefore sensitive to steric effects. The closer the bond is to 180° the stronger is the bond. The angle must be at least 140° for hydrogen bonding to occur. (Grant and Higuchi 1990)

Another classification of forces often used employ the terms polar forces and dispersive forces. Polar forces arise from molecules with permanent or induced dipoles including polarizable molecules and dispersive forces arise from fluctuation of charge inside a molecule

3.1.4 Linear free energy relationships (LFERs)

The linear free energy relationships are the basis of the HPLC method as used for the determination of octanol water partition coefficients in this study (see chapter 6) and describes the relationship between partition coefficients in two systems. For the sake of relevance, a HPLC system consisting of an aqueous mobile phase and a stationary phase and the octanol-water system has been used as example.

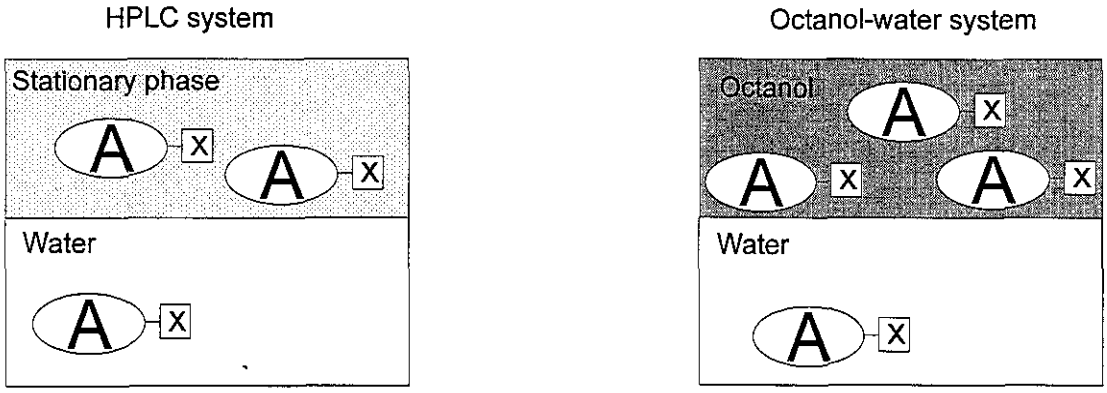


Fig 3.1. *Schematic representation of a HPLC system and the octanol-water system. A is the mother part of a compound i and x is the substituent part of a compound i.*

In the two model systems: stationary phase-water (sw) and octanol-water (ow), the excess free energy of a compound (i) in each system can be written as the sum of the excess free energy of a mother compound (A) and the additional excess free energy of the number (n) of substituents (x).

$$\Delta G^{i}_{ow} = \Delta G^{A}_{ow} + n\Delta G^{x}_{ow}$$
$$\Delta G^{i}_{sw} = \Delta G^{A}_{sw} + n\Delta G^{x}_{sw} \qquad \text{eq 3.18}$$

Where ΔG^{i}_{sw} and ΔG^{i}_{ow} are the excess free energies of the compound i in the two systems, ΔG^{A}_{sw} and ΔG^{A}_{ow} are the excess free energies of the mother compound A in the two systems, ΔG^{x}_{sw} and ΔG^{x}_{ow} are the additional free energies of the mother compound caused by substituent x and n is the number of substituents.

If the mother compound A is defined so that $\Delta G^{A}_{ow} = 0$ the equations above can be rearranged to:

$$\Delta G^{i}_{sw} = \frac{\Delta G^{x}_{sw}}{\Delta G^{x}_{ow}} \Delta G^{i}_{ow} + \Delta G^{A}_{sw} \qquad \text{eq 3.19}$$

which relates the partitioning in the HPLC system to the partitioning in the octanol-water system. If the HPLC stationary phase and the octanol phase interacts with solutes in a similar way, that is with the same mechanisms in the same proportion, the relationship between the additional excess free energy of the mother compound caused by any substituent x in the octanol-water system and the stationary phase-water system will be constant and the equation can be expressed as a linear relationship between partition coefficients.

$$\log K_{sw} = a \log K_{ow} + b \qquad \text{eq 3.20}$$

For a linear free energy relationship to exist, the structural dissimilarities of compounds should be similarly reflected in the excess free energy/activity coefficients for the two systems. This is normally the case for non-polar compounds where the activity in the organic phase is small and where the activity in the water is governed mostly by size

and not by specific interactions, but for polar compounds specific interactions, especially hydrogen bonding, can be present in one system while it plays no role in another.

3.2 Partition coefficients

Partition coefficients are, as stated elesewhere, valuable in the evaluation of the environmental compartmentazation of a compound, and this study has been concerned with three environmentally important partition coefficient, namely the octanol-water partition coefficient, the organic matter-water partition coefficient and the soil sorption coefficient. In this chapter these partition coefficient are defined and their nature is discussed.

Partition coefficients, as normally used, are defined as

$$K_{sw} \equiv \frac{C_s}{C_w} \qquad \text{eq 3.21}$$

at equilibrium, where C_s is the volume concentration in the organic (solvent) phase and C_w is the concentration in the water phase. The partition coefficients as normally used are therefore slightly different from the partition coefficients based on molar fraction (K'_{12}) that were derived from the Boltzmann equation. However, also the volume concentration based partition coefficient can be related to excess free energy (Scwarzenbach et al. 1993).

$$\ln K_{sw} \equiv \frac{C_s}{C_w} = \ln \frac{\frac{x_{i,s}}{V_s}}{\frac{x_{i,w}}{V_w}} = \frac{\Delta g^e_{i,sw}}{RT} + \ln \frac{V_w}{V_s} = \frac{\Delta G_{sw}}{RT} \qquad \text{eq 3.22}$$

3.2.1 The octanol water partition coefficient K_{ow}

In dealing with the distribution and transport of compounds in the environment several physical properties are valuable. These are especially partition coefficients that quantify the partitioning between two phases of which, for environmental purposes, one is usually water and the other is some organic phase, for instance soil organic matter. The partitioning of compounds in the environment is complex and in most cases the compound partition between many phases at the same time. In groundwater aquifers for instance, a chemical will be partitioned between minerals, different kinds of organic matter and water. One way of treating this complexity is to use overall sorption coefficients such as K_d, the soil sorption coefficient, where the soil is treated as a whole. The problem with this holistic approach is that it is very specific and measured K_ds will only be applicable to the soil for which it was measured and only under the same conditions. Another way of treating the complexity is to go into detail with the mechanisms and for instance investigate the sorption to different parts of the soil like minerals and different kinds of organic matter. This gives more universal knowledge generally applicable, but as many chemicals exists and the measurements of partition coefficients are difficult and very time consuming it is unrealistic to complete all detailed measurements for all compounds. Therefore, a solution is to use more simple standardized measurable partition coefficients, that are available for many compounds,

and relate them to the specific environmental partition coefficients of interest with linear free energy relationships (LFERs).

One standardized partition coefficient is the 1-octanol water partition coefficient. The octanol water partition coefficient K_{ow} is the most widely used standard partition coefficient for environmental purposes, because it has been shown to correlate with many descriptors of environmental partitioning phenomena (Schwarzenbach et al. 1993). K_{ow} has been shown to be correlated with the bio concentration factor (BCF) in rainbow trout for PCBs (Chiou et al. 1977) with uptake of PAC from sediments to muscle of fin fish (Hellou et al. 1995) and other bioaccumulation phenomena (Dorsey and Khaledi 1993). Also in the modelling of plant uptake and transport, K_{ow} has been used successfully (Trapp et al. 1990, Simonich and Hites 1995, Polder et al. 1995).

The octanol water partition coefficient is defined as:

$$K_{ow} = \frac{C_{oct}}{C_{water}} \left(\frac{mol \cdot L_{oct}^{-1}}{mol \cdot L_{w}^{-1}}\right) \qquad \text{eq 3.24}$$

The partition coefficient should be independent of concentration, that is the compounds should obey Henrys law. In the "real world" this means that concentrations must be kept low. The partition coefficient is defined for the same species of a compound in both phases, normally the neutral, and therefore ionization must be taken into account for acids and bases. Temperature dependency is rather small for environmental purposes, approximately ± 0.01 log K unit/degree Kelvin (Sangster 1989). For molecules that tend to self associate in either of the phases, concentration dependency of the partition coefficient is important, but only at high concentrations, where there are enough molecules to meet and self associate (Bohra et al. 1994).

In the octanol water system there is a mutual solution of the opposite phase in that there is 0.008 % mol/mol octanol (4.5 mol/m3 (Leo et al. 1971)) in the water phase and 21 % mol/mol (2,3 mol/L Leo et al. 1971) water in the octanol phase (Schwarzenbach et al. 1993, Miller et al. 1985). This means that n-octanol is a so called cosolvent in the water phase. The dissolved octanol will be in water lined cavities as single molecules, but these cavities can possibly be shared by the solute giving the solute a lower excess energy and therefore a higher concentration in the water phase. However, Miller et al. (1985) found that there was no measurable difference between the activity of solutes in water and in octanol saturated water for mainly PCBs and PAHs, whereas Chiou et al. (1977) found differences of 1.8 (hexachlorobenzene) and 2.6 (DDT) in activity. In theory polar compounds should benefit less from a cosolvent than nonpolar compounds, in regards to water solubility, as polar compounds already have a lower activity in the water phase than the nonpolar compounds and would create less strong interactions with octanol than the nonpolar compounds. The large amount of water present in the octanol phase could be expected to influence the activity of solutes in the octanol phase, but according to Miller et al. (1985) PCBs and PAHs had nearly the same activity in water-free and water-saturated octanol. This could be due to the non polarity of the compounds tested and possibly there is a difference between the activity of a hydrogen bonding solute in water-free and water saturated octanol.

The solvatochromic approach

Using a solvatochromic approach Abraham and Rosés (1994) investigated octanol water partitioning

The solvation equation applied was

$$\log K_{ow} = c_a + r_a R_2 + s_a \pi_2^H + a_a \sum \alpha_2^H + b_a \sum \beta_2^H + v_a V_x \qquad \text{eq. 3.25}$$

Where c,r,s,a,b and v with subscript a are adjustable coefficients describing the relative importance of the different molecular interactions between solute and the molecules in the octanol phase and in the water phase.

The solvatochromic parameters of the solute are
R_2 is the excess molar refraction if the solute
π_2^H is the dipolarity/polarizability of the solute
α_2^H is the hydrogen bond donor ability of the solute
β_2^H is the hydrogen bond acceptor ability of the solute
V_x is the volume of the solute (McGowan)

The equation (3.25) was fitted by Abraham and Rosés (1994 b) using 613 compounds with known solvatochromic parameters, and they found that log K_{ow} could be described with the following equation (3.26).

$$\log K_{ow} = 0.088 + 0.562 R_2 - 1.054 \pi_2^H + 0.034 \sum \alpha_2^H - 3.460 \sum \beta_2^H + 3.814 V_x \quad \text{eq. 3.26}$$

A slightly different equation , without the molar refraction term, has been used by Pagliara et al. (1995) and they found very similar results.

The solvatochromic results can be summarized in the following table.

Solute property	Importance for octanol water partitioning	
dipolarity/polarizability	Important	Favours water
hydrogen bond acceptor ability	Important	Favours water
molar volume	Important	Favours octanol
excess molar refractivity	Little important	Favours octanol
hydrogen bond donor ability	Not important	

Table 3.1. *Importance of solute properties for octanol-water partitioning.*

This is what could be expected from the knowledge of general solvation theory, as outlined by Grant and Higuchi (1990), except for the unimportance of hydrogen bond donor ability of the solute. This unimportance was also found by the above mentioned Pagliara et al. (1995) using two different sets of data from the literature. From the equation above it can be seen that octanol and water has more or less the same hydrogen bond basicity and this is why it becomes unimportant for partitioning. From solvation theory it would be expected that hydrogen bond donors would be favoured by the water phase as the hydrogen bond basicity of water, according to solvatochromic

measurements, is supposed to be higher than that of octanol. As described above, this is not what was found by Abraham et al. (1994 b) and according to Abraham et al. (1994 b) the explanation for the discrepancy is not the large amount of water in the water saturated octanol but rather that the solvatochromic measurements are incorrect. The explanations for the sign of the other coefficients are that since the cohesive energy density (the molar energy of vaporization/molar volume) for water (16.65-23.4 $Cal^{1/2}cm^{-3/2}$) is greater than for octanol (10.2 $Cal^{-1/2}cm^{-3/2}$) (Grant and Higuchi 1990), increasing size favors the octanol phase. Conversely, polar and polarizable molecules are favored by the water phase as water is more polar than octanol. And as the hydrogen bond donor ability of water is greater than that of octanol, hydrogen bond acceptors will be favored by the water phase.

3.2.2 The organic matter-water partition coefficient K_{om}

In difference from the octanol-water partition coefficient, the organic matter water partition coefficient is not unambiguous in that many types of organic material exists. K_{om} is mainly used in relation to sorption to soil, but may as well be used for sorption to organic material in rivers, plants, air particles etc.

$$K_{om} = \frac{C_{om}}{C_{w,neut}} \left(\frac{mol \cdot kg_{om}^{-1}}{mol \cdot L_w^{-1}}\right) \qquad \text{eq 3.27}$$

K_{om} is difficult to measure. First the organic material of question must be isolated, which can be a complicated task (Grøn et al. 1996), and followingly an experiment, to determine partitioning must be made. Alternatively, K_{om} can be calculated from the soil sorption coefficient and the amount of organic matter in the soil, under the assumption that all sorption is caused by the organic material. However, the amount of organic matter in soil is difficult to measure and the assumption, that all sorption is caused by the organic material of the soil, may not be justified.

3.2.3 The organic carbon-water partition coefficient K_{oc}

The organic carbon-water partition coefficient is a normalized version of K_d or K_{om} with regards to organic carbon. It has been found practical to use this normalization because it gives more universal and easier comparable values and furthermore, the amount of organic carbon is easy to measure. If K_d is transformed to K_{oc} it is implied that all sorption is caused by organic matter, which may not be the case.

$$K_{oc} = \frac{C_{oc}}{C_{w,neut}} \left(\frac{mol \cdot kg_{oc}^{-1}}{mol \cdot L_w^{-1}}\right) \qquad \text{eq 3.28}$$

and

$$K_{oc} = \frac{m_{om}}{m_{oc}} K_{om} \left(\frac{mol \cdot kg_{oc}^{-1}}{mol \cdot L_W^{-1}}\right) \qquad \text{eq 3.29}$$

3.2.4 The soil-water partition coefficient

A very useful but also very complex partition coefficient is the soil-water partition coefficient K_d. K_d for a given compound is related to all sorptive properties of a soil and it is therefore very specific which means that a measured K_d only can be applied for the soil for which it was measured. The relationship between sorbed concentration and

aqueous concentration is not necessarily linear and a Freundlich isotherm is therefore often used to describe the relationship.

$$C_s = K_d \cdot C_w^n \qquad \text{eq 3.30}$$

If n is larger than 1, a higher proportion of the solute is sorbed at higher aqueous solute concentrations. This could be the case if for instance the sorption of the first solutes makes the sorption of the next solutes easier. If n is smaller than 1, a smaller proportion of the solute is sorbed at higher aqueous solute concentrations. This could be the case if for instance a limited number of sorption sites are available as the likelihood of a solute meeting a sorption site would decrease with increasing solute concentration.

The mechanistic complexity of K_ds can be illustrated by the formula given by Schwarzenbach et al. (1993) for an ionizable compound:

$$K_d = \frac{C_{om} \cdot f_{om} + C_{\min} \cdot A + C_{ie} \cdot \sigma_{ie} \cdot A + C_{rxn} \cdot \sigma_{rxn} \cdot A}{C_{w,neut} + C_{w,ion}} \quad (\frac{mol \cdot kg_{soil}^{-1}}{mol \cdot L_w^{-1}}) \qquad \text{eq 3.31}$$

Where
C_{om} is concentration of solute in the organic matter of the soil
f_{om} is the amount of organic matter in the soil
C_{min} is the concentration of sorbate bound to mineral surfaces
A is the area of mineral surface
C_{ie} is the concentration of ioinized sorbate bound to sites of opposite charge on the solid surface
σ_{ie} is the concentration of charged sites on the solid surface
C_{rxn} is the concentration of solute bound in a reversible reaction to the solid surface (not sorbed)
σ_{rxn} is the concentration of reaction sites on the solid surface
$C_{w,neut}$ is the concentration of the neutral solute in water
$C_{w,ion}$ is the concentration of the ionized solute in water
(Schwarzenbach et al. 1993)

It is clear that if sorption to minerals (solid surface) is of little importance K_d can easily be related to the organic matter.

3.3 Summary

In this chapter it has been shown that partitioning can thermodynamically be related to chemical potential that is a function of activity or excess energy created by non ideality. The non ideality is created by intermolecular forces like Van der Waals forces and hydrogen bonding which ultimately are responsible for partitioning. The validity of Linear free energy relationships were theoretically shown to be dependent on the molecular interactions in the systems of question. The octanol-water partition coefficient was defined together with the soil sorption coefficient, the organic matter-water partition coefficient and the organic carbon-water coefficient that is a normalization of the organic matter partition coefficient. All these partition coefficients are valuable when the environmental distribution of a compound shall be evaluated and there are several ways that the partition coefficients can be measured which is the subject of the following chapter.

4. Methods for determination of partition coefficients

4.1 Methods for determining octanol water partition coefficients

There are two main ways of determining octanol water partition coefficients experimentally, the direct and the indirect approach. In the direct approach an experiment is set up in which the solute of interest is equilibrated between mutually saturated octanol and water phases. The solute is followingly quantified in one or both phases and the partition coefficient is calculated. Even though this may seem simple many problems exists which have lead to the development of several more or less complicated methods. Despite the problems the direct approach must be used when great accuracy is needed. The shake flask method, the slow stirring method and the generator column method described below are all direct methods. The indirect approach is usually chosen when partition coefficients of many compounds are to be determined in a short period of time, and it can be either experimental or based on theory but in both cases previously established data are used for calibration. In the experimental indirect approach some measurable parameter is related to partition coefficients via calibration using a calibration set of solutes. The parameter is measured for the solute of interest and the partition coefficient is calculated from the calibrated relationship. The more similar the measured parameter is to octanol water partitioning the more accurate will determinations be. Obviously, the accuracy is dependent on the accuracy and availability of directly measured values. If experimental work is not an option an indirect theoretical approach can be used. In the indirect theoretical approach partition coefficients are predicted using molecular properties. Again, directly measured values must be used for calibration and this approach is therefore also dependent of the accuracy and availability of directly measured values.

4.1.1 Direct measurement of K_{ow}

The main problem in direct measurement of K_{ow} is the low water concentrations obtained in the experiments for the very hydrophobic compounds as the low water concentrations can be difficult to measure. However, also other problems like micelle formation, sorption to glassware and impurities may distort measurements and many methods have been developed with the aim of circumventing these problems.

The shake flask method

The shake flask method, adopted by the OECD guidelines for testing of chemicals, is a simple method where octanol and water are shaken with the solute of interest in a flask until equilibration has occurred. After the phases have separated, the solute is quantified in each phase by a suitable analytical method and the partition coefficient is calculated. (OECD 1981). According to Bruijn et al. (1989), the method is not suitable for log K_{ow} > 4.5 because octanol micelles form in the water phase during shaking. The larger the K_{ow}, the larger will the problem with micelles be. The shake flask method is fast, simple and non laborious, but only low K_{ow} can be measured because of the low water concentration obtained and micelle formation. Furthermore, the method is sensible to impurities (Leo et al. 1971).

The slow stirring method

The slow stirring method is an improved version of the shake flask method, designed to avoid the formation of octanol micelles in the water phase. A carefully set up octanol-water system including the solute is stirred gently with strict temperature control until equilibrium is obtained. The solute is quantified in the two phases and the partition coefficient is calculated. (Bruijn et al. 1989). A study by van Haelst et al. (1994) showed that although high hydrophobicities of isomeric tetrachlorobenzyltoluenes could be measured, addition of the solutes as a mixture to the slow stirring system for simultaneous determination of K_{ow}s gave results incompatible with individual determinations. The advantage of the method is that high K_{ow} can be measured as no micelles are formed, but the method is still limited by the low water concentration obtained, the difficult setup and the sensibility to impurities.

The generator column method

The generator column method is a method in which several of the problems from the shake flask method and the slow stirring method are solved. The method utilizes two columns, a generator column and an extractor column. The generator column is a column of dimethyl chlorosilane-treated silica that is coated with water saturated octanol in which the solute of interest has been dissolved. The generator column is slowly eluted with octanol saturated water and at the outlet of the column the solute is partitioned between the two phases according to the equilibrium partitioning constant. The water eluent from the generator column is lead through the extractor column, normally a C_{18} column, where the solute is retained. After sufficient water has been lead through the columns the extractor column is disconnected purged and dried with nitrogen and eluted with hexane in which the solute concentration is determined. The solute concentration in octanol is considered unchanged. (DeVoe et al. 1981, Woodburn et al. 1984). The advantages are that high K_{ow} can be measured as the problems of micelle formation and low water concentrations are avoided. Problems with adsorption to glass ware are also avoided as the system is equilibrated before measurements are begun. The disadvantages are the complicated setup which makes the method laborious and time consuming. Like the other direct methods, the generator column method is sensible to impurities.

4.1.2 Indirect experimental measurement of K_{ow}

Indirect measurements of K_{ow} have the advantage of increased productivity compared with direct methods. With indirect methods easily measurable parameters are related to partition coefficients via linear free energy relationships (see chapter 3.1.4) using a calibration set of solutes for which K_{ow} previously have been determined.

Liquid liquid chromatography using octanol coated columns

The most direct of the indirect methods is liquid liquid chromatography with octanol coated C_8 or C_{18} columns. A set of solutes with known K_{ow}s are chromatographed on the octanol columns using water as an eluent and the measured retentions are related to known K_{ow}s. To determine an unknown K_{ow} the solute of interest is chromatographed and the partition coefficient is calculated from the established relationship between retention and K_{ow} (Mirrlees et al. 1976). The correlation between retention and partition coefficients are excellent on these columns but the columns are unstable and suffer from loss of octanol during operation.

Solvent generated liquid liquid chromatography SGLLC

Solvent generated liquid liquid chromatography is an improvement of the traditional octanol column method that suffers from erosion. The SGLLC method uses the mobile phase, octanol saturated water, to load the column with octanol. In this way a steady state octanol coating is obtained and the problem of erosion during operation is eliminated. In other regards the method is identical to the liquid liquid chromatography using octanol columns (Cichna et al. 1995).

Countercurrent chromatography

In counter current chromatography a biphasic liquid system is used for chromatography. The stationary phase liquid (octanol) is kept stationary by centrifugal forces while the mobile phase (water) is pushed through it. This liquid-liquid system only enables measuring of octanol water partition coefficients of 0.003-300 (Berthod et al. 1992, Berthod et al. 1996).

Co-current chromatography

Co current chromatography is an extension of the counter current chromatography so that it is possible to measure K_{ow} of up to 20000 ($\log K_{ow} = 4.3$) . In co-current chromatography both the octanol and the water moves in the same direction at different rates (Berthod et al. 1996).

The HPLC method

Reversed phase high performance liquid chromatography offers the possibility of fast determination of partition coefficients when a large number of partition coefficients are to be determined. The method has been widespread (Ruepert et al. 1985, Thus and Krak 1985, OECD 1989, Whitehous and Cooke 1982, Unger and Chiang 1981, Yamagami and Takao 1992, Eadsforth 1986, Rapaport and Eisenreich 1984, Minick et al. 1989) and some suggest that retention on RP-HPLC columns are more suitable for correlation with biological processes than the octanol water partition coefficient. (Dorsey and Khaledi 1993). Using this method a number of reference compounds are chromatographed and the retention time is related linearly to reference K_{ow} values. The solutes with unknown K_{ow} are followingly chromatographed and K_{ow} are predicted from the retention. There are many possibilities for selecting operational conditions and HPLC column material.

The drawbacks of the method are mainly, as with all indirect methods, the dependability of accurate reference data and the validity of the linear relationship coupling retention with octanol water partitioning.

4.1.3 Theoretical calculation of K_{ow}

From water solubility

As the activity of many non polar organic solutes are much larger in water than in an organic phase like octanol it is possible to relate their octanol-water partitioning well to water solubility. For series of homologues, $\log K_{ow}$ and $\log C_s$ has been related linearly, but different series will have different linear relationships.(Hansch et al. 1968, Chiou et al. 1977, Miller et al. 1985, MacKay et al. 1980, Niimi 1991). A large problem is the availability of water solubilities which, for azaarenes, are limited.

Solvatochromatic approach

The solvatochromatic approach is an interesting approach to calculation of partition coefficients. The solvatochromatic approach uses the knowledge of molecular electronic and physical properties of both solute and solvents to calculate partitioning. The molecular properties of interest are, polarizability, size, dipole moment and hydrogen bonding ability of solute and solvent as these properties are responsible for the sizes of the intermolecular forces (Kamlet et al. 1988). Unfortunately the availability of solvatochromic parameters is limited and the method is mostly applied for fundemental mechanistic studies (Abraham and Rosés 1994, Abraham et al. 1994 a and b)

Group contribution methods

A large number of group contribution methods exists, but they are all based on the same methodology. In group contribution methods, hydrophobicity is considered to be largely additive in that a compound can be split up into groups or fragments which each contribute with a certain amount of lipophilicity to the total lipophilicity of the compound. The fragmental lipophilicity values are determined from very large dataset by datafitting. Beside fragmental lipophilicity values the methods also utilizes correction factors to account for special situations such as sterical hindrance and proximity effects. Examples of group contribution methods are Rekker and Mannholds *f*-fragment method, Meylan and Howards fragment contribution method (Meylan and Howard1995), the π-system of Fujita et al. (1964) and ClogP (Leo 1993).

UNIFAC method

UNIFAC is an acronym for UNIQUAC functional-group activity coefficients and is, as the name implies, and extention of the UNIQUAC method. In UNIFAC the activity coefficients of a solute in different solutions, e.g. octanol and water, are estimated or measured and from these, solubilities or partition coefficients can be calculated. The great advantage of going through activity coefficients is that a long range of parameters can be calculated from the same activity coefficients. Beside solubilities and partition coefficients, henry law constants and chromatographic retention can be calculated. (Grant and Higuchi 1990).

4.1.4 Choice of methods for K_{ow} determination in this study

As the objective of this study has been concerned with determination of octanol water partition coefficients for a large groups of compounds, azaarenes and polar PACs, rather than individual compounds, it was advantageous to use mainly indirect methods. Of these, the experimental HPLC method and the theoretical Rekker and Mannholds *f*-fragment method together with ClogP was tested. The HPLC method is the fastest of the experimental methods if a large number of partition coefficients are to be determined and, once calibrated, has the potential of easy future determinations. The HPLC method requires only a minimum of solute and is insensitive to solute impurities. For the basic azaarenes there is also the advantage that protonated species will not influence results. The availability of directly measured octanol water partition coefficients of azaarenes is limited and as directly determined values are the basis of any indirect determination, the octanol water partition coefficient was determined directly for three azaarenes. For this purpose the generator column method was applied as the generator column method has been demonstrated to be successful for determinations of very high K_{ow}s (Woodburn et al. 1984). The two group contribution methods tested was chosen because they represent the most used theoretical methods designed to be applicable for all kind of compounds.

The other theoretical methods mentioned all suffer from lack of data and are not readily applicable for azaarenes or polar PACs.

4.2 Methods for the determination of organic matter-water partition coefficients

As with the octanol water partition coefficient both direct and indirect methods exists for experimental determination of K_{om}. However, the methods are quite new and several problems were experienced with the fluorescence method and the dialysis bag method when these methods were used in connection with this study.

4.2.1 Direct methods for determination of K_{om}

Fluorescence measurement

The fluorescence method (Gauthier et al. 1986) can only be used with highly florescent solutes and is based on the assumption that unassociated solute is fluorescent while associated (sorbed) is not, which was indirectly confirmed by experiments (Gauthier et al. 1986). The fluorescence of a range of aqueous solutions with different concentrations of organic material or solute is measured and from comparison with blinds the sorbed fraction can be calculated. Unfortunately, correction of inner filter effects must be made even when weak solutions are used, which complicates measurements together with problems of dissolved oxygen (Danielsen et al. 1995).

Dialysis bag

The dialysis bag method (McCarthy and Jimenez 1985) utilizes closed dialysis tubes that are semipermeable. They have a certain molecular weight cut off (a certain distribution of pore sizes) and can therefore retain large molecules while smaller molecules can move through. For the measurement of K_{om}s a solution of the organic material is placed in the tubes which are closed and put into a water solution containing the solute of interest. The solutions with the dialysis bags are shaken until equilibrium is obtained where after the solute concentration in the solution outside the dialysis tube is measured. The amount of solute sorbed is calculated as the difference between blinds (identical but with no organic material) and the equilibrated solutions. Several concentrations of solute or of organic material are used and from these it can be determined if the sorption is linear or nonlinear.

4.2.2 Indirect methods for determination of K_{om}

HPLC method with chemically immobilized organic matter

Like it is possible to use octanol coated columns for the measurement of K_{ow}, columns chemically immobilized organic material can be used for indirect determination of K_{om}. A set of calibration solutes with known K_{om} are chromatographed and the linear relationship is subsequently used for prediction of unknown K_{om}s from measured capacity coefficients. The advantage of this method is the similarity of the column material and the sorbate of interest, i.e. humic acid. The only differences will be those caused by the bonding procedure, such as steric alterations or chemical modifications. As the organic material is bound chemically to the column material, the columns are reasonably stable.

4.2.3 Theoretical methods

Polarity curves

Xing et al. (1994), have developed a system to predict K_{oc} from a polarity index of the sorbate (PI= (O+N)/C) and of the sorbent. The polarity of the sorbent was calculated from the polarity of the sorbate, its K_{ow} and measured K_{oc}.

Group contribution methods

Meylan et al. (1992) and Ames and Grulke (1995) have developed group contribution methods, similar to those described for K_{ow}. However, the data material is much smaller in the case of K_{oc} which limits the validity of this kind of methods.

4.2.4 Choice of methods for K_{om}/K_{oc} determination in this study

For this study, the HPLC method with a chemically bonded humic acid has been chosen for determination of K_{oc}, because it is the only experimental method that makes determination of K_{oc} for a large number of compounds possible in a relatively short period of time.

4.3 Methods for determination of K_d

4.3.1 Direct methods for determination of K_d

The soil column method

Breakthrough curves from soil column experiments can be used to calculate K_d by fitting a suitable model in which K_d is a coefficient and it has been shown that results comply with batch experiment results.(Allen et al. 1993, Johnson and Farmer 1993). However, the method is complicated in that all problems related to column experiments, such as preferential flow and degradation, will influence the K_d determinations.

The batch method

The batch method is a simple setup where soil is shaken with water and the solute of interest. After equilibration has occurred, solute concentration in the two phases is determined. The drawbacks of the batch method are difficulties in determination of sorbed amount, potential biotic degradation during experiments, inhomogeneity of the soil samples and slow equilibration.

4.3.2 Theoretical methods for determination of K_d

Molecular topology

Sabljic (1987) have demonstrated that the correlations between K_{ow} and K_d are difficult to trust. Instead he advocates for using a molecular topology model utilizing first order connectivity indices and good results were obtained for a wide selection of compounds.

4.3.3 Choice of method for determination of K_d in this study

For this study, it was chosen to determine K_d by the use of batch experiments. The soil column method seems more susceptible to errors than the batch method and the setup is more complicated. The use of a batch method limits the number of K_ds that can be

determined, but if no values previously have been measured for the soil and solute type of question, indirect methods are not viable.

4.4 Summary

As has been shown, a large number of methods exists for the determination of octanol water partition coefficients as well as for the determination of organic matter water partition coefficients. The specific nature of soil sorption coefficients limits the number of indirect methods available for determination of K_d. For experimental determinations of K_{ow} for polar PACs, both a direct method and an indirect method were chosen, namely the generator column method and the HPLC method. For determination of K_{oc} a HPLC method with an chemically immobilized humic acid was chosen and a batch method was chosen for determination of K_d. As the potential possibilities of theoretical predictions are appealing, two group contribution methods, the *f*-fragment method and ClogP, were selected for testing.

5. Determination of K_{ow} with the generator column method

5.1 Introduction

The generator column method, shortly described in chapter 4, is a method for direct measuring of K_{ow} developed by DeVoe et al. (1981). The method, as developed by DeVoe et al. (1981) is suitable for very lipophilic/hydrophobic compounds but the method was extended to even more lipophilic compounds by Woodburn et al. (1984).

The general concept of the generator column is that an equilibrium distribution of the solute is created between a stationary octanole phase and a mobile aqueous phase in a column. The large octanol-water interfacial area of the column facilitates fast equilibration of the solute between the phases. Mutually saturated octanol and water phases are applied to diminish the loss of stationary phase. For measurements, the generator column is a glass column with a silanized silica support material. Water saturated octanol containing the solute of interest is applied to the support of the glass column where it is sorbed. Followingly, octanol saturated water is lead through the generator column and an equilibrium solute-water concentration, corresponding to the partition coefficient is created. The eluent water is lead through an reversed phase extractor column, where the solute is retained and when sufficient water has been lead through the extractor column, the extractor column is disconnected, purged and dried with nitrogen. Finally the solute is extracted from the extractor column with hexane and quantified, where after the solute-water concentration can be calculated.

The generator column method has several advantages. The problem of sorption of solute to glassware and other surfaces are minimized as the system is equilibrated by letting octanol saturated water run through the system for a period of time before the extractor column is connected. Very small concentrations of solute in the water phase can be measured, because large amounts of water-eluent can be lead through the extractor column and micelles are avoided because of the slow flow rates used.

5.2 Experimental

The generator column experiments were conducted according to Woodburn et al. (1984) and K_{ow}s of dibenz[a,c]acridine, dibenz[c,h]acridine and 10-azabenzo[a]pyrene were determined. The generator column system consisted of a HPLC pump, the thermostated generator column, an extractor column and a UV-detector for detection of accidental breakthroughs (see Fig 5.1).

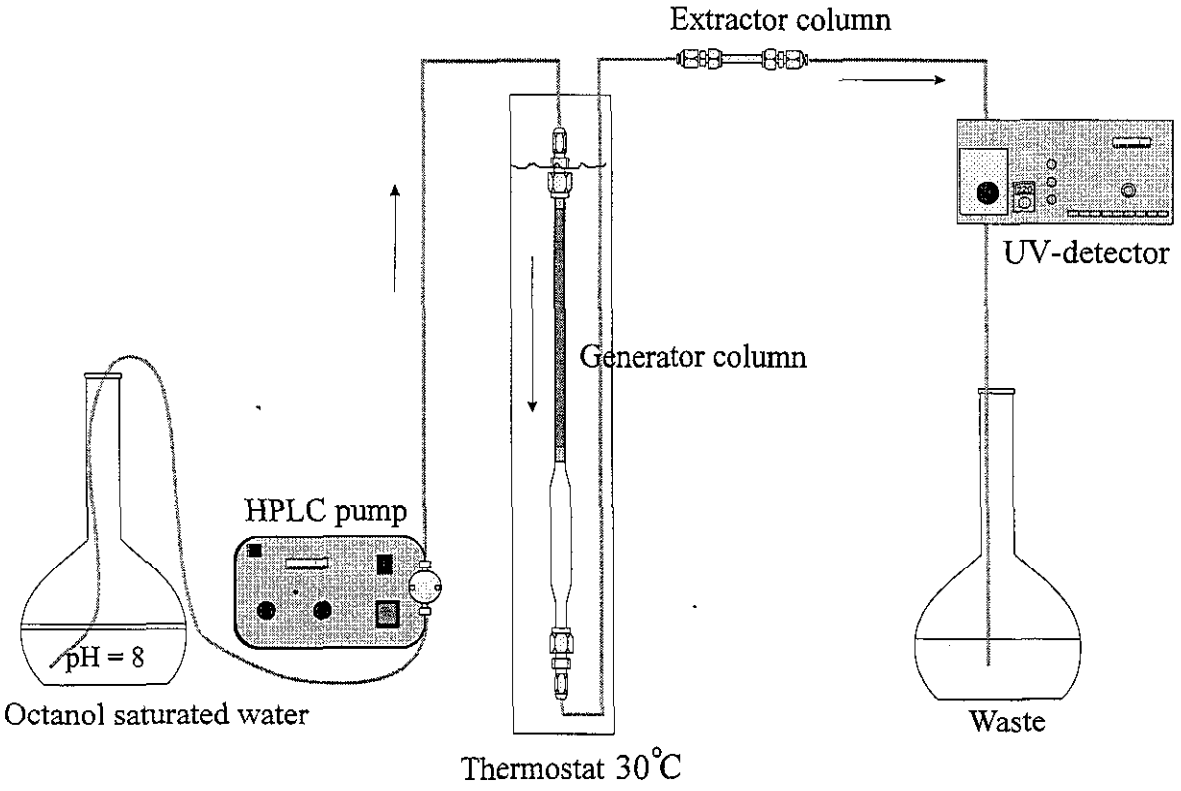


Fig 5.1. *The generator column setup.*

5.2.1 Materials

The generator column was a 240 mm 4mm ID glass column with an enlargement near one end, see Fig 5.1. The small diameter part of the column was hand packed, by tapping and rotation, with dry DCMS (dimethyl chlorosilane)-treated and acid washed Chromosorb W, mesh size 60/80 from Johns Manville. The 80 mm, 4.6 mm ID stainless steel extractor column was packed with polygosil 35-42 octadecyl silica material from Machery-Nagel. The flow of the system was provided with either a Kontron LC pump or a Waters 410 LC pump. A LDC UV detector was used for detection of accidental breakthroughs. All tubing was clean teflon tubing and Peek ferrules were used for connections. The 1-octanol (for synthesis [GC] >99 %) was purchased from Merck. Laboratory grade ion exchanged water, extra purified on a Millipore-Q water purification system was used for all experiments. The water was buffered to pH 8 with Na_2HPO_4 and NaH_2PO_4 Both Merck (for synthesis). The testcompounds dibenz[a,c]acridine, dibenz[c,h]acridine and 10-azabenzo[a]pyrene were obtained as pure compounds from Commision of the European Communities, Community Bureau of Reference Materials. n-hexane for extraction was from Merck (for synthesis).

Quantitation was done with a Shimadzu LC10-HPLC system with PDA detector, thermostated column oven and autoinjector. Isocratic elution with water/methanol was used on a 50 mm x 4.6 mm ID, Phenomenex, Prodigy ODS II, 5 µm, column.

5.2.2 Procedure

Approximately 10 mg of the solute was dissolved in 100 ml octanol that was saturated with water by stirring for 14-18 h. The octanol was isolated and used for coating the generator column by drawing it through the column with gentle suction until the octanol

could be seen above the support. The octanol was left for 24 h. on the column and excess octanol was then purged with octanol saturated buffered water with the enlarged end of the column upwards. When no more free phase octanol left the column, approximately after 500 mL water had been lead through, the generator column was turned upside down, so that the enlarged end now pointed downwards, see Fig 5.1. The enlargement should then trap any accidentally released free phase octanol. Finally, the outlet of the generator column was connected with the extractor column with clean teflon tubing and the experiment was begun. The temperature of the generator column was kept at 30^0C in a thermostated water bath. About 1-4 l of octanol saturated buffered water was pumped through the system for each determination with a speed of 0.5-1 ml min^{-1} and collected in a tared weighing flask. After an appropriate amount of water had been pumped through the column system, the extractor column was disconnected and dried with nitrogen to remove the water. The solute was then extracted with 10 ml *n*-hexane. For the dibenz[c,h]acridine and 10-azabenzo[a]pyrene the n-hexane was concentrated to 1 ml by evaporation under nitrogen and the solute was finally quantified on HPLC by injecting 20 mL in the cases of dibenz[c,h]acridine and 10-azabenzo[a]pyrene and 100 µL hexane sample in the case of dibenz[a,c]acridine which were not evaporated. The HPLC measurements were done at 290 nm for dibenz[c,h]acridine and 10-azabenzo[a]pyrene and 282 nm for dibenz[a,c]acridine. The generator column was loaded twice for each compound tested and for each loading two determinations of log K_{ow} were made. Between loadings the generator column was cleaned with methanol and dried with nitrogen.The amount of eluted water was determined by weighing the tared weighing flask.

5.3 Results and discussion

The solute concentration in the water phase can be calculated from the collected amount of solute and the amount of water eluted and as the solute concentration in the octanol can be considered unchanged throughout the experiment, the partition coefficient can be calculated. The hexane was analyzed on HPLC in triplicate. The standard deviations in determination of solute concentration in hexane were in general 0-5 %. The results of the generator column determinations are given in Table 5.1.

	dibenz[a,c]acridine		**dibenz[c,h]acridine**		**10-azabenzo[a]pyrene**	
	eluent	log K_{ow}	eluent	log K_{ow}	eluent	log K_{ow}
1st loading	820 g	5.657	2696 g	6.315	3818 g	5.383
	916 g	5.708	3877 g	6.634	4361 g	5.682
2nd loading	752 g	5.608	3854 g	6.415	3076 g	1.540*
	-	-	-	-	4098 g	5.796
Average		**5.658 ± 0.050**		**6.455 ± 0.163**		**5.533 ± 0.212**

Table 5.1. *Results from generator column experiments. The result marked * is not included in the average log K_{ow} value (recommended). The temperature was 30^0C.*

As can be seen from Table 5.1, second determinations gave results similar to first determinations, for the same loading, which indicates that the column had not been depleted of solute. At pH 8 the dibenz[c,h]acridine (pKa = 3.6) is speciated with neutral to protonated as 25120:1. If all of the protonated fraction is in the water phase this would give a log K_{ow} of 4.4 which is much lower than what was found in this study. The reason for the higher log K_{ow} found in these experiments seems to be that the protonated species is not collected on the extractor column, due to its high water solubility and

therefore does not influence the results which then can be considered representative for the neutral compounds.

6. Determination of K_{ow} with the HPLC method

6.1 Background

The general HPLC method is based on a linear free energy relationship (LFER) between the partitioning in a HPLC stationary phase-eluent system and the partitioning in an octanol-water system.

$$\log K_{ow} = a \cdot \log k' + c \qquad \text{eq 6.1}$$

The linear coefficients a and b are estimated from sets of known log K_{ow} and experimentally determined HPLC capacity coefficients k`. Using the calibrated expression, unknown log K_{ow}s can subsequently be predicted from measured capacity coefficients.

As explained in Chapter 3 the LFER, coupling retention in a HPLC column system to octanol water partitioning, is only valid if *the solute undergoes the same types of intermolecular interactions in the two systems* !. For a HPLC system to be suitable for prediction of K_{ow} the retention in the HPLC system should therefore be governed by the same mechanisms that govern partitioning in the octanol water system. In other words the HPLC system should mimic the octanol-water system and for this a bonded organic stationary phase together with a water based eluent is the natural choice, but several commercially available bonded stationary phases and several normally used organic modifiers exist. Dorsey and Cooper (1994) concludes that the latest studies concerning RP-HPLC show that "retention is governed by a partitioning process rather than by adsorption", which suggests that the activity of a solute in both HPLC phases will be important for retention.

To aid in the selection of suitable stationary and mobile phase, the interactions between solute and system phase molecules in an octanol-water system and in a reversed phase HPLC system can be compared as done below.

The octanol water system The solute will interact-	**Chromatographic system with a nonpolar phase and water based eluent** The solute molecules will interact-
With water molecules in the water phase by van der Waals forces by hydrogen bonding if the solute is a hydrogen bond donor or acceptor	With water molecules in the eluent by van der Waals forces by hydrogen bonding if the solute is a hydrogen bond donor or acceptor
With octanol molecules in the water phase Cosolvency	With organic modifier molecules in the eluent by van der Waals forces by hydrogen bonding between organic modifier molecules and solute if the solute and the modifier are hydrogen bond donors or acceptors.
	With solvated molecules (organic modifier) in the stationary phase by van der Waals forces by hydrogen bonding if organic modifier and solute are hydrogen bond donors or acceptors.
With octanol molecules in the octanol phase by van der Waals forces by hydrogen bonding between octanol and solute if the solute is a hydrogen bond donor or acceptor	With the stationary phase functional groups by van der Waals forces by hydrogen bonding if stationary phase and solute are hydrogen bond donors or acceptors.
	With the stationary phase structure by steric interactions
With water molecules in the octanol phase. by hydrogen bonding if solute is hydrogen bond donor or acceptor.	With residual hydroxy groups on the stationary phase by hydrogen bonding if solute is hydrogen bond donor or acceptor. By dipole-dipole interactions if the solute is polar With water molecules bound by residual hydroxy groups on the stationary phase by hydrogen bonding if solute is hydrogen bond donor or acceptor. By dipole-dipole interactions if the solute is polar

Table 6.1. *Conceptual ordering of solute interactions with the phases in the octanol-water system and in a HPLC system.*

The ordering of interactions across from each other in the table does not implicate equivalence between mechanisms but is merely used as a basis of discussion of the differences and similarities between the two systems as follows below.

1. The interactions between solute and water molecules in the mobile phase and water of the octanol-water system are considered identical.

2. The organic modifier in the mobile phase does not have any immediate equivalent in the water phase of the octanol-water system and the interactions between solute and organic modifier in the mobile phase will be special to the RP-HPLC system. In the water phase of the octanol-water system octanol acts as a cosolvent but the importance of this mechanism seems to be limited and especially so for polar compounds, see chapter 3. The organic modifier is not a cosolvent in the mobile phase as the fraction of organic modifier usually is to high for all organic modifier molecules to be solvated by

water. In stead, the organic modifier will act as a solvent itself and solvate solutes in proportion to its volume fraction (Schwarzenbach et al. 1993). The thermodynamic benefit (lower activity in water) for a nonpolar solute from being solvated by organic modifier molecules would probably not be as large as that obtained with octanol.

3. The stationary phase of the RP-HPLC system will be solvated by organic modifier molecules due to dispersive forces and/or polar forces depending on functional group and organic modifier (Scott 1993, Tchapla et al. 1993). If a 100% MeOH mobile phase was used with a C_{18} column, 65 ±15 % volume of the stationary phase was alkyl chains whereas the remaining 35 ±15 % volume was methanol (Dorsey and Cooper 1994). There is no immediate equivalent to this in the octanol-water system, but if the organic modifier resembles water, the large amount of water present in the octanol phase of the octanol-water system could be likened to the organic modifier in the stationary phase.

4. The interactions between a polar solute and octanol in the octanol-water system includes hydrogen bonding as well as van der Waals forces and to have a direct equivalent in the RP-HPLC system, the functional groups of the stationary material must also be able to interact through the same forces. Of course this would be expected to be especially important for polar hydrogen bond forming compounds.

5. An important difference between RP-HPLC systems and the octanol-water system is the difference in steric structure. The octanol is a bulk liquid phase whereas the RP-HPLC phase is a structured phase, more or less well defined depending on type (monomeric/polymeric), production method and operation conditions. In a theory of retention mechanisms in RP-HPLC it is stated that the partial ordering of the grafted stationary phase chains, at sufficiently high bonding density, could lead to entropic expulsion of a solute, leading to less retention than expected and that the importance of the mechanism increases with increasing chain density (Dorsey and Cooper 1994). This is very unlike the octanol-water system where there is no equivalent mechanism. The structure of the stationary phase can result in shape discrimination not occurring in the octanol-water system and this is especially the case for polymeric columns (Sander et al. 1991). In examinations of monomeric phases with a high bonding density using homologue series it has been found that there is a certain solute length above which the solute-stationary chain interactions change and the relationship between number of carbons and retention change. Polymeric phases do not show this behavior (Tchapla et al. 1993).

6. The large amount of water in the octanol of the octanol-water system, possibly important for the partitioning of polar solutes, could have its equivalent in a water like organic modifier as already mentioned above. The amount of water in RP-HPLC stationary phases is usually very small, independent of organic modifier concentration and controlled by the amount and types of residual silanols (Dorsey and Cooper 1994, Jaroniec et al. 1993) so the amounts of water will not be equal in the organic phases of the two systems. Residual silanophilic interactions have been found to play an important role in the chromatographic process as they interact with polar and hydrogen bonding solutes, either directly or through bound water and while the importance of water for solute solubility in water saturated octanol is uncertain, the residual silanols definitely are important for retention (Dorsey and Cooper 1994). From chromatographic reasons, tailing and low resolution, large amount of silanols has traditionally been unpopular and the amount is often reduced by end capping.

From the above comparison and discussion some major points can be gathered concerning the mimicking of the octanol-water system with RP-HPLC.

- There is the possibility of hydrogen bonding between polar solutes and the octanol organic phase. This may be mimicked in a HPLC system..
- There are inherent structural differences between a RP-HPLC stationary phase and octanol.
- There is an organic modifier in the RP-HPLC stationary phase whereas there is no organic modifier in the octanol phase of the octanol water system.
- There is a large amount of organic modifier in the mobile phase whereas there is little octanol in the water phase of the octanol water system.

And most important, the differences between the two systems depend on solute properties, especially hydrogen bonding ability and polarity, and on the nature of stationary and mobile phase.

6.2 The choice of RP-HPLC systems for determination of K_{ow}

From the points made, it would seem an advantage not to use any organic modifier at all, but with normal RP-HPLC columns this is not viable as retention times would become prohibitively long. However, it is clear that the organic modifier that has properties closest to those of water should be preferred for prediction of K_{ow}s. Methanol is the most polar and the most water like of traditional organic modifiers. Methanol can form hydrogen bonds like water and is similar in size. A mobile phase with water and methanol will therefore have properties as close to those of water as possible. Methanol will solvate the stationary phase which possibly can correspond to the water in water saturated octanol and the curvature in the relationship between k' and φ (fraction of organic modifier in the mobile phase) is less for methanol-water than for both acetonitrile-water and tetrahydrofuran-water (Lambert 1993) which could be an advantage if extrapolation to 100 % water is used. In this work, methanol water eluents have consequently been used throughout for prediction of partition coefficients.

The choice of stationary phase is more complicated. There are at least two possibilities of mimicking the octanol phase of a octanol water system. The first is to use a bonded phase which groups resemble octanol very closely. That is they should have a polar part with the ability of making H-bonds and at the same time have a nonpolar part responsible for dispersion forces. The second possibility is to use a nonpolar phase responsible for dispersion forces and rely on a solvating organic modifier, residual silanols and residual silanol bound water for the hydrogen bonding in the stationary phase. In this work both approaches have been tested in that a **Diol stationary phase** was chosen together with a traditional **C_{18} stationary phase.**

The columns applied were monomeric or "brush type" as monomeric phases are more reproducible (Dorsey and Cooper 1994) and properties will be more consistent. Furthermore, the shape selectivity of the polymeric columns is not a desireable quality when mimicking the octanol-water system. The C_{18} columns were endcapped and identical retention of benzene and toluene when a nonpolar mobile phase was used, confirmed that the amount of residual silanole groups was minimal. The endcapped columns were preferred because residual silanols seemed to be a rather less well defined

component of the system to be relied on for hydrogen bonding. The only way a fully end capped column will be able to form hydrogen bonds is through solvating organic modifier molecules.

6.2.1 The Diol column

The Diol column functional groups posses two hydroxy moieties and a short alkyl part (see Fig 6.1) In the normal phase mode, the Diol column has been shown to participate in hydrogen bonds with azaarenes (Siouffi et al. 1986) which means, that since there is no water in the stationary phase during normal phase mode, the hydroxy moieties of the functional groups are able to interact with hydrogen bonding solutes. The normal synthetic route followed in the preparation of Diol columns may, dependent on synthesis conditions like pH, temperature and solvent, lead to the occurrence of different species on the silica surface. Glycol ethers, polyaddition poducts and oligosilanes has been observed and different Diol columns can therefore have quite different hydrophilic and hydrophobic properties. (Pesek and Matyska 1994). A new pathway resulting in a diol phase without side products has been suggested by Pesek and Matyska (1994) but unfortunately these are not yet commercially available. No work on the use of Diol column for prediction of partition coefficients has previously been reported.

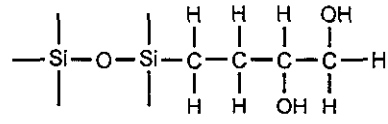


Fig 6.1. *The bonded group of the Diol column.*

6.2.2 The C_{18} column

The bonded groups in a C_{18} column are octadecylsilanes and a C_{18} column is also called an ODS column. The ODS bonded alkyl chains are entirely nonpolar. The chains are flexible ((Lochmüller et al. 1993) and depending on the mobile phase they will be more or less extended. Unlike the Diol column, the ODS columns have been used frequently for prediction of partition coefficients (Ruepert et al. 1985, Thus and Krak 1985, OECD 1989, Whitehouse and Cooke 1982, Unger and Chiang 1981, Yamagami and Takao 1992, Eadsforth 1986, Rapaport and Eisenreich 1984, Minick et al. 1989)

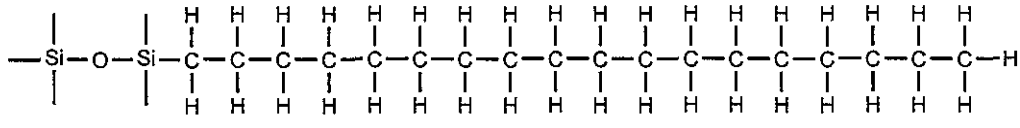


Fig 6.2. *The octadecylsilane of the ODS or C_{18} column.*

Using a solvatochromic approach Abraham and Rosés (1994) compared a reversed phase HPLC system (ODS3) with the octanol water system (see chapter 3).

The solvation equations applied were

$$\log K_{ow} = c_a + r_a R_2 + s_a \pi_2^H + a_a \sum \alpha_2^H + b_a \sum \beta_2^H + v_a V_x \qquad \text{eq. 6.2}$$

$$\log k' = c_b + r_b R_2 + s_b \pi_2^H + a_b \sum \alpha_2^H + b_b \sum \beta_2^H + v_b V_x \qquad \text{eq. 6.3}$$

Where c,r,s,a,b and v with subscripts a or b are adjustable coefficients describing the relative importance of the different molecular interactions between solute and the system phase molecules.

The solvatochromic parameters of the solute are
R_2 is the excess molar refraction if the solute
π_2^H is the dipolarity/polarizability of the solute
α_2^H is the hydrogen bond donor ability of the solute
β_2^H is the hydrogen bond acceptor ability of the solute
V_x is the volume of the solute (McGowan)

Abraham and Rosés (1994) applied the equation (eq. 3.25) to capacity coefficients measured by Smith and coworkers on a spherisorp ODS 3 (C_{18}) phase with several methanol-water, acetonitrile-water and tetrahydofuran-water mobile phases. They found that the stationary phase were more polarizable but less dipolar than the mobile phases. The mobile phases were stronger hydrogen bond acceptors than the stationary phase and the mobile phases were also much stronger hydrogen bond donors than the stationary phase. The stationary phase was, not surprisingly, much more hydrophobic than the mobile phases. Like for the octanol water system the sovatochromic findings can be summarized for the ODS-HPLC system as done in Table 6.2.

Solute property		
dipolarity	Important/not important	Favours mobile phase
polarizability	not important	Favours mobile phase
hydrogen bond acceptor ability	Very Important	Favours mobile phase
molar volume	Very Important	Favours stationary phase
excess molar refractivity	Little important	Favours mobile phase
hydrogen bond donor ability	Important	Favours mobile phase

Table 6.2. *The importance of solute properties for retention on a ODS HPLC column.*

Abraham and Rosés (1994) subsequently compared the K_{ow} and k′ results for different eluents by adjusting all the coefficients of the k′equations so that the v coefficient was identical to that of the K_{ow} equation. They found that 70 % MeOH/30 % water gave the closest match to the K_{ow} equation also mentioned in chapter 3.

$$\log k'_{70/30} = c + 0.78 R_2 - 1.65 \pi_2^H - 1.25 \sum \alpha_2^H - 3.48 \sum \beta_2^H + 3.81 V_x \qquad \text{eq 6.4}$$

$$\log K_{ow} = 0.088 + 0.562 R_2 - 1.054 \pi_2^H + 0.034 \sum \alpha_2^H - 3.460 \sum \beta_2^H + 3.814 V_x \qquad \text{eq 6.5}$$

But as can be seen even in this best case, there are large differences between the r coefficients and the a coefficients. The same discrepancy between hydrogen bond acceptor ability of a C_{18} HPLC system with a water-methanol mobile phase and of the octanol water system was found by Pagliara et al. (1995) using a slightly different solvatochromic equation and extrapolated k'_w values. This indicates that when using a

C_{18} column, compounds that differ in hydrogen bond donor ability or polarity/dipolarity could give inconsistent relationships between partition coefficients and capacity coefficients. Yamagami et al. (1994) found more or less the same regarding solute H donor ability with a smaller data material. For ester and amide derivatives of heteroaromatic compounds, strong hydrogen bond donors partition relatively less into a C_{18} phase than into octanol giving underestimation of K_{ow}, but they also found that strong hydrogen bond acceptors partition relatively more into a C_{18} phase than into octanol giving overestimation of K_{ow}. Best predictions was found at 50 % MeOH. (Yamagami et al. 1994). Also Park et al. (1994) have applied the solvatochromic approach to RP-HPLC columns including C_{18}, C_8, phenyl and cyano columns using 40 % v/v MeOH, methanol water eluents. They found for C_{18}, C_8 and phenyl columns, that solutes with hydrogen bond donor abilities and especially hydrogen bond acceptor abilities would be less retained than non H-bonding solutes as could be expected and that the hydrogen bond donor ability of the solute was more important than the hydrogen bond acceptor ability. In contrast to Abraham and Rosés (1994) they found that the polarity of the column was of no importance in retention, but this may be due to the relatively small data material. Even though findings differ slightly, the results reported here do predict problems with K_{ow} predictions for hydrogen bond donors using C_{18} columns and possibly also with polar compounds.

6.2.3 The amount of methanol in the mobile phase

As stated above it is impossible to use pure water as an eluent because of the long retention times but it could be suspected that a minimum of organic modifier would give the give the best HPLC system for mimicking the octanol water system Things are however more complicated and not even fully understood.

The decrease in volume by mixing methanol with water derives from a complex formation between water and methanol. Therefore it is supposed that there is at least three phases in the water-methanol eluent: W+[WO]+O, where W is water, O is organic modifier and WO is the water organic modifier complex. The amount of complex can be determined from measurement of the decrease in volume by mixing. In the simple model the complex is considered to be a 1:1 complex. (Jaroniec et al. 1993) In accordance with this, an investigation of the ternary system of methanol water eluents showed that when one moves from 0% to 100% methanol different mechanisms are taking place.
0-40 % (v/v)Methanol: Binary mixture of water and methanol-water complexes (No free methanol)
40-80% (v/v)Methanol: Ternary mixture of water, methanol and water-methanol complexes
80-100% (v/v)Methanol: Binary mixture of methanol an methanol-water complexes (No free water)
(Scott 1993)
This means that mobile phase not only change in proportion between components when organic modifier content is changed, but the actual nature of the components change.

To complicate this further, the nature of the stationary phase also changes. Tchapla et al. (1993) suggest that when using water rich eluents with C_{18} columns, organic modifier molecules are inserted between the stationary phase chains making solute penetration/partitioning into the stationary phase possible, but when water rich eluents are used, the monomeric stationary phase will expel the inserted organic modifier

creating an unordered phase. For very low concentrations of organic modifier (1-2 % w/w) the monomeric brush type material reacts opposite to expected. Normally, retention decreases with higher organic modifier content of the mobile phase but for the low concentrations of organic modifier the retention increases with organic modifier content. The explanation for this seems to be that the C_{18} chains collapse at low organic modifier content and as the organic modifier is increased they extend and retention is actually decreased. Only after the chains has been fully extended, when there is sufficient free phase methanol, does the retention decrease with increased organic modifier content (Scott 1993). This theory is supported by the fact that the phenomena of reversed organic modifier fraction - chromatographic retention is not observed for bulk type rigid polymeric stationary phases (Scott 1993). Wirth (1994) states that organic modifiers like methanol and n-propanol mainly is situated on the C_{18} chain-water interface, but this do not comply with the observations of Scott (1993).

The change of stationary phase structure with organic modifier content in the mobile phase may be responsible for the sovatochromic findings mentioned above, where a methanol content of 70 % v/v was found to yield the HPLC system with properties closest to those of the octanol-water system (Abraham and Rosés 1994). Yamagami et al. (1994) found that 40 % MeOH was optimal for mimicking the octanol-water system with a C_{18} column using polar substituted heterocycles.

Taking the complicated mechanisms described into account it is very likely that different columns will have different optimal mobile phase mixtures for the prediction of K_{ow} and it was decided to try several within the practical possible range.

6.2.4 Extrapolation to 100 % water

Some investigators have found that they could improve their results by extrapolating to capacity coefficients corresponding to a mobile phase of 100 % water while others have not (Yamagami and Takao 1992). Valkó et al. (1993) states that "there is probably no one best equation for extrapolating all retention data to 0 % organic modifier for purposes of predicting log K_{ow} from chromatographic data" but linear and quadratic expressions have been used. According to Schoenmakers and Tijssen (1993) and Lambert (1993), the relationship between the volume fraction of organic modifier in the eluent and capacity coefficient k' is quadratic

$$\log k' = A\varphi^2 + B\varphi + C \qquad \text{eq 6.6}$$

where φ (volume fraction of organic modifier)

but in some cases, for aromatic compounds and with methanol as the organic modifier, it has been found that the quadratic term could be neglected (Hammers et al. 1982) resulting in a linear relationship.

$$\log k' = B\varphi + C \qquad \text{eq 6.7}$$

However, Jaroniec (1993) found that the linear relationship were only valid when there was a free phase of methanol. This means that it will be invalid with mobile phases below approximately 40 % MeOH. From any of the relationships a theoretical capacity coefficient at 100 % water could be calculated but as different environments and therefore different mechanisms are at hand in the column at different concentrations of

organic modifier (Scott 1993) no simple mathematical relationship can be expected to hold and as mentioned above, there is no physical reality behind the extrapolated number k'_w. Furthermore, if extrapolations are to be made from a limited range of φ. i.e. 30-70, the value of k'_w will be uncertain. Therefore the capacity coeffficients k' have not been extrapolated to a theoretical k' at 100 % water eluent in this study.

Dead time markers

There is no generally accepted standard for dead time markers and several approaches can be used. Hafkensheid and Tomlinson (1986) mentions the following methods:

1. Pure diluted mobile phase components
2. Differential weighing of the column
3. Linearization of the retention of a homologues series
4. Radio labelled mobile phase components
5. Anions(nitrite, nitrate, dichromate)

According to Hafkensheid and Tomlinson (1986) 1-5 give over estimations of t_0 and even pure water may lead to considerably higher values of t_0 than that obtained using a dilute solution of water in the mobile phase. However $NaNO_3$ has been used in this study as suggested by Nowotnik and Narra (1993). Also pure water has been used and gave similar results to those of $NaNO_3$.

6.2.5 Summary

Investigation of mechanisms in octanol water partitioning and HPLC reveresed phase column retention mechanisms held together with the theory of linear free energy relationships has shown that two types of HPLC system may be successful when applied for prediction of K_{ow} with the HPLC method. One type has bonded phase functional groups that can create polar interactions with a solute and the other type relies on solvating organic modifier for mimicking of the hydrogen bonding of octanol. A Diol column was chosen to represent the first approach whereas an ODS column was chosen to represent the second approach.

6.3 Experimental determination of log K_{ow} for azaarenes, PAHs and two S,O-heterocycles with the HPLC method

6.3.1 Introduction

The goal of these experiments was to evaluate the applicability of the Diol and the ODS HPLC systems for prediction of K_{ow} for polar POM compounds especially azaarenes and to predict K_{ow}s for azaarenes with unknown K_{ow}. The experiment was done by measuring capacity coefficients for a range of relevant test compounds at different methanol water compositions and relating them to octanol water partition coefficients from the literature.

6.3.2 Experimental

Test compounds.

Two groups of test compounds were used, namely azaarenes and PAHs. The azaarene test compounds were chosen to represent the monoazaarenes occurring in the

environment as described in chapter 2. Together with the azaarenes, homologue PAHs were included in the test set as a comparison between the PAH and the azaarenes could illustrate the importance of the nitrogen atom and furthermore the PAH could help in the understanding of chromatographic mechanisms.

Materials

The HPLC system used was a low pressure gradient Shimadzu LC10-HPLC system with PDA detector, thermostated column oven and autoinjector. The ODS columns used were Phenomenex Prodigy ODS-2 columns (150 mm x 4,6 mm ID and 50 mm x 4,6 mm ID) with a particle size of 5 µm. pore size 150 Å, surface area 310 m2/g, carbon load 18.4 %. This column is similar to Inertsil. The ODS columns were end capped and experiments with n-hexane as an eluent showed no difference in the retention times of benzene and nitro-benzene, confirming a negligible amount of residual silanol groups in accordance with the claim of the manufacturer.

The Diol column (250 mm x 4,6 mm ID) was slurry packed with Nucleosil 7 OH (Diol) from Macherey-Nagel, particle size 7 µm. The Diol column was packed using a high pressure slurry technique. The column material was suspended in a 70 % 2-propanole 30 % methanol mixture and pumped onto the column with methanol at a pressure of 350 bar. The eluents were mixed from laboratory grade ion exchanged water, extra purified on a Millipore-Q water purification system, and LiChrosolv® methanol 99.8 % (GC) from Merck or Lab Scan HPLC methanol.

The test compounds were obtained from several sources as pure compounds (see appendix Table A1). Their identity was confirmed by UV absorption spectra. Light was kept from entering the test compound solutions by covering the flasks or keeping them in the dark. It was evident that many of the compounds photolyze quite easily. The test compounds were dissolved in methanol with a typical concentration of 0.04 g l^{-1} and from these solutions, mixtures were made with 3-5 compounds in each mixture. The mixtures were designed so that the compounds eluted at suitable intervals.

Chromatographic procedure

20 µL was injected in all cases with the autoinjector using a partially filled loop technique.
All measurements were repeated three times. The average standard deviation in retention time between replicated injections was 0.7 %. A flowrate of 1 mL/min was used and the temperature was kept at 30^0 C for all measurements.

The dead time of the system t_0, used for calculating the capacity coefficient: $k' = (t_r - t_0)t_0^{-1}$, was determined by chromatographing $NaNO_3$. This was done in connection with each set of chromatographic runs. During the experimental period the dead time had a variation of 3 % on the ODS columns (two identical columns) and 1.5 % on the Diol columns. Water was also tested as a deadtime marker and gave similar results to those of $NaNO_3$.

It was not possible, from a practical point of view, to use less than 65 % MeOH in the eluent for the ODS column due to long retention times of the most hydrophobic compounds. For the Diol system on the other hand, a minimum of 50 % v/v water had

to be used in order to get separate retention times for the least retained compounds. Therefore 50 and 35 % v/v methanol was used with the Diol column (Diol-50 and Diol-35) and 85, 75 and 65 % v/v methanol was used for the ODS column (ODS-85, ODS-75 and ODS-65).

The shape of the peaks obtained was in general symmetrical with a slight tailing on both column types. The very long retention times obtained in some cases for the larger compounds, resulted in quite distorted peaks. A few examples of typical chromatograms are given below in fig 6.3, 6.4 and 6.5.

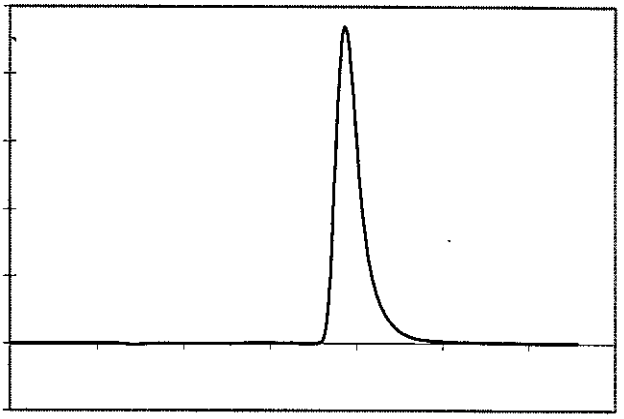


Fig 6.3. Quinoline on the ODS column, 65 % MeOH/35 % Water at 228 nm

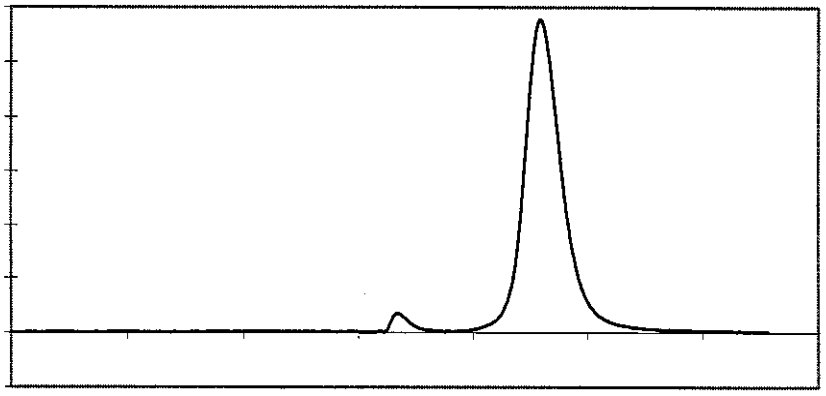


Fig 6.4. Naphthalene on the Diol column, 35 % MeOH/65 % water at 249 nm.

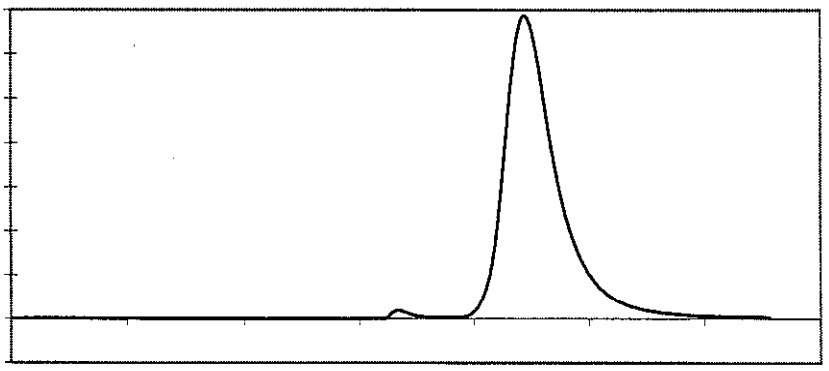


Fig 6.5. Quinoline on the Diol column, 35 % MeOH/65 % water, at 228 nm

6.3.3 Results

Chromatographing the test compounds using the ODS and the Diol column, at different eluent compositions, gave a large number of capacity coefficients, see Table 6.3, which together with the reference log K_{ow} values, formed the data sets used for evaluation of the HPLC systems and the linear models applied for predictions.

Compound	diol 35 k'	diol 50 k'	ods 65 k'	ods 75 k'	ods 85 k'	Reference log K_{ow}
quinoline	0.396	0.282	0.859	0.605	0.353	2.065
isoquinoline	0.407	0.210	0.904	0.543	0.307	2.08
4-azaflourene	0.698	0.426	1.409	1.038	0.389	
9H-carbazole	0.856	0.559	4.024	1.895	0.681	3.505
acridine	0.891	0.484	2.847	1.271	0.621	3.345
phenanthridine	1.014	0.432	2.816	1.584	0.699	3.47
benzo[f]quinoline	1.069	0.537	3.964	1.718	0.846	3.4
benzo[h]quinoline	0.959	0.525	5.174	1.723	0.824	3.6
dibenz[a,c]acridine	6.586	2.204	74.187	19.107	6.433	5.66
benz[a]acridine	2.282	1.000	7.965	3.045	1.249	
10-azabenzo[a]pyrene	5.854	2.042	24.508	7.808	3.419	5.533
dibenz[a,h]acridine	6.019	2.023	73.462	19.004	5.626	
dibenz[a,j]acridine	5.554	1.195	24.118	8.299	2.525	
dibenz[c,h]acridine	9.191	2.620	154.716	33.619	12.449	6.45
dibenzofuran	0.754	0.437	10.981	4.191	1.585	4.12
dibenzothiophene	1.191	0.719	15.932	6.709	2.157	4.435
naphtalene	0.418	0.276	5.291	2.706	1.110	3.296
flourene	0.763	0.499	14.328	5.921	2.316	
anthracene	1.377	0.736	18.498	6.646	2.423	4.475
phenanthrene	1.374	0.774	17.456	5.570	1.865	4.46
benz[a]anthracene	4.431	1.677	60.326	14.951	4.352	5.79
pyrene	2.862		26.475			4.88
benzo[a]pyrene	9.707	2.872	105.130	27.667	7.246	5.97
dibenz[a,c]anthracene	14.859	3.839	165.882	35.852	9.159	6.17
dibenz[a,h]anthracene	17.121	4.225	193.757	39.891	11.235	6.5
dibenz[a,j]anthracene	14.131	3.650	193.180	39.214	10.947	

Table 6.3. *Capacity coefficients for the different HPLC systems.The references to the log K_{ow} values are given in Table 6.7.*

Models

Considering the 5 chromatographic systems used, a multitude of models can be set up for prediction of K_{ow}. Firstly, data from each system can be used independently, which gives one model for each system and secondly, combination models can be formulated based on data from two systems, the Diol and the ODS system. If these models shall provide additional description, the characteristica of the two systems should be as different as possible. On this basis the following models have been tested.

Name	Model
	Single system models
Diol-35	$\log K_{ow} = a \cdot \log k'_{Diol\text{-}35} + b$
Diol-50	$\log K_{ow} = a \cdot \log k'_{Diol\text{-}50} + b$
ODS-65	$\log K_{ow} = a \cdot \log k'_{ODS\text{-}65} + b$
ODS-75	$\log K_{ow} = a \cdot \log k'_{ODS\text{-}75} + b$
ODS-85	$\log K_{ow} = a \cdot \log k'_{ODS\text{-}85} + b$
	Two-system models
Diol-35,ODS-65	$\log K_{ow} = a \cdot \log k'_{Diol\text{-}35} + b \log k'_{ODS\text{-}65} + c$
Diol-50,ODS-65	$\log K_{ow} = a \cdot \log k'_{Diol\text{-}50} + b \log k'_{ODS\text{-}65} + c$
Diol-35,ODS-85	$\log K_{ow} = a \cdot \log k'_{Diol\text{-}35} + b \log k'_{ODS\text{-}85} + c$

Table 6.4. *Models for prediction of K_{ow} from HPLC retention data.*

Calibration

Three kinds of datasets were used for calibration of the models, a dataset containing data from polar compounds (azaarenes), a dataset containing data from nonpolar compounds (the PAH and two S,O-heterocycles) and a pooled dataset containing all the data from both sets. In the following, the number given behind the model designation tells which dataset has been used for calibration. 1 refers to the "polar dataset", 2 to the "nonpolar dataset" and 3 to the pooled dataset. For instance ODS-65-2 refers to the ODS-65 model calibrated using only data from nonpolar compounds. The data were fitted with the models of Table 6.4 using the least squares method and the resulting models are given in Table 6.5.

Model name	**Model with fitted coefficients**
Diol-35-1	$\log K_{ow} = (2.963 \pm 0.131) \log k'_{Diol\text{-}35} + (3.420 \pm 0.066)$
Diol-50-1	$\log K_{ow} = (3.826 \pm 0.209) \log k'_{Diol\text{-}50} + (4.538 \pm 0.083)$
ODS-65-1	$\log K_{ow} = (1.930 \pm 0.112) \log k'_{ODS\text{-}65} + (2.336 \pm 0.122)$
ODS-75-1	$\log K_{ow} = (2.423 \pm 0.144) \log k'_{ODS\text{-}75} + (2.877 \pm 0.103)$
ODS-85-1	$\log K_{ow} = (2.698 \pm 0.174) \log k'_{ODS\text{-}85} + (3.699 \pm 0.091)$
Diol-35,ODS-65-1	$\log K_{ow} = (0.723 \pm 0.360) \log k'_{ODS\text{-}65} + (1.882 \pm 0.549) \log k'_{Diol\text{-}35} + (3.009 \pm 0.212)$
Diol-50,ODS-65-1	$\log K_{ow} = (0.919 \pm 0.390) \log k'_{ODS\text{-}65} + (2.050 \pm 0.772) \log k'_{Diol\text{-}50} + (3.497 \pm 0.447)$
Diol-35,ODS-85-1	$\log K_{ow} = (0.564 \pm 0.721) \log k'_{ODS\text{-}85} + (2.358 \pm 0.785) \log k'_{Diol\text{-}35} + (3.476 \pm 0.098)$
Diol-35-2	$\log K_{ow} = (1.813 \pm 0.130) \log k'_{Diol\text{-}35} + (4.256 \pm 0.085)$
Diol-50-2	$\log K_{ow} = (2.546 \pm 0.163) \log k'_{Diol\text{-}50} + (4.887 \pm 0.063)$
ODS-65-2	$\log K_{ow} = (1.955 \pm 0.084) \log k'_{ODS\text{-}65} + (2.046 \pm 0.134)$
ODS-75-2	$\log K_{ow} = (2.526 \pm 0.158) \log k'_{ODS\text{-}75} + (2.431 \pm 0.174)$
ODS-85-2	$\log K_{ow} = (2.981 \pm 0.214) \log k'_{ODS\text{-}85} + (3.437 \pm 0.134)$
Diol-35,ODS-65-2	$\log K_{ow} = (2.617 \pm 0.645) \log k'_{ODS\text{-}65} + (-0.629 \pm 0.607) \log k'_{Diol\text{-}35} + (1.302 \pm 0.730)$
Diol-50,ODS-65-2	$\log K_{ow} = (2.764 \pm 0.966) \log k'_{ODS\text{-}65} + (-1.063 \pm 1.266) \log k'_{Diol\text{-}50} + (0.862 \pm 1.407)$
Diol-35,ODS-85-2	$\log K_{ow} = (1.492 \pm 1.079) \log k'_{ODS\text{-}85} + (0.923 \pm 0.657) \log k'_{Diol\text{-}35} + (3.849 \pm 0.319)$
Diol-35-3	$\log K_{ow} = (2.447 \pm 0.190) \log k'_{Diol\text{-}35} + (3.760 \pm 0.112)$
Diol-50-3	$\log K_{ow} = (3.311 \pm 0.224) \log k'_{Diol\text{-}50} + (4.649 \pm 0.088)$
ODS-65-3	$\log K_{ow} = (1.846 \pm 0.069) \log k'_{ODS\text{-}65} + (2.303 \pm 0.095)$
ODS-75-3	$\log K_{ow} = (2.291 \pm 0.106) \log k'_{ODS\text{-}75} + (2.803 \pm 0.098)$
ODS-85-3	$\log K_{ow} = (2.708 \pm 0.117) \log k'_{ODS\text{-}85} + (3.640 \pm 0.068)$
Diol-35,ODS-65-3	$\log K_{ow} = (1.444 \pm 0.160) \log k'_{ODS\text{-}65} + (0.598 \pm 0.221) \log k'_{Diol\text{-}35} + (2.600 \pm 0.137)$
Diol-50,ODS-65-3	$\log K_{ow} = (1.361 \pm 0.182) \log k'_{ODS\text{-}65} + (0.949 \pm 0.335) \log k'_{Diol\text{-}50} + (2.926 \pm 0.234)$
Diol-35,ODS-85-3	$\log K_{ow} = (2.035 \pm 0.260) \log k'_{ODS\text{-}85} + (0.684 \pm 0.244) \log k'_{Diol\text{-}35} + (3.648 \pm 0.058)$

Table 6.5. *Fitted models for prediction of K_{ow} from HPLC capacity coefficients.*

For each of the calibrated models the average squared error (MSE) was calculated and the result is shown on Fig 6.3 below.

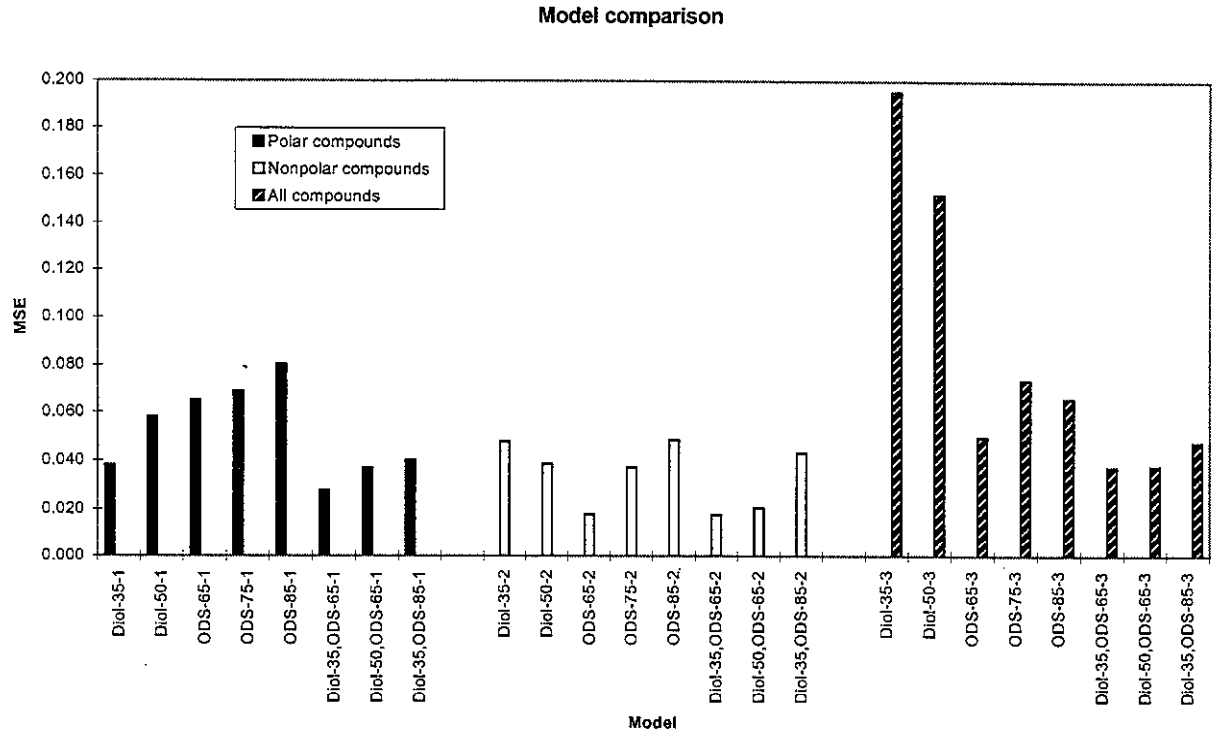


Fig 6.3. *The mean squared error for different chromatographic systems and different calibration datasets.*

As can be seen from Fig 6.3, there are large differences in the errors obtained with the different models, and this is discussed in the following.

Single system models

When considering single system models, the plot of mean square errors shows, that for azaarenes (polar compounds) the smallest mean square error is obtained when the Diol-35 system is used applying only data from polar compounds in the calibration. For the PAH (nonpolar compounds), on the other hand, the best predictions are obtained with the ODS-65 using only data from nonpolar compounds for calibration. The largest possible amount of water in the mobile phase gave the best predictions for both columns.

If the deviations between predicted values and reference values for individual compounds are considered it can be seen from Fig 6.4, that for the polar compounds, the ODS-65-1 model gives the closest prediction for 6 out of 10 compounds, whereas the Diol-35-1 model gives the closest prediction for the remaining four compounds. However, the very large error in the prediction of log K_{ow} for 10-azabenzo[a]pyrene using the ODS-65-1 model makes the Diol-35-1 model the most accurate on an overall basis when azaarenes are concerned.

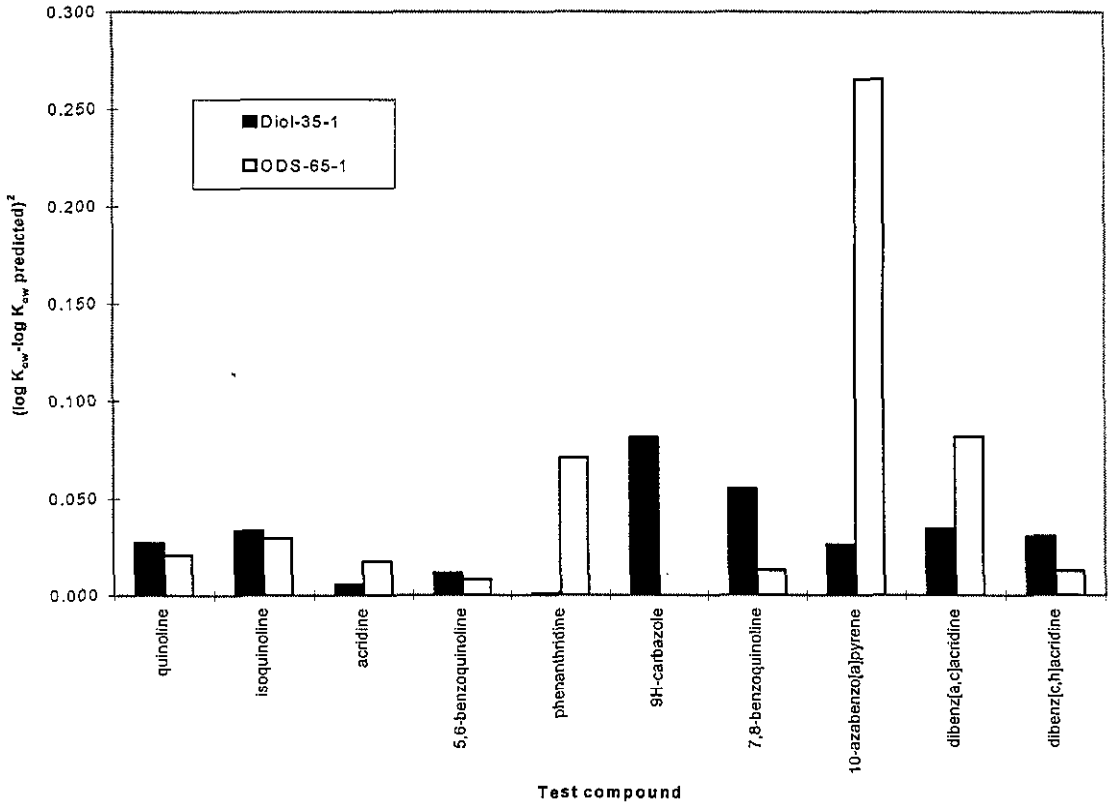


Fig 6.4. *The squared error for individual compounds using the Diol-35-1 and the ODS-65-1 models.*

Pooled dataset

If the pooled dataset is used for calibration the error increases compared to when using the split datasets and there is consequently no advantage in using the pooled dataset for prediction of K_{ow} for azaarenes (see Fig 6.3). In between the single system models calibrated with the pooled dataset it can be noted that the Diol-35-3 and Diol-50-3 models give much less precise predictions than the ODS-65-3, ODS-75-3 and ODS-85-3. The reason for this seems to be that polar and nonpolar compounds react quite differently in the Diol systems whereas they react similar in the ODS systems. This can be verified by looking at the relationship between capacity coefficients and partition coefficients.

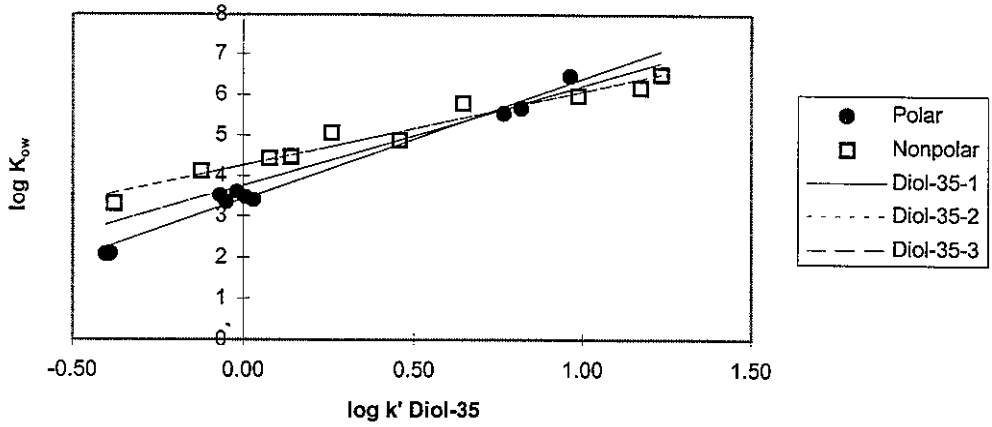


Fig 6.5. *The relationship between capacity coefficient and log K_{ow} for the Diol-35 chromatographic system.*

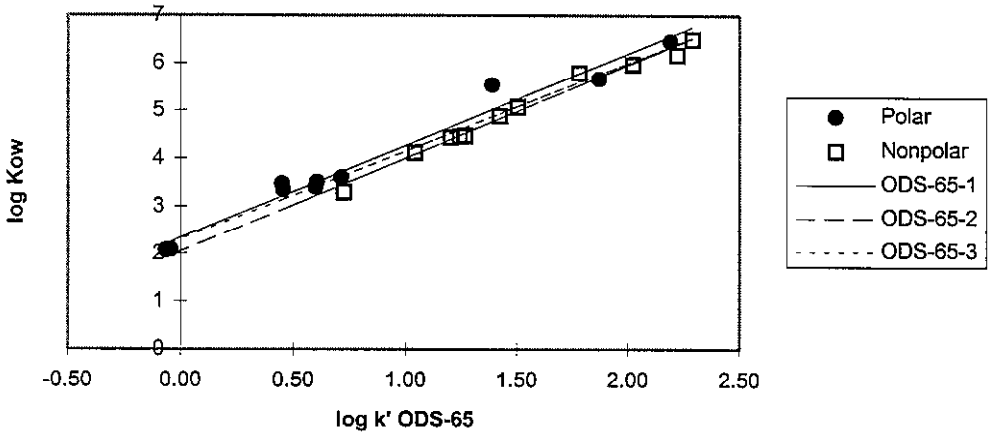


Fig 6.6. *The relationship between capacity coefficient and log K_{ow} for the ODS-35 chromatographic system.*

From Fig 6.5 it is evident that, on the Diol column, the polar compounds do not follow the same linear free energy relationship (LFER) as do the nonpolar compounds. On the ODS column, on the other hand, the difference between LFERs of the polar and the nonpolar compounds is very small as can be seen from Fig 6.6. This means that the ODS system have mechanisms closer resembling those of the octanol-water system than the Diol-system.

The difference in retention mechanisms of the Diol and the ODS columns is also demonstrated in Fig 6.7 below where it can be noted that the relationship between retention on the ODS and the Diol column are different for polar and nonpolar compounds. From Fig 6.7 it can be seen that the Diol column retain the polar compounds relatively more than the ODS column when compared to the retention of the nonpolar compounds which is most likely the effect of hydrogen bonds between the OH groups of the Diol material and the nitrogen of the azaarenes.

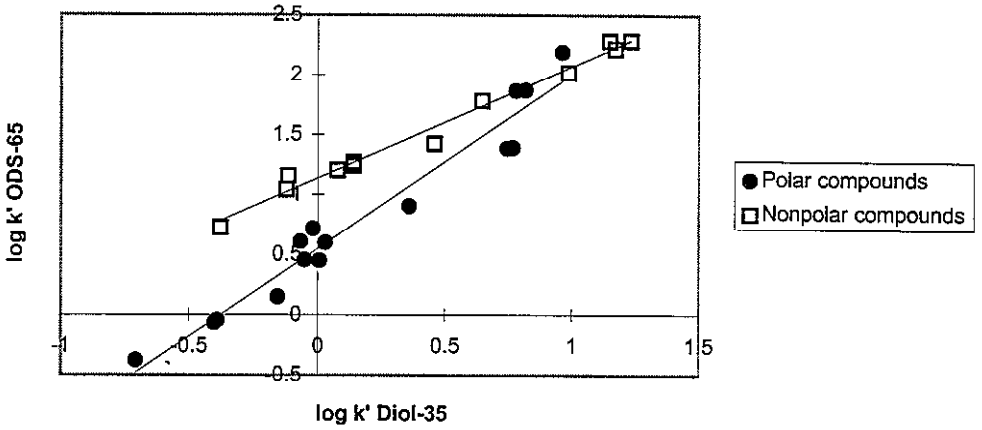


Fig 6.7. *The relationship between capacity coefficients in the ODS-65 and the Diol-35 chromatographic systems.*

Even though the different LFERs for polar and nonpolar compounds on the Diol column result in inprecise predictions when a pooled dataset is used for calibration, it can be noted that in the ODS-65-3 model, most of the variation originates from predictions for the polar compounds, whereas for the Diol-35-3 model, most of the variation can be attributed to predictions for the nonpolar compounds (see Fig 6.8). This confirms the findings that the Diol column is more suitable for prediction of K_{ow} for azaarenes than the ODS column.

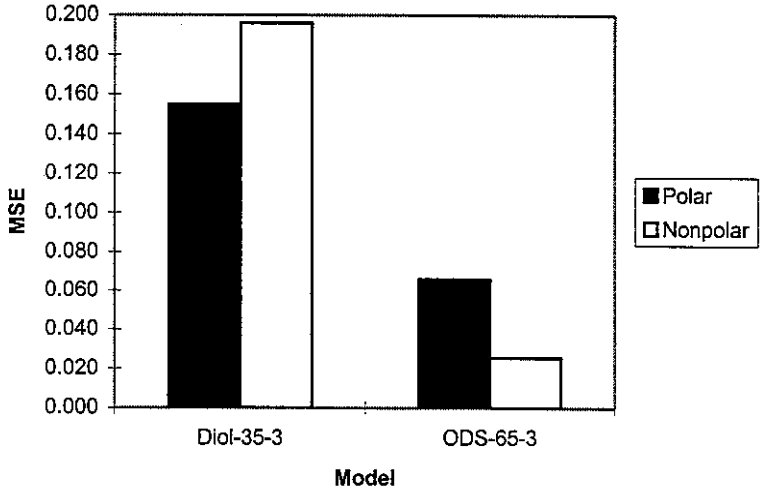


Fig 6.8. *The mean squared error calculated separately for polar and nonpolar compounds.*

To illustrate the problems in predicting K_{ow} for one kind of compounds using a model calibrated with a different kind of compounds, errors in prediction of log K_{ow} can be calculated individually for polar and nonpolar compounds as illustrated on Fig 6.9. Fig 6.9 shows that for instance the use of the Diol-35-2 model (calibrated with nonpolar compounds) for prediction of log K_{ow} for azaarenes would result in substantial mispredictions.

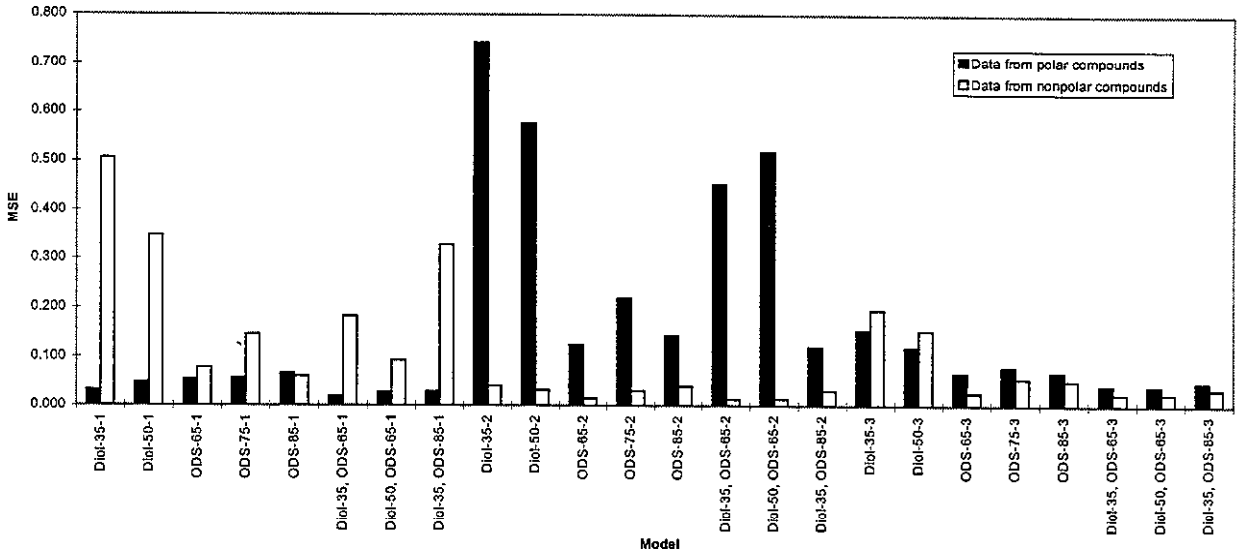


Fig 6.9. *The mean squared error calculated separately for polar and nonpolar compounds for all models.*

Two-system models

As mentioned above there is a possibility of using the data from two different chromatographic systems simultaneously in the prediction of K_{ow} and the Diol system and the ODS system could well be a combination worthwhile exploring as these columns have partly different retention mechanisms. In the ODS column the interactions between a solute and stationary material are mainly due to dispersive forces while the interactions between a solute and the stationary material of the Diol column beside dispersive forces also include polar interactions and hydrogen bonding.

The three two-system models investigated were the *Diol-35,ODS-65*, the *Diol-50,ODS-65* and the *Diol-35,ODS-85* combinations. The first was chosen because the Diol-35 and ODS-65 system data isolated gave the best predictions and the two other models were chosen to be combinations of low and high organic modifier eluent content in the in the two systems. The importance of the interactions with the stationary phase for retention is supposed to be stronger at low organic modifier content in the eluent than at high organic modifier content in the eluent and by choosing to use data from high organic modifier content from one column with data from low organic modifier content from the other column, the relative importance of different retention mechanisms, for the model, could possibly be optimized.

From Fig 6.3 it can be seen that for the polar compounds there seems to be an immediate advantage in using the best two-system model. For the nonpolar compounds the two-system models are not better than the best single system model, but for the pooled data there again seems to be an advantage in using an two-system model, either the Diol-35,ODS-65-3 or the Diol-50,ODS-65-3 model. However if the statistics are closely investigated the two-system models only seem to be valuable when using a pooled dataset for calibration, in that the addition of a second parameter (ODS data or Diol data) in the other cases do not improve the models at a 95 % level according to a sequential t-test (Montgomery 1991). The statistical findings are given below in table 6.6.

	Relative importance		Relative importance	
Model	ODS-65		Diol-35	
Diol-35,ODS-65-1	0.26	not significant	0.74	significant
Diol-35,ODS-65-2	0.94	significant	0.06	not significant
Diol-35,ODS-65-3	0.92	significant	0.08	significant
	ODS-65		Diol-50	
Diol-50,ODS-65-1	0.44	not significant	0.56	significant
Diol-50,ODS-65-2	0.92	significant	0.08	not significant
Diol-50,ODS-65-3	0.87	significant	0.13	significant
	ODS-85		Diol-35	
Diol-35,ODS-85-1	0.06	not significant	0.94	significant
Diol-35,ODS-85-2	0.49	not significant	0.51	not significant
Diol-35,ODS-85-3	0.89	significant	0.11	significant

Table 6.6. *The relative importance and statistical significance at 95 % of the parameters in the two-system models. The relative importance has been calculated from the relative reduction in sums of squares of regression caused by addition of the second parameter when the other parameter was already in the model (Montgomery 1991).*

The statistics show that when the pooled data are used for calibration the ODS data are the most important in prediction of K_{ow}. The ODS data can be considered to be responsible for the description of the dispersive forces in K_{ow} whereas the Diol column will contribute with additional description in regard to polar and hydrogen bonding interactions.

Predicted K_{ow} values for azaarenes, PAH and two S,O heterocycles

From the above it is clear that for azaarenes the Diol-35-1 model should be used for prediction of K_{ow} and for the PAH the ODS-65-2 model should be used. Applying these models the predicted log K_{ow} are as given in table 6.7 below.

	log K_{ow}			
	Diol-35-1	ODS-65-2	Literature	Reference
Azaarenes				
quinoline	2.229 ± 0.503	1.917 ± 0.329	2.065	Sangster (1989) De Voogt (1990)
isoquinoline	2.263 ± 0.501	1.960 ± 0.328	2.08	Sangster (1989)
9H-carbazole	3.220 ± 0.478	3.228 ± 0.319	3.505	Rogers and Cammarata 1969 Sangster (1989)
4-azaflourene	2.958 ± 0.483	2.337 ± 0.325		
acridine	3.271 ± 0.477	2.934 ± 0.321	3.345	Hansch and Fujita (1964), Unger and Chiang (1983) De Voogt (1990)
benzo[f]quinoline	3.506 ± 0.475	3.215 ± 0.320	3.4	De Voogt et al. (1988)
benzo[h]quinoline	3.366 ± 0.476	3.441 ± 0.318	3.6	De Voogt et al. (1988)
phenanthridine	3.438 ± 0.475	2.925 ± 0.321	3.47	
benz[a]acridine	4.482 ± 0.476	3.807 ± 0.317		
10-azabenzo[a]pyrene	5.694 ± 0.506	4.761 ± 0.315	5.533	This study
dibenz[a,c]acridine	5.846 ± 0.512	5.702 ± 0.315	5.66	This study
dibenz[a,j]acridine	5.627 ± 0.504	4.748 ± 0.315		
dibenz[a,h]acridine	5.730 ± 0.507	5.693 ± 0.315		
dibenz[c,h]acridine	6.275 ± 0.530	6.326 ± 0.317	6.45	This study
PAHs				
naphthalene	2.296 ± 0.500	3.460 ± 0.318	3.296	Sangster (1989)
flourene	3.073 ± 0.480	4.306 ± 0.315		
anthracene	3.832 ± 0.473	4.523 ± 0.315	4.475	Sangster (1989) Hansch and Fujita (1964)
phenanthrene	3.829 ± 0.473	4.473 ± 0.315	4.46	Hansch and Fujita (1964)
pyrene	4.774 ± 0.481	4.827 ± 0.315	4.88	Nikaitani, and Hansch, Pomona College, unpublished analysis
benz[a]anthracene	5.336 ± 0.494	5.526 ± 0.315	5.79	Wang et al. (1986)
benzo[a]pyrene	6.345 ± 0.533	5.998 ± 0.316	5.97	Mallon and Harrison (1984)
dibenz[a,c]anthracene	6.893 ± 0.561	6.385 ± 0.318	6.17	Gould, and Hansch, Pomona College, unpublished analysis
dibenz[a,h]anthracene	7.075 ± 0.571	6.517 ± 0.318	6.5	Sangster (1989)
dibenz[a,j]anthracene	6.828 ± 0.558	6.514 ± 0.318		
S,O-heterocycles				
dibenzofuran	3.056 ± 0.481	4.080 ± 0.316	4.12	Abraham et al. (1994 a)
dibenzothiophene	3.645 ± 0.474	4.396 ± 0.315	4.435	De Voogt (1990)

Table 6.7. *The predicted values of log K_{ow} for azaarenes, PAH and two S,O,-heterocycles using the Diol-35-1 model and the ODS-65-2 model. The values are given with prediction intervals (Montgomery 1991).*

The predictions from both models are rather similar as long as divided datasets are used for calibration as can be seen from Fig 6.10 and Fig 6.11.

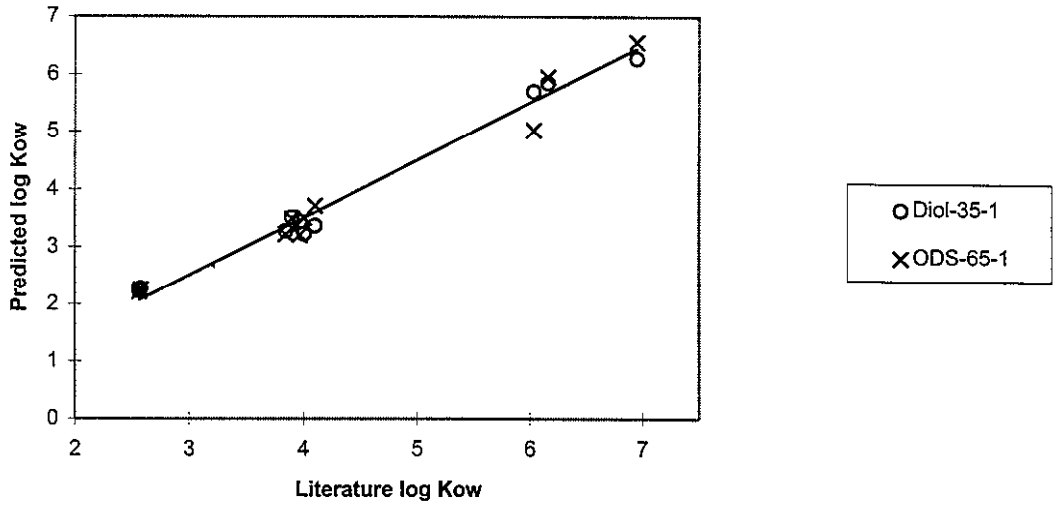


Fig 6.10. *The relationship between predicted and literature log K_{ow} values for azaarenes.*

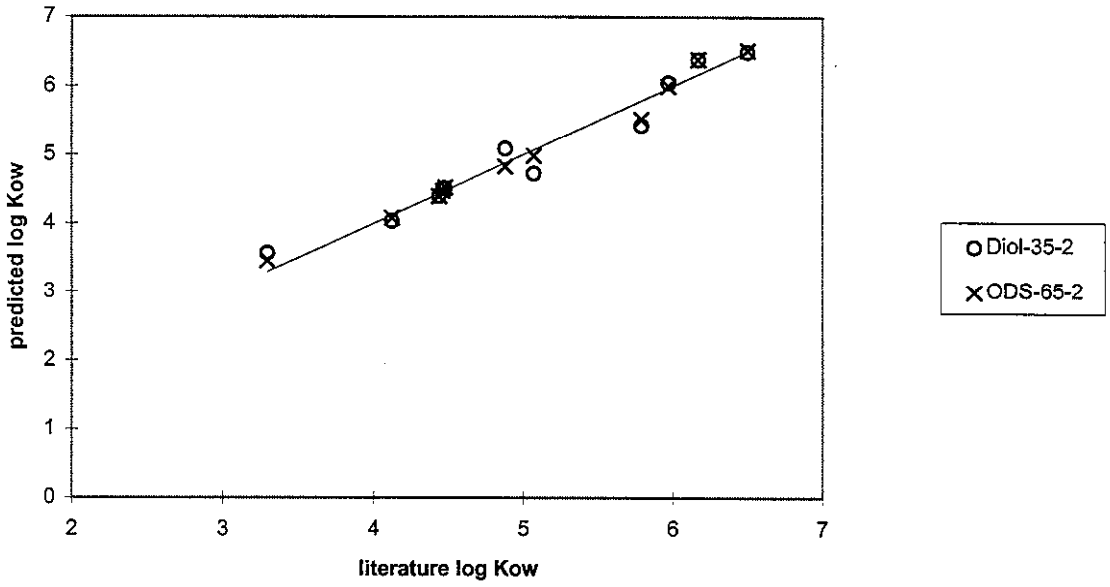


Fig 6.11. *The relationship between predicted and literature log K_{ow} values for PAHs and two S,O-heterocycles.*

The deviations between literature and predicted values can be attributed to inaccuracy of HPLC measurements, inaccuracy of literature values and inherent differences in the mechanisms of HPLC retention and octanol-water partitioning. The standard deviation of measured retention times caused by pump inaccuracy, column instability/degradation and temperature variation is less than 1 % so mechanical inconsistencies does not explain the deviations. It is not possible to evaluate the accuracy of the literature values as, for most compounds, there is only one value available, but, variations of 0.1-0.4 log

K_{ow} values has been reported. It is likely that the higher K_{ow} values will have larger uncertainties than the lower K_{ow} values because the difficulty of experimental determination increases with increasing lipophilicity of the solute and this could play a major part for the errors obtained. However, the higly correlated relationship between measured HPLC retention and literature K_{ow} values The remaining cause for deviations is the difference in mechanisms of the two systems as has been discussed and this can be reduced by grouping compounds of the same type for calibration and prediction. Only if the accuracy of reference log K_{ow} values was known, would it be possible to determine the actual error arising from the model.

The mechanisms of octanol-water partitioning of azaarenes

The calculation of partition coefficients, as given in Table 6.7, shows that in general K_{ow} increases with increasing molecular weight. Azaarenes have lower log K_{ow}s than the analogue PAHs and the differences in log K_{ow} are in the range of 1.1-1.3 independent of molecular size. A statistical t-test showed no significant difference at a 95 % confidence level between the slopes of the molecular weight- log K_{ow} relationships for the unshielded (see below) azaarenes and analogue PAHs (see Fig 6.12).

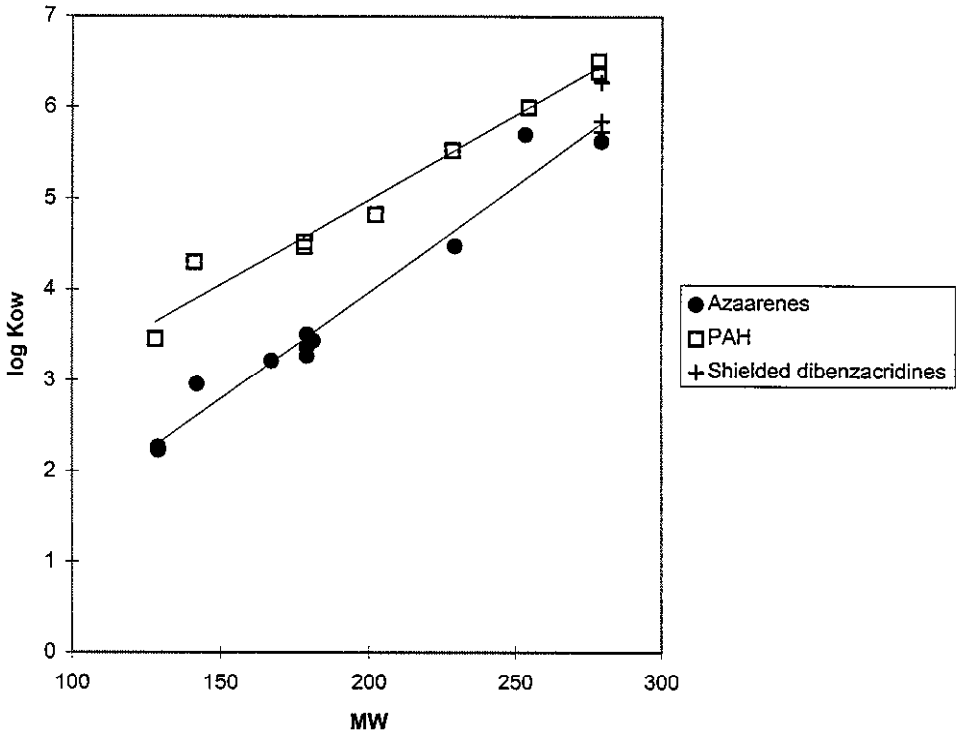


Fig 6.12. *Log K_{ow} against molecular weight for unshielded azaarenes and equivalent PAH. There was no statistical difference, at a 95 % level, between the slope of the two lines.*

For azaarenes, K_{ow} increases if the nitrogen atom is shielded by benzene rings as shielding reduces the nitrogen lonepair availability for hydrogen bonding. This can be seen with the dibenzacridines where K_{ow} diminishes through: dibenz[c,h]acridine > dibenz[a,c]acridine~dibenz[a,j]acridine > dibenz[a,j]acridine in accordance with the

number of shielding rings (see Fig 6.13). With the benzoquinolines a similar ordering, log K_{ow} of benzo[h]quinoline > benzo[f]quinoline, can be obtained according to literature values and according to the ODS-65-1 values, but not when predictions from the Diol column are used.

To evaluate the results from the two columns in relation to the chemical structure of the compounds, the relative difference,

$$\Delta \log K_{ow} = (\log K_{ow,Diol\text{-}35\text{-}1} - \log K_{ow,ODS\text{-}65\text{-}1}) / \log K_{ow,Diol\text{-}35\text{-}1}, \qquad \text{eq 6.8}$$

for the estimated log K_{ow} values, as given in Table 6.7, have been calculated for the four dibenzacridines (see Fig 6.13). The relative difference increases with accessibility of the nitrogen atom for hydrogen bonding which again indicates, that the Diol column does facilitate hydrogen bonding between the azaarene and the hydroxy groups in the column material.

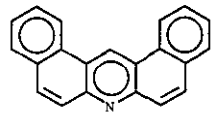
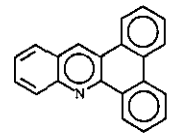
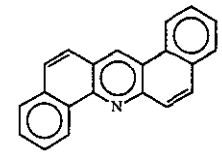
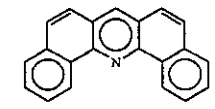
Compound	dibenz[a,j]acridine	dibenz[a,c]acridine	dibenz[a,h]acridine	dibenz[c,h]acridine
				
log K_{ow}	5.63	5.85	5.73	6.28
Δ log K_{ow}	-11%	2%	3%	5%

Fig 6.13. *Formulas, predicted log K_{ow} (Diol-35-1) and relative differences* $\Delta \log K_{ow} = (\log K_{ow,Diol\text{-}35\text{-}1} - \log K_{ow,\ ODS\text{-}65\text{-}1})/\log K_{ow,Diol\text{-}35\text{-}1}$ *for the dibenzacridines.*

The effect of endocyclic sulphur and oxygen, as in dibenzothiophene and dibenzofurane, on K_{ow} is much smaller, +0.06 and -0.23 log K_{ow} units respectively, than the effect of nitrogen as in carbazole, -1.09 log K_{ow} units, when compared to fluorene. This supports the statement made in chapter two, that S and O heterocyclics has partitioning properties more like the PAHs than like the N-PACs or azaarenes.

6.3.4 Summary

The polar and the nonpolar compounds do not follow the same LFER on the Diol column while they do on the ODS column. This causes the Diol column to be unsuitable for predictions of mixtures of polar and nonpolar compounds and shows that the retention mechanisms of the Diol column are different from the mechanisms of octanol-water partitioning. It seems likely that the difference shall be found in the balance between lipophilic and polar interactions and that the Diol column most likely have a stronger hydrogen bonding than the octanol relative to the lipophilic interactions. Other investigators have also found that different LFERs apply to different types of solutes (Hammers et al. 1982, Abraham et al. 1994 a). The mechanisms of retention on the ODS column is closer to the mechanisms of octanol-water partitioning than the mechanisms of retention on the Diol column, but the most accurate predictions of K_{ow} for azaarenes was obtained with the Diol column. Average errors were as low as 0.18 log K_{ow} units whereas the maximum error was 0.28 log K_{ow} units. The ODS column, although more similar to the octanol-water system, had a larger average error of prediction (0.23 log K_{ow} units) which mainly was caused by the large maximum error of 0.52 log K_{ow} units

obtained for 10-azabenzo[a]pyrene. For practical determinations of log K_{ow} for azaarenes, both columns will be applicable as long as a calibration dataset of azaarenes is used. A two-system model including capacity coefficients from both a Diol column and an ODS column was statistically advantageous when predicting K_{ow} for a dataset including both polar and nonpolar compounds. However, the two system model is less practical as the double number of chromatographings has to be performed. With the columns tested, the highest possible amount of water in the mobile phase gave the most accurate predictions.

For the azaarenes, K_{ow} increases with molecular weight and decreases with the availability for hydrogen bonding, as shown for the dibenzacridines where neighbouring benzene rings diminished the hydrogen bond availability of the nitrogen atom. In general log K_{ow} for azaarenes are 1.1-1.3 lower than log K_{ow} for the analogue PAHs and the difference is independent of size within the range of the test compounds.

6.4 Determination of K_{ow} for polar substituted PACs

As described in chapter 2, a large variety of polar substituted PACs can be found in the environment and they may be environmentally important. Therefore, K_{ow} was determined for a number of environmentally relevant polar substituted PACs by the HPLC method to study the effects of different polar substituents on K_{ow}. As testcompounds, representing polar substituted PACs, substituted anthracenes and pyrenes were chosen. For prediction of K_{ow} for the polar substituted PACs, the two system model Diol-35,ODS-65-3, developed in chapter 6.3, was used because the polar substituted PACs include compounds that differ in properties like polarity and hydrogen bonding as the calibration set of the Diol-35,ODS-65-3 model also does.

6.4.1 Experimental

The experimental part was limited to the measuring of capacity coefficients on the ODS-65 and the Diol-35 system and the measurements for the polar substituted PACs were done together with the measurements for azaarenes and PAHs. Details of equipment and materials are therefore exactly as described in chapter 6.3. The polar substituted PAC test compounds were obtained as pure compounds from different sources (see Table A1 in appendix) and their identities were confirmed by UV-absorption spectra.

6.4.2 Results and discussion

log K_{ow}s for the substituted polycyclic aromatic compounds were calculated from the linear combination of measured HPLC capacity coefficients from the ODS-65 and the Diol-35 systems, with the ODS-65,Diol-35-3 model developed in chapter 6.3.

$$\log K_{ow} = (1.444 \pm 0.160) \cdot \log k'_{ODS\text{-}65} + (0.598 \pm 0.211) \cdot \log k'_{Diol\text{-}35} + (2.600 \pm 0.137) \quad \text{eq 6.9}$$

$$n = 16,\ r^2 = 0.98,\ \text{standard deviation on log } K_{ow} = 0.193$$

The resulting predictions are given below in table 6.8. Unfortunately, it is not possible to calculate the actual errors obtained because very few literature values exist for these compounds. The literature values given in Table 6.8 for the substituted PACs are mainly unpublished results.

		log K_{ow}			
Compound	Diol-35,ODS-65-3	Rekker-ar	Rekker-al	Lit.	Reference
anthracene	4.515 ± 0.430			4.475	Hansch & Fujita 1964 Sangster 1989
9-anthrone	3.252 ± 0.427	3.860	2.765	3.66	Debnath & Hansch*
9,10 anthraquinone	3.294 ± 0.443			3.39	Pratesi et al. 1979
9-anthracenecarbonitrile	3.974 ± 0.420	4.155	3.279	4.26	Debnath & Hansch*
9-nitroanthracene	3.702 ± 0.437	4.271	3.395	4.78	Debnath & Hansch*
9-methylanthracene	4.925 ± 0.437	5.034	5.034	5.07	Kenaga & Goring 1978 Karickhoff et al. 1979
9-anthracenecarboxamide	2.279 ± 0.474	3.175	2.299		
9-acetylanthracene	3.601 ± 0.423	4.058	3.401		
9-anthracenecarboxylicacid-methylester	3.823 ± 0.424	4.491	3.834		
9-anthracenecarboxaldehyde	3.772 ± 0.428	3.977	3.320		
9-methoxyanthracene	4.343 ± 0.429	4.584	3.489		
9-chloroanthracene	5.225 ± 0.439	5.243	4.367		
9-bromoanthracene	5.317 ± 0.445	5.444	4.568		
9,10-dibromanthracene	6.208 ± 0.451	6.374	4.622		
9-hydroxyanthracen		4.323	3.228		
pyrene	4.929 ± 0.419			4.88	Rekker and Mannhold 1992 Nikaitani & Hansch*
1-pyrenealdehyd	4.217 ± 0.421	4.392	3.735		
1-acetylpyrene	4.161 ± 0.423	4.473	3.816		
2-acetylpyrene	4.259 ± 0.420	4.473	3.816		
4-acetylpyrene	4.148 ± 0.422	4.473	3.816		
1-pyrenecarboxylicacid-methylester	4.693 ± 0.419	4.906	4.249		
2-pyrenecarboxylicacid-methylester	4.813 ± 0.421	4.906	4.249		
4-pyrenecarboxylicacid-methylester	4.697 ± 0.419	4.906	4.249		
1-bromopyrene	5.746 ± 0.429	5.859	4.983		
2-bromopyrene	5.734 ± 0.431	5.859	4.983		
4-bromopyrene	5.769 ± 0.429	5.859	4.983		
1,3-dibromopyrene	6.752 ± 0.456	6.789	5.037		
1,6-dibromopyrene	6.591 ± 0.451	6.789	5.037		
1,8-dibromopyrene	6.628 ± 0.451	6.789	5.037		
N-methylquinolinium iodide	-0.151 ± 0.734				
Quinoline-N-oxide	1.211 ± 0.518				
2-hydroxyquinoline	1.631 ± 0.464	1.6578276	0.563		

Table 6.8. *Predicted log K_{ow} values and prediction intervals for polar substituted PACs using the two system model Diol-35,ODS-65-3. Rekker-ar and Rekker-al log K_{ow} values have been calculated as described below. The references marked * are unpublished results from Pomona college log P database.*

The effect of the substituents on log K_{ow} can be calculated as the difference between log K_{ow} of the substituted PAH and log K_{ow} of the parent PAH as shown on Fig 6.14

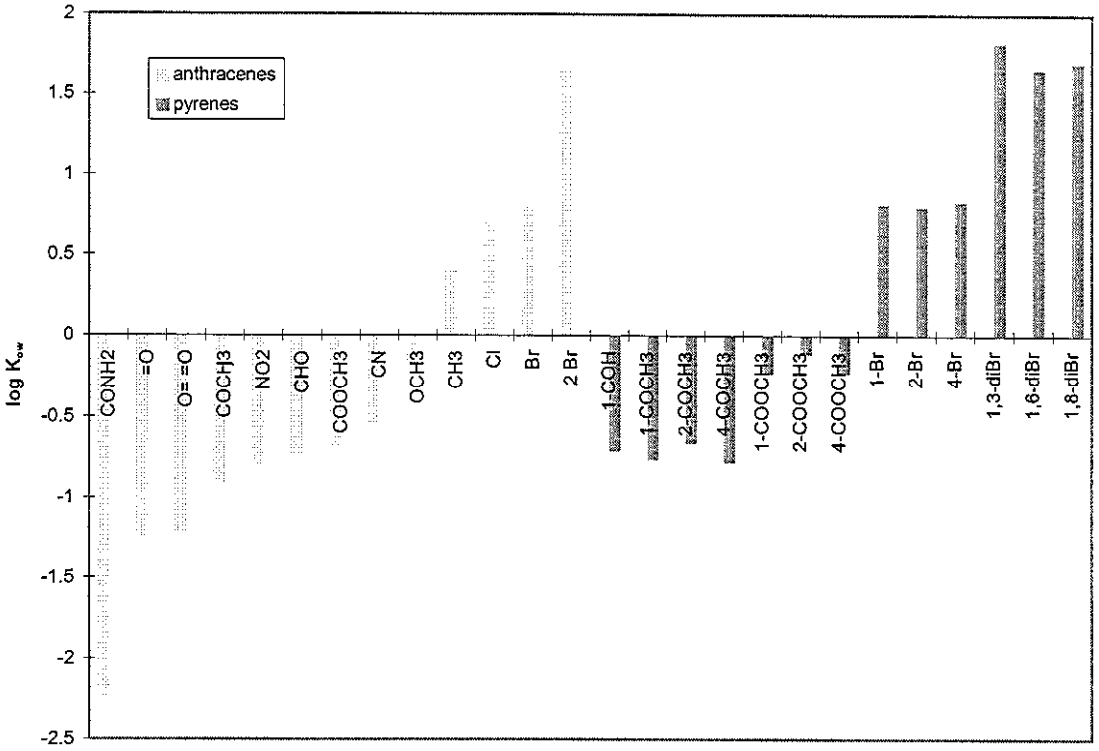


Fig 6.14. *The effect of different substituents on log K_{ow} as predicted by the HPLC method.*

The addition of the hydrogen bonding substituents =O, CN, NO_2, CHO, $COCH_3$, $COOCH_3$ and $CONH_2$ to anthracene or pyrene causes a decrease in log K_{ow} while the addition of the non hydrogen bonding substituents CH_3, Cl and Br causes an increase in K_{ow} due to the increase in size. The very low K_{ow} determined for $CONH_2$-anthracene is most likely due to the substituent being both a hydrogen bond donor and a double hydrogen bond acceptor. The addition of the OCH_3 substituent to anthracene has only a small effect on lipophilicity. The methoxy group is electron donating according to the Hammet constant σ = -0.12 (Chapman and Shorter 1978) and the oxygen is therefore not especially rich in electrons, which limits its hydrogen bond acceptor ability. At the same time the methyl group increases hydrophobicity and these two counteracting mechanisms seemingly cause the effect of the substituent on log K_{ow} to be close to zero for 9-methoxyanthracene.

Although HPLC columns often exhibit shape selectivity (Sentell and Dorsey 1989), the columns used here do not discriminate distinctly between the isomeric bromopyrenes and the variation in log K_{ow} between the three monobromopyrenes is therefore much smaller than the span of prediction intervals. The same applies for the dibromopyrenes. As the Br substituent exhibits approximately the same effect on lipophilicity when attached to anthracene as when attached to pyrene and as the increase in lipophilicity is the same from unsubstituted to monosubstituted and from monosubstituted to disubstituted pyrenes and anthracenes, the effect of bromine on lipophilicity is additive, see Table 6.9.

	log K_{ow} difference from mother compound	Substituent effect on log K_{ow}
1,3-dibromopyrene	1.823	0.912
1,6-dibromopyrene	1.662	0.831
1,8-dibromopyrene	1.699	0.850
1-bromopyrene	0.817	0.817
2-bromopyrene	0.805	0.805
4-bromopyrene	0.841	0.841
9-bromoanthracene	0.802	0.802
9,10-dibromoanthracene	1.694	0.847

Table 6.9. *The effect of bromine on log K_{ow}*

6.4.3 Comparison with theoretical models

The experimentally predicted HPLC log K_{ow} values have been compared with values predicted from the theoretical *f*-fragment method of Rekker and Mannhold (1992) as this could set the HPLC results in perspective and as the applicability of theoretical group contribution methods for prediction of K_{ow} for polar substituted PAH thereby could be evaluated.

The *f*-fragment method is developed for the prediction of log K_{ow} for a large variety of compounds and has wide predictive abilities (Rekker and Mannhold 1992). A given compound is split up into chemical groups or fragments, each of which is assumed to contribute with a certain amount of lipophilicity to the total lipophilicity of the compound. This is formalised in the formula given by Rekker.

$$\log K_{ow} = \sum \underline{a}_n f_n + b \cdot C_M \qquad \text{eq 6.10}$$

f_n represents the lipophilicity contribution of a constituent part of a structure to the total lipophilicity, $\underline{a}_n$ is a numerical factor indicating the incidence of a given fragment in the structure and C_M is called the magic constant. C_M is used to account for different special cases by being added once or more times to the expression, according to rules given by Rekker and Mannhold (1992). The values of the different fragment constants used here for prediction of log K_{ow}s, were estimated by Rekker using a set of 1100 compounds with known log K_{ow}s (Rekker and Mannhold 1992). With the polar substituted anthracenes and pyrenes, log K_{ow} was calculated by using the HPLC determined values of anthracene and pyrene, subtracting Rekker and Mannholds H fragment value and adding the fragment value of the substituent in question. The fragment values are given in Table 6.10 below and the resulting predictions were given in Table 6.8.

Substituent	Aromatic fragment value	Aliphatic fragment value
Br	1.134	0.258
Cl	0.933	0.057
OH	-0.353	-1.448
$CONH_2$	-1.135	-2.011
NO_2	-0.039	-0.915
CHO	-0.333	-0.99
COOH	-0.942	-0.066
$-O-CH_3$	0.274	-0.821
CH_3	0.724	0.724
CN	-0.155	-1.031
CO_2CH_3	0.181	-0.476
=O	-0.45	-1.545
$COCH_3$	-0.252	-0.909
H	0.204	0.204

Table 6.10. *Log K_{ow} fragment values according to Rekker and Mannhold (1992).*

As discussed earlier and as can be noted from the HPLC results, the free lonepairs of oxygen and nitrogen can act as hydrogen bond acceptors and the occurrence of these atoms in a molecule decrease lipophilicity. However, the ability of the hydrogen bond acceptors to participate in hydrogen bonds is reduced if the electron system is delocalized. In the derivation of fragmental constants it has therefore been necessary to calculate fragmental constants for both aliphatic bound substituents (no conjugation) and aromatic bound substituents (conjugation with aromatic π system) and this has been done in the *f*-fragment system (Rekker and Mannhold 1992). The log K_{ow}s calculated from aliphatic and aromatic *f*-fragments are both given in Table 6.8. The HPLC predicted log K_{ow} values for substituted anthracenes have been compared with *f*-fragment calculated log K_{ow} values in Fig 6.15.

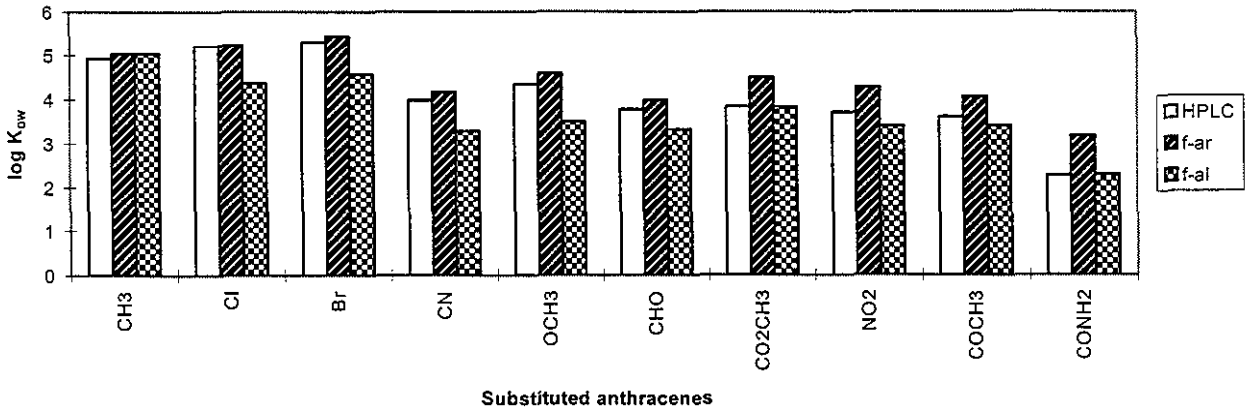


Fig 6.15. *Predicted log K_{ow} values for polar substituted anthracenes using either HPLC (Diol-35,ODS-65-3 model) or aromatic or aliphatic f-fragments.*

From Fig 6.15 it can be seen that the HPLC K_{ow} values are close to the aromatic fragmental K_{ow} values for the 9-substituted CH_3-, Cl-, Br-, CN- and OCH_3-anthracenes while for the 9-substituted $COOCH_3$-, NO_2-, $COCH_3$- and $CONH_2$-anthracenes, the HPLC K_{ow} values are close to the aliphatic fragmental K_{ow} values. The HPLC K_{ow} value for 9-CHO-anthracene is in between the aromatic and the aliphatic fragmental values. The mechanism responsible for this distribution seems to be intramolecular steric hindrance. Molecular mechanics calculations, supported by ^{17}O chemical shifts, have shown that peri hydrogens in position 1 and 8 (see Fig 6.16) forces the 9 position substituents $COOCH_3$, $CONH_2$ (Baumstark et al. 1987) and $COCH_3$ (Oakley and Boykin 1986) to twist out of plane with anthracene in an angle of approximately 70° while the plane of NO_2, using X-ray techniques (Trotter1959), was found to be at an angle of 85° to the plane of anthracene. Consequently, the overlap between the π system of these substituents and the aromatic π system is almost zero and the addition of the substituents causes a decrease in lipophilicity almost as large as what would have been found if the substituents were bound to aliphatic compounds. In 9-CHO-anthracene (Boykin et al. 1987) the angle has been calculated to be 39° and this explains why this substituent has a K_{ow} that lies in between the K_{ow} values that can be predicted by aromatic and aliphatic fragments. In 9-substituted Cl-, Br-, CH_3-, OCH_3- and CN-anthracene no conformational variation exists and the aromatic fragmental log K_{ow} predictions for these substituents are close to the values determined by HPLC.

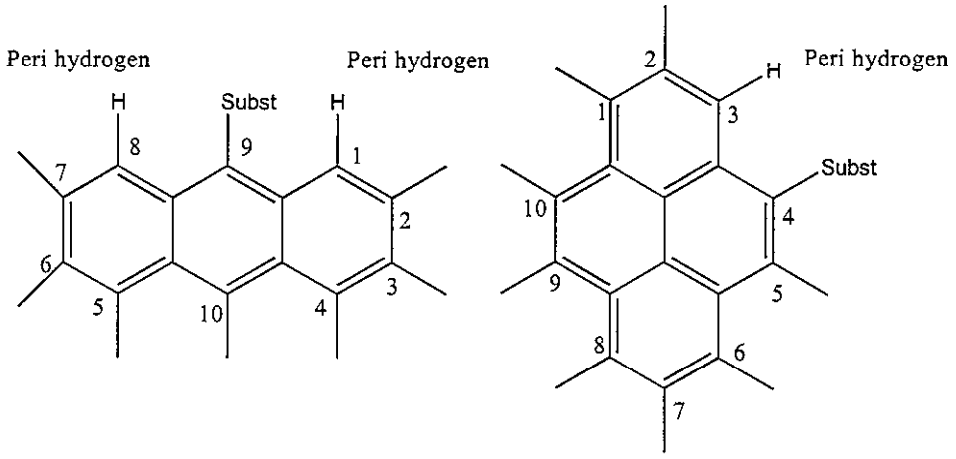


Fig 6.16. *Illustration of anthracene substituted in the 9 position with two peri hydrogens and pyrene substituted in the 3 position with one peri hydrogen.*

The large difference between the effect of carboxylic acid methylester on K_{ow} of pyrene and of anthracene is due to the very small reduction in K_{ow} that the carboxylic acid methylester causes in the pyrenes. Although there is one peri hydrogen in the 1- and 4-pyrenecarboxylic acid methylesters, see Fig 6.16, they can assume conformations where there is little steric hindrance (Hansen et al. 1977, Hansen and Berg 1983) and so the effect on K_{ow} is minimized.

Investigation of CHO, $COCH_3$ and $COOCH_3$ substituted pyrenes showed that the decrease in K_{ow} caused by addition of these substituents are smaller for pyrene than for anthracene which confirms the role of peri hydrogens in octanol water partitioning of substituted PACs. The decrease caused by acetyl is 0.9 log K_{ow} units for anthracene and approx. 0.7 for pyrenes while the decrease caused by carboxylic acid methylester is 0.7 for anthracene and only 0.12-0.24 for pyrenes (see Table 6.8). This corresponds to the

fact that the 9-substituted anthracenes have 2 peri hydrogens while the 1-, 4- and 2-substituted pyrenes have only 1, 1 and 0 peri hydrogens respectively (see Fig 6.16). The fewer peri hydrogens of pyrene, compared to anthracene, cause the substituents to be less out of plane with the aromatic system for the substituted pyrenes (Hansen et al. 1977, Hansen and Berg 1983) than for the 9-substituted anthracenes and the overlap between π systems to be larger. Among the substituted pyrenes the number of peri hydrogens is also important as the unhindered 2-substituted $COOCH_3$ and $COCH_3$ pyrenes (no peri hydrogens) have higher log K_{ow} values than the corresponding 1- and 4- substituted $COOCH_3$ and $COCH_3$ substituted pyrenes (1 peri hydrogen), see Table 6.8 and Fig 6.17.

Because of the smaller sterical hindrance in the substituted pyrenes, compared to the 9-substituted anthracenes, the K_{ow} values calculated from aromatic fragment values are closer to the measured HPLC values than those calculated from aliphatic fragment values for all $COOCH_3$ substituted pyrenes, and especially so for the 2-substituted. For the 1- and 4- $COCH_3$-substituted pyrenes the HPLC log K_{ow} values are right between the aromatic and aliphatic fragment values, while the HPLC log K_{ow} value for the 2-substituted isomer is closer to the aromatic value. For 1-pyrenecarboxaldehyde the reduction in K_{ow} is reasonably well predicted by the aromatic fragment value as it is for 9-anthracenecarboxaldehyde, see Fig 6.17.

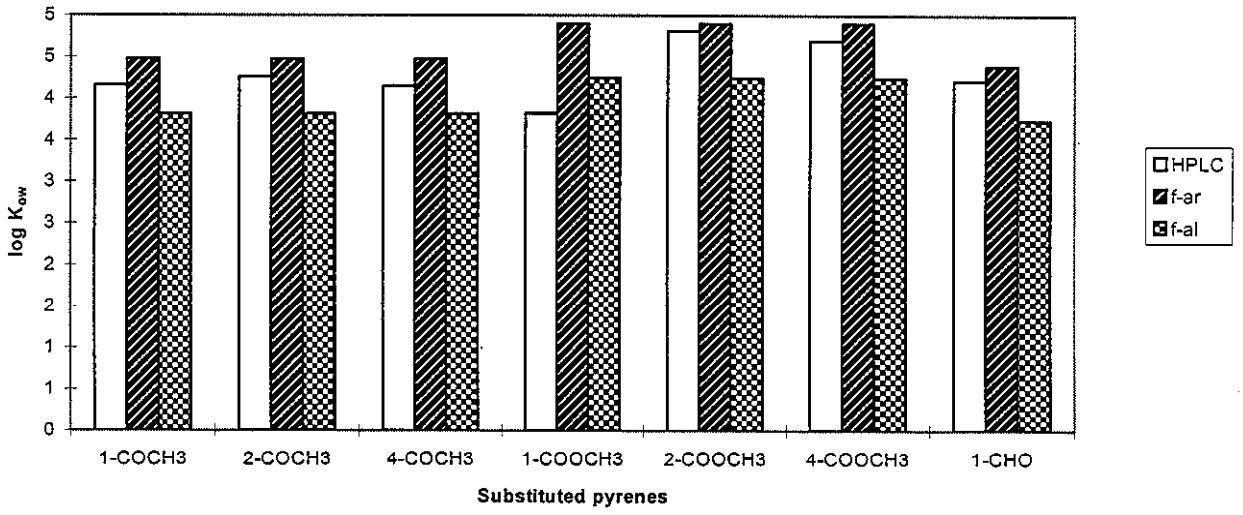


Fig 6.17. *Predicted log K_{ow} values for polar substituted pyrenes using either HPLC (Diol-35,ODS-65-3 model) or aromatic or aliphatic f-fragments.*

For the bromopyrenes a good agreement between the HPLC measured K_{ow} values and the K_{ow} values calculated from aromatic fragments is found.

9-anthrone is a tautomer in equilibrium with 9-hydroxyanthracene with 9-anthrone being the most abundant in water at neutral pH and according to the HPLC measurements, 9-anthrone has a relatively low K_{ow} (log K_{ow} = 3.25), while the *f*-fragment log K_{ow} value for 9-hydroxy anthracene is 0.6 higher (log K_{ow} = 3.92). This

indicates that the lonepair electrons of the oxygen atom are more available for hydrogen bonding in anthrone than in hydroxyanthracene and that the hydrogen bond donor ability of the hydroxy group is of little importance for octanol-water partitioning.

Quinoline and the three quinoline derivatives N-methylquinolinium ion, quinoline-N-oxide and 2-hydroxyquinoline have very different K_{ow}s as determined by HPLC. Especially K_{ow} of the N-methyl quinolinium ion is low, but with the high water solubility caused by its ionic nature the low K_{ow} seems reasonable. However, in the fitting of the Diol-35,ODS-65-3 model no data from ionic compounds were included, as the partitioning mechanism for these compounds is quite different, and the determined K_{ow} value for the N-methyl quinolinium ion must therefore be regarded as a guiding value rather than an absolute one. Quinoline N-oxide has a log K_{ow} value of about half the value of neutral quinoline and this reduction in K_{ow} is probably caused by the lonepairs of oxygen and the strong dipole between the positively charged nitrogen and the negatively charged oxygen. The log K_{ow} value of 2-hydroxyquinoline, calculated with the aromatic *f*-fragment constant, fits the value determined by HPLC well.

6.4.4 Summary

Log K_{ow} has been estimated for a range of substituted polycyclic aromatic compounds using the HPLC method and the variation in log K_{ow} could qualitatively be explained by molecular size and electronic and steric structure. Comparison with K_{ow} calculated from theoretical fragmental values showed that careful consideration should be observed when using fragment methods. With the $COOCH_3$, $COCH_3$, NO_2 and $CONH_2$ substituents in the 9 position of anthracene, steric conditions called for the use of aliphatic fragmental values instead of aromatic fragmental values as conjugation was hindered due to group twisting caused by peri hydrogens. HPLC measurements of K_{ow} for $COOCH_3$, $COCH_3$ and CHO substituted pyrenes, confirmed the role of peri hydrogens in octanol water partitioning for substituted PACs. The increase in lipophilicity caused by halogen substituents was shown to be additive.

7. Determination of K_{ow} for azaarenes, PAHs and two S,O-heterocycles with theoretical Models

7.1 Introduction

The potential advantages of theoretical models for the prediction of physical chemical paramaters are obvious. Time consuming and expensive laboratory work can be avoided and fast answers can be gained. The problem with models are validity and accuracy but in many cases, there is no alternative, for instance if a large amount of compounds has to be evaluated. It is estimated that between 50 and 100.000 compounds is found in the environment in an extent and concentration that could have an impact on human health. In the EU there was 100.106 commercially sold chemicals listed on the EINECS list in 1981 and the environmental risk of the majority of these compounds has not been evaluated (Teknologi-rådet 1996/2).

Theoretical models for prediction of K_{ow} can be of many kinds. Some models, like the Unifac method (Campbell and Luthy 1985, Kan and Thomson 1996) utilize activity coefficients others solvatochromic parameters like polarizability, molar volume and hydrogen bonding ability, electronic parameters like the Homo-Lumo gap (Balasubramanian 1994) or topological parameters like connectivity indices(Güsten et al. 1991, Sabiljic 1987 a). Other models are almost purely empirical like the fragmental methods of Rekker and Mannhold (1992), Fujita et al. (1964) and Meylan et al. (1992).

In this study, three models have been tested for prediction of log K_{ow} for azaarenes. A simple specifc model that has been developed from the knowledge acquired from the HPLC experiments and two non specific models, Rekker and Mannholds *f*-fragment method and the ClogP method of Leo (1993).

7.2 The simple specific models

From the HPLC experiments, and general solvation theory, it is clear that especially three factors are important for the K_{ow} of the azaarenes. These factors are:

- Size of the molecule
- Nitrogen lone pair
- Shielding of nitrogen lonepair

The molecular size is very important for lipophilicity as described in chapter 3 and confirmed by HPLC findings. The nitrogen lone pair causes hydrogen bonding and exchange of a C atom with a nitrogen reduces log K_{ow} with 1.2 log K_{ow} units for PACs irrespective of molecular size and finally it was found in this study that the shielding of the nitrogen atom by neighbouring benzene rings caused a decrease in hydrogen bonding ability of the nitrogen molecule, transferring into an increase in log K_{ow}.

Three types of parameters, describing these molecular properties, were consequently defined and the following simple linear model was set up:

$$\log K_{ow} = a \cdot \text{size descriptor} + b \cdot \text{N occurrence} + c \cdot \text{N lonepair availability} + d$$

To quantify the factors with model parameters, different indices are used. Nitrogen occurrence is described with a N-index (NI), that can assume the value of 1 or 0 indicating nitrogen or no nitrogen respectively and nitrogen lone pair un-availability is described as the number of shielding rings, where the shielding factor is 0 for compounds not containing ring nitrogen atoms.

For quantification of size, several size descriptors are tried: the first and second order connectivity indices, which have been known to correlate well with surface area, the Wiener index (Kier and Hall 1986) and the molecular weight (MW). The information of atom types, in the form of valence corrected indices, is not included in the connectivity indices as the connectivity indices are only used as descriptors of size in this investigation. The advantage of keeping atom type and molecular size separated in the model is, that the statistical significance of each parameter can be evaluated.

The first and second order connectivity indices and the Wiener index are calculated from molecular graphs consisting of vertices and edges. Each atom, apart from hydrogens, is represented by a vertice and bindings between atoms are represented by edges. From the molecular graph a connectivity matrix, describing which atoms are directly connected by bonds, can be written and from this a distance matrix, containing the distances between atoms, can be calculated. In this investigation all bonds are set to have unity length and the various indices are calculated from the connectivity and distance matrices. The Wiener index (W) is half the sum of the elements d_{ij} in the distance matrix, see below. The first order connectivity index ($^1\chi_R$) is the sum of the products of valences in both ends of an edge (bond) for all the edges in a graph and the second order connectivity index ($^2\chi_R$) is the sum of the products of valences in all subgraphs of length 2 (3 vertices, 2 edges) (Trinajstic 1983).

$$\text{Wiener index: } W(G) = \frac{1}{2}\sum_{(i,j)} d_{ij}(G) \qquad \text{eq 7.1}$$

$$\text{First order connectivity index: } {}^1\chi_R(G) = \sum_{s=1}^{s_e} (D_i D_j)_s^{-1/2}, i \neq j \qquad \text{eq 7.2}$$

$$\text{Second order connectivity index: } {}^2\chi_R(G) = \sum_{s=1}^{s_L} (D_i D_j D_k)_s^{-1/2}, i \neq j \neq k \qquad \text{eq 7.3}$$

G is a graph, d_{ij} is the shortest distance between vertice i and j, D_i is valence of vertice no. i, s_e is the total number of edges and s_L is the total number of paths of length 2. A computer program, based on graph theoretical algorithms of Nielsen (1993), was written in Pascal for calculation of the three connectivity indices. The programme was tested against literature values of Kier and Hall (1986) and gave identical results. The calculated indices are given in appendix Table A9.

7.2.1 Models and calibrations

From the descriptors a number of models were constructed and calibrated. The tested models are given in Table 7.1 together with the calibration datasets used. The compounds of the calibration dataset are given in Table 7.2.

Model	Calibration dataset
$\log K_{ow} = a \cdot MW + b$	Polar, Nonpolar, Pooled
$\log K_{ow} = a \cdot 1st\ CI + b$	Polar, Nonpolar, Pooled
$\log K_{ow} = a \cdot 2nd\ CI + b$	Polar, Nonpolar, Pooled
$\log K_{ow} = a \cdot WI + b$	Polar, Nonpolar, Pooled
$\log K_{ow} = a \cdot MW + b \cdot SI + c$	Polar
$\log K_{ow} = a \cdot 1st\ CI + b \cdot SI + c$	Polar
$\log K_{ow} = a \cdot 2nd\ CI + b \cdot SI + c$	Polar
$\log K_{ow} = a \cdot MW + b \cdot NI + c$	Pooled
$\log K_{ow} = a \cdot 1st\ CI + b \cdot NI + c$	Pooled
$\log K_{ow} = a \cdot 2nd\ CI + b \cdot NI + c$	Pooled
$\log K_{ow} = a \cdot WI + b \cdot NI + c$	Pooled
$\log K_{ow} = a \cdot MW + b \cdot NI + c \cdot SI + d$	Pooled
$\log K_{ow} = a \cdot 1st\ CI + b \cdot NI + c \cdot SI + d$	Pooled
$\log K_{ow} = a \cdot 2nd\ CI + b \cdot NI + c \cdot SI + d$	Pooled

Table 7.1. *Simple specific models tested and calibration dataset used. 1st CI = first order connectivity index , 2nd CI = second order connectivity index, WI = Wiener index, NI = nitrogen occurrence index and SI = shielding index.*

For the models and calibration dataset given here, mean square errors (MSEs) were calculated and are shown in Fig 7.1.

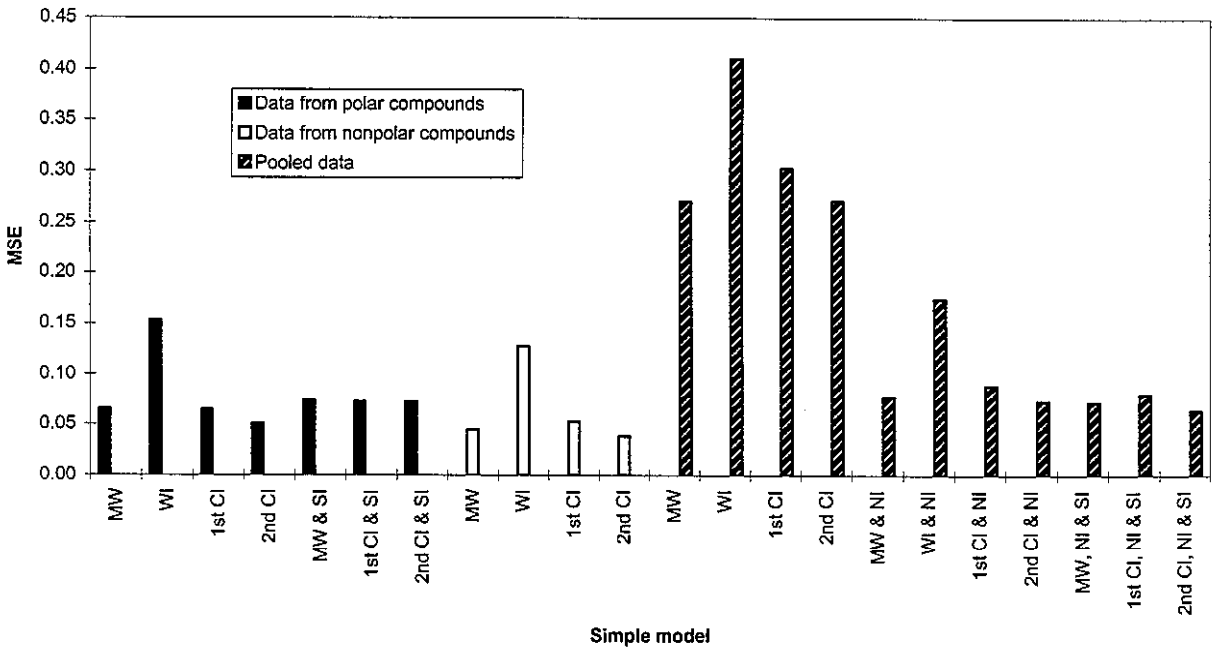


Fig 7.1. *The mean square error in prediction of log K_{ow} using different models and calibration* datasets. Each model has only been used for prediction of log K_{ow} for the compounds that constituted the calibration dataset. The coefficients of the models are given in appendix, Table A11.

From Fig 7.1 it can be seen, that the second order connectivity index are the best size descriptor closely followed by the first order connectivity index and molecular weight,

while the use of the Wiener index results in much larger errors. The errors given in Fig 7.1 show, not surprisingly, that the size of the compounds alone is not sufficient for accurate prediction of K_{ow} when the pooled datset is considered. Here the predictions are greatly improved when the N-index is added as a parameter in the model. A modified stepwise regression significance test (Montgomery 1991) also shows, that the N-index contributes significantly at a 95 % level to the description of log K_{ow} in the case of the *2nd CI & NI* model.

The addition of the shielding parameter in the models for azaarenes (polar), increases error as can be seen for from Fig 7.1, and it only reduces the MSE slightly for combined data. The contribution to the *2nd CI & NI* model is not significant at a 95 % level (only significant at a 92 % level). The reason for this seems to be that only few of the test compounds have shielding groups. However, as the parameter is crucial for the differentiation between the dibenzacridines and as it does not deteriorate the three parameter models, the best simple specific model is considered to be the *2nd CI,NI and SI* model.

7.2.2 The accuracy of predictions

To examine the predictive qualities of the *2nd CI, NI and SI* model, a cross validation was performed. The model was experimentally calibrated 100 times using all combinations of data from 18 out of the 20 compounds available, while the data from the last two compounds, one polar and one nonpolar, were used for validation. This gave 10 predictions for each compound, as there was 10 polar and 10 nonpolar compounds in the dataset. The average error of prediction (average of absolute value of residuals), for the compounds not included in the calibrations the cross validation errors were 0.27 log K_{ow} units in average or 6 %. The maximum absolute cross validation error was 0.76 log K_{ow} units which was obtained for dibenz[c,h]acridine. This relatively large error was obtained because dibenz[c,h]acridine is one of the few test compounds with shielding and the only one with a shielding index of 2. When dibenz[c,h]acridine was not included in the calibration, the model coefficient of the shielding parameter was poorly determined and predictions for compounds with shielding became inaccurate. The model is therefore sensitive to removal of compounds with nitrogen shielding from the calibration set.

compound	Literature Log(K_{ow}) Refs. in Table 6.7	Simple model log K_{ow}	Error log K_{ow}	Error in %	Cross validation error log K_{ow}	Cross validation error in %
Nonpolar						
naphtalene	3.296	3.217	0.08	2%	0.04	1%
dibenzofuran	4.12	4.161	0.04	1%	0.11	3%
dibenzothiophene	4.435	4.161	0.27	6%	0.26	6%
phenanthrene	4.46	4.351	0.11	2%	0.08	2%
anthracene	4.475	4.426	0.05	1%	0.02	0%
9-methylanthracene	5.07	4.752	0.32	6%	0.34	7%
benz[a]anthracene	5.79	5.604	0.19	3%	0.25	4%
benzo[a]pyrene	5.97	6.186	0.22	4%	0.18	3%
dibenz[a,c]anthracene	6.17	6.674	0.50	8%	0.53	9%
dibenz[a,h]anthracene	6.5	6.753	0.25	4%	0.20	3%
Polar						
quinoline	2.065	2.359	0.29	14%	0.32	16%
isoquinoline	2.08	2.359	0.28	13%	0.30	15%
acridine	3.345	3.568	0.22	7%	0.16	5%
benzo[f]quinoline	3.4	3.493	0.09	3%	0.34	10%
benzo[h]quinoline	3.6	3.493	0.11	3%	0.22	6%
phenanthridine	3.47	3.493	0.02	1%	0.07	2%
9H-carbazole	3.505	3.303	0.20	6%	0.32	9%
10-azabenzo[a]pyrene	5.533	5.328	0.21	4%	0.53	10%
dibenz[a,c]acridine	5.66	5.816	0.16	3%	0.30	5%
dibenz[c,h]acridine	6.45	5.895	0.55	9%	0.76	12%
average			0.21		0.27	

Table 7.2. *Test compounds together with literature log K_{ow} values, simple model log K_{ow} predictions (log K_{ow} = (0.654 ± 0.037) · 2nd CI - (0.974± 0.130)·NI + (0.257 ± 0.138) · SI + d), simple model log K_{ow} prediction errors and cross validation log K_{ow} errors.*

7.3 Rekkers f-fragment method

Rekkers and Mannholds *f*-fragment method (Rekker and Mannhold 1992) is used here as an example of a simple non-specific model approach. The method is described in chapter 6. As large fragments as possible have been used in this investigation. For instance in the calculation of log K_{ow} for dibenzanthracene, anthracene is preferred as a starting fragment rather than using three benzene rings or C and H to build the same fragment. The scheme for calculating log K_{ow} by *f*-fragments are shown in Table A10 in appendix and the predicted log K_{ow} values are given in Table 7.3.

7.4 The ClogP system

Another well established system of log K_{ow} prediction is the ClogP system developed by Leo (1993). This is a practical computerized system and it is sold commercially via Biobyte Corp. The ClogP method works much in the same manner as the *f*-fragment method but the method is fully computerized and more structural and electronic considerations are taken into account when calculating log K_{ow}. Furthermore the

fragments of ClogP are based on the largest log K_{ow} database available, namely the star list database of Pomona College containing 8162 selected log K_{ow} values. The predictions of the ClogP are given table 7.3 and the smiles notation, used as input in the ClogP program, are given in appendix, Table A12.

Compound	log K_{ow} ClogP	log K_{ow} *f*-fragment
quinoline	2.03	2.096
isoquinoline	1.82	2.096
9H-carbazole	3.52	3.71
4-azaflourene	2.85	3.068
acridine	3.41	3.316
benzo[f]quinoline	3.2	3.316
benzo[h]quinoline	3.2	3.316
phenanthridine	3.2	3.347
benz[a]acridine	4.59	4.686
10-azabenzo[a]pyrene	4.84	4.983
dibenz[a,c]acridine	5.76	5.972
dibenz[a,h]acridine	5.76	5.972
dibenz[a,j]acridine	5.76	5.972
dibenz[c,h]acridine	5.76	5.972
naphtalene	3.32	3.389
flourene	4.13	4.251
phenanthrene	4.49	4.666
anthracene	4.49	4.666
pyrene	4.95	4.895
benz[a]anthracene	5.66	5.916
dibenz[a,c]anthracene	6.84	7.202
dibenz[a,h]anthracene	6.84	7.202
dibenz[a,j]anthracene	6.84	7.202
dibenzofuran	4.09	4.035
dibenzothiophene	4.56	4.584

Table 7.3. *Predicted log K_{ow} values using Rekker and Mannholds f-fragment method or ClogP.*

7.5 Comparison between Models and the HPLC method

For the azaarenes and PAH + two S,O-heterocycles, predictions obtained by the simple specific model and by Rekker and Mannholds fragment method can be compared with those obtained with the HPLC method by studying the errors as seen on Fig 7.2 and Fig 7.3.

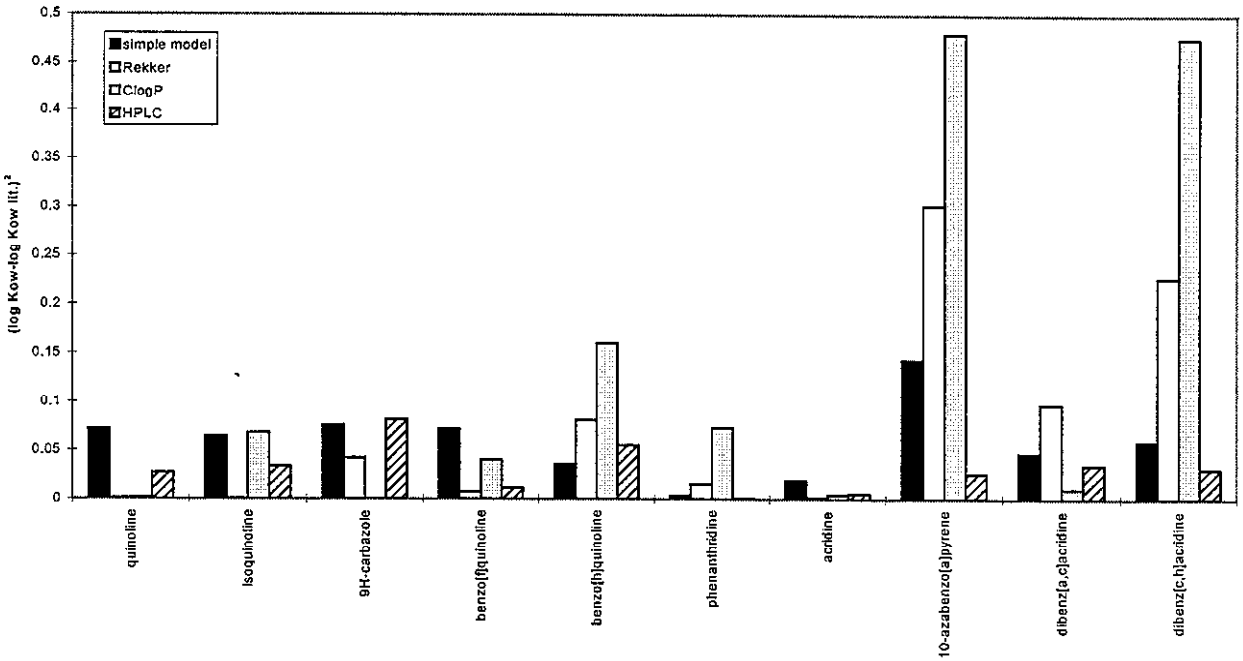


Fig 7.2. *Squared errors in log K_{ow} for azaarenes using the best simple specific model, Rekker and Mannholds aromatic f-fragment method, ClogP or HPLC (Diol-35-1) for predictions.*

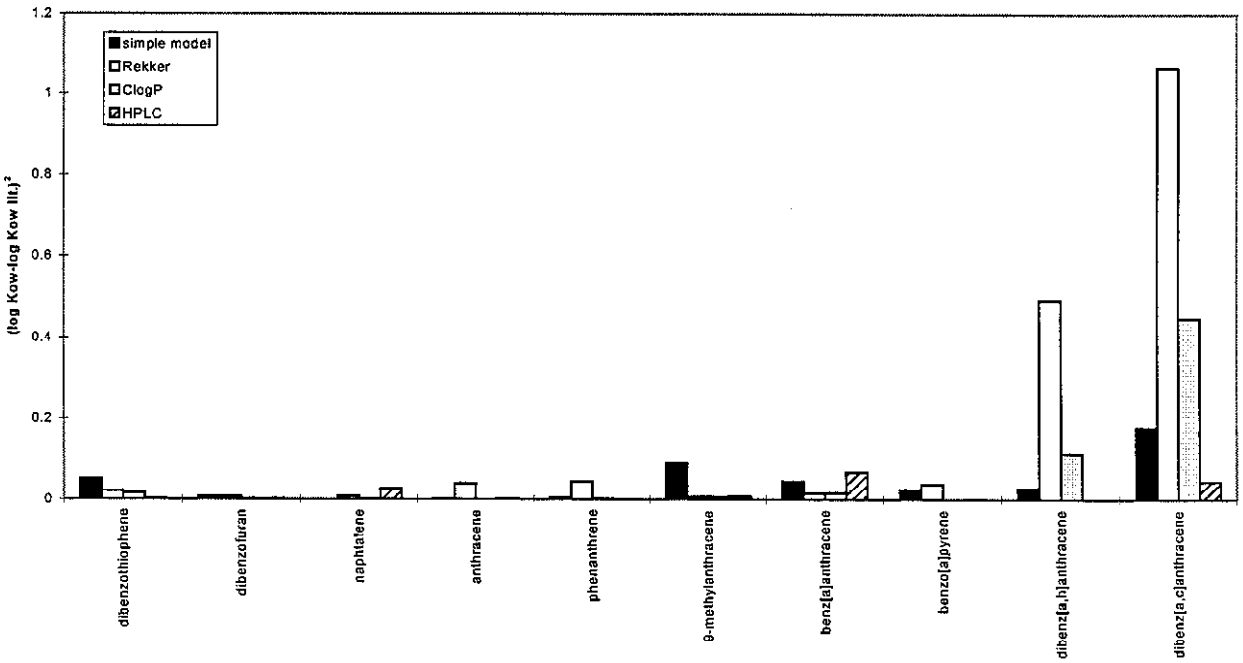


Fig 7.3. *Squared errors in log K_{ow} for nonpolar compounds using the best simple specific model, Rekker and Mannholds aromatic f-fragment method, ClogP or HPLC (ODS-65-2 model) for predictions.*

From Fig 7.2 and Fig 7.3 it can be seen that in general smaller errors are obtained by the HPLC method than by any of the theoretical methods. This is confirmed by calculations of the average squared errors as shown in Fig 7.4.

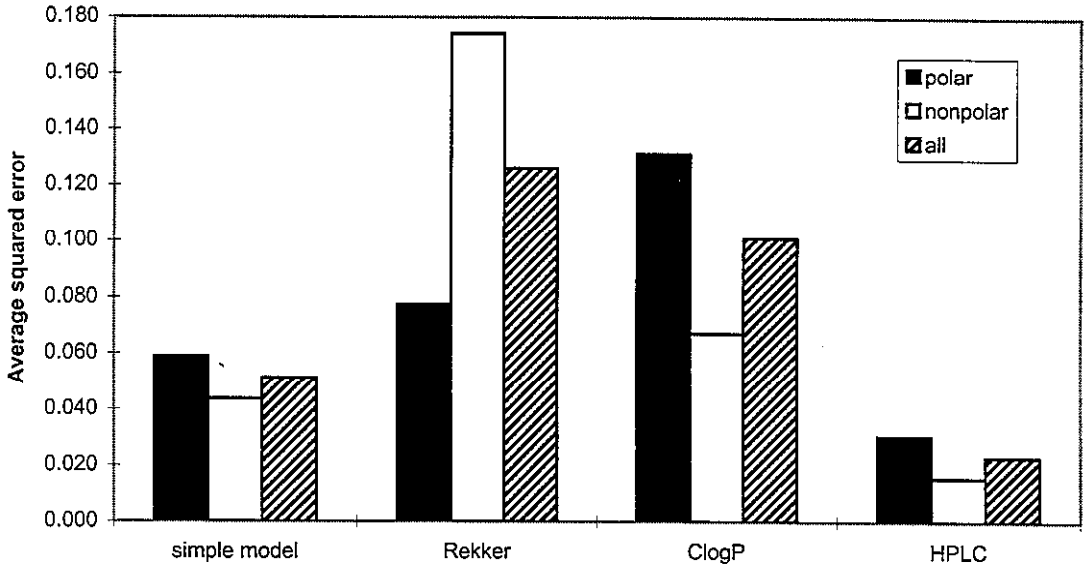


Fig 7.4. *Calculation of average squared errors for the different models. The error has been calculated for the group of polar and the group of nonpolar compounds as well as for all compounds together.*

For Rekker and Mannholds *f*-fragment method the MSE of the predictions is 0.126 and the average error is 0.250 log K_{ow} units. The errors are unevenly distributed and are larger, i.e., more than 1 log K_{ow} unit, for the compounds with high log K_{ow}. The predictions by the ClogP method are slightly more accurate than those of Rekker and Mannholds method if both polar and nonpolar compounds are considered MSE = 0.209, but for the azaarenes alone Rekker and Mannholds method is more accurate with an average log K_{ow} error of 0.226 versus 0.281 for ClogP.

Of the theoretical models tested, the simple specific model gave the most accurate predictions of MSE = 0.051 and an average error of 0.199 log K_{ow} units. Even when the error of the simple specific model was calculated with cross validation (0.21 log K_{ow} units), it was smaller than the error of the other two models. The HPLC method is superior to the theoretical models, but the best simple specific model is almost as accurate. The HPLC method and the simple specific models are based on compounds similar to those for which the predictions are to be valid while Rekker and Mannholds method, on the other hand, is based on all kinds of different compounds, some of which are very different from N-PACs and PAHs. This may explain why Rekker and Mannholds method give the poorest estimates even though it is based on a large data set..

In the HPLC method the column and eluent system experimentally mimics the octanol-water system. The advantage of this approach, in comparison with theoretical methods, is that eventual unknown mechanisms will be taken into account if these mechanisms are similar in the two systems. The shielding of the nitrogen atom in dibenz[c,h]acridine is an example of an unknown factor affecting K_{ow}, that was reflected likewise in retention and octanol water partitioning and where K_{ow} could be successfully predicted using HPLC.

Rekker and Mannholds approach and the ClogP method does, in many cases, not distinguish between different molecular configurations and they both predict the same log K_{ow} for the 4 dibenzacridines in the test set. This is a major drawback as log K_{ow} values vary 0.65 log K_{ow} units between dibenz[a,j]acridine and dibenz[c,h]acridine due to shielding.

In the simple specific model, the N-index parameter assumes the same importance of the nitrogen atom, regardless of the size of the molecule in which it is incorporated, as Rekker and Mannholds method also does. The HPLC results confirm the validity of this assumption, since a plot of log K_{ow} against molecular weight gave two lines of equal slope for non shielded N-PACs and PAH (see Fig 6.12). In the simple specific models, no advantage was found in using the more complicated connectivity indices as size descriptors compared to molecular weight. This is probably a consequence of the test compounds used. The connectivity indices are calculated in such a way, that the value of the indices becomes smaller for compact molecule structures than for extended molecule structures of the same molecular weight. Especially the higher order of indices give information about the compactness of molecules, but as all the test compounds are equally compact ring systems, the additional information is to limited to be of importance.

7.6 Summary

The best simple specific models, with the parameters second order connectivity index or molecular weight, N-index and shielding, predict log K_{ow} of tested azaarenes almost as well as the HPLC method and with much less work, not requiring any experiments. The HPLC method yields the most accurate predictions for the test-compounds: azaarenes, PAHs and two S,O-heterocycles. Rekker an Mannholds *f*-fragment method and the ClogP method, based on a large data sets, do not address the problem of shielding, which plays a major role in the partitioning behaviour of some azaarenes. Additionally, they both give inaccurate predictions for large compounds, e.g., dibenz[a,h]anthracene. Therefore, Rekker and Mannholds *f*-fragment method and the ClogP method are not considered good choices for the prediction of K_{ow} for polycyclic aromatic compounds and it seems that in general, careful considerations should be made before using simple non-specific models for prediction of K_{ow}.

8. Determination of K_{oc} for azaarenes, PAHs and polar substituted PACs

8.1 Introduction

As described in chapter 3, K_{oc} is an environmentally important physical chemical parameter that describes the partitioning of a compound between organic material and water normalized with respect to organic carbon. In this study, K_{oc} was determined indirectly by the use of a HPLC column with chemically immobilized humic acid material. This is a fast way of determining K_{oc} and it was therefore possible to determine K_{oc} for a large range of polar polycyclic aromatic compounds. The humic acid used was Aldrich humic acid which is the only commercial humic acid available in large amounts. Aldrich humic acid is for this reason very often used in studies of organic material water partitioning and a number of directly measured K_{oc} values have been reported which can be used for calibration of the HPLC method.

8.2 Experimental

8.2.1 Materials

The test compounds, given in Table 8.1, are the same as those for which K_{ow} has been determined in this study (see chapter 6). The test compounds were obtained from several sources as pure compounds (see appendix Table A1) and their identity was confirmed by UV absorption spectra. Light was kept from entering the test compound solutions by covering the flasks or keeping them in the dark. It was evident that many of the compounds photolyze quite easily. The HPLC system used was a low pressure gradient Shimadzu LC10-HPLC system with PDA detector, thermostated column oven and autoinjector. The eluents were mixed from laboratory grade ion exchanged water, extra purified on a Millipore-Q water purification system, buffered to pH 7 with 0.01M phosphate buffer, and LiChrosolv® methanol 99.8 % (GC) from Merck or Lab Scan HPLC methanol.

8.2.2 Preparation of chemically bonded humic acid column.

The silica gel with chemically bonded humic acid was prepared according to the method of Bulman and Szabo(1995) A suspension of silica gel (10 g) Nucleosil-Si-50-10 (Machery Nagel), toluene (50 ml)and 3 aminopropyl triethoxysilane (2,5 ml) was refluxed by stirring under argon for 4 h. The crude aminopropyl silica gel was removed by filtration through a sintered glass filter (G4). The product was washed successively with 2 * 50 mL of each of the following solvents: Toluene, methanol, water and methanol and dried at 50 ^{0}C. The resulting suspension of the aminopropyl silica gel (10.47 g) and a 5 % water solution of glutardialdehyde (230 mL) (Merck zur synthesis) was stirred at room temperature under argon in a 500 mL round bottomed flask. The reaction mixture was filtered through a sintered glass filter (G4) and washed with water (350 mL) several times. The activated silica gel was dried at 50 ^{0}C over night and the yield was 11.98 g. Subsequently, humic acid (1.0 g) (Aldrich)was dissolved in water (100 mL), the activated silica gel was added and reaction suspension was stirred at room temperature under argon for 7.5 h. he product was pressed as dry as possible and suspended in a 0.1 M water solution of 2-aminoethanol (Merck) buffered with

phosphoric acid to pH 7.5 (total volume 100 mL). The suspension was stirred at room temperature under argon for 3.5 h. and filtered through a sintered glass filter (G4). The filtrate was washed with water (5*50 mL) and dried at 50 C overnight yielding 11.7 g. The HPLC humic acid column was packed in methanol at 350 bar.

8.2.3 Chromatographic procedure

The test compounds were dissolved in methanol with a typical concentration of 0.04 g l^{-1} and from these solutions mixtures were made with 3-5 compounds in each mixture. The mixtures were designed so that the compounds eluted at suitable intervals. 20 μL was injected in all cases with the autoinjector using a partially filled loop technique. All measurements were repeated three times. The standard deviation between replicated injections was 0.7 %. The temperature was kept at 30^0 C for all measurements.
An eluent of 65 % v/v methanol and 35 % v/v water was used in all cases. Flow rate was 1 mL/min for all measurements. The dead time of the system t_0, used for calculating the capacity coefficient: $k' = (t_r-t_0)t_0^{-1}$, was determined by chromatographing water. This was done in connection with each set of chromatographic runs.

8.3 Results

The determined capacity coefficients are given in Table 8.1.

Compound		Predicted	Measured
Azaarenes	k'	log K_{oc}	log K_{oc}
quinoline	0.216	2.933 ± 0.441	3.05 [a]
isoquinoline	0.219	2.944 ± 0.440	
4-azaflourene	0.629	3.602 ± 0.333	
acridine	1.179	3.994 ± 0.284	
phenanthridine	1.279	4.045 ± 0.278	
benzo[f]quinoline	1.327	4.068 ± 0.276	
benzo[h]quinoline	1.435	4.117 ± 0.272	
9H-carbazole	3.555	4.683 ± 0.248	
benz[a]acridine	6.365	5.047 ± 0.263	
10-azabenzo[a]pyrene	27.984	5.971 ± 0.378	
dibenz[a,c]acridine	40.223	6.198 ± 0.416	
dibenz[a,h]acridine	37.917	6.161 ± 0.410	
dibenz[a,j]acridine	26.805	5.944 ± 0.374	
dibenz[c,h]acridine	46.171	6.284 ± 0.431	
S,O-heterocycles			
dibenzofuran	1.478	4.135 ± 0.270	
dibenzothiophene	2.932	4.563 ± 0.248	
PAH			
naphthalene	0.720	3.686 ± 0.321	3.32[b]
flourene	1.629	4.196 ± 0.265	
anthracene	3.291	4.635 ± 0.248	4.87±0.26[b,c,d]
phenanthrene	3.317	4.640 ± 0.248	4.81±0.16[c,d,e]
benz[a]anthracene	16.641	5.647 ± 0.329	5.48[b]
benzo[a]pyrene	46.554	6.289 ± 0.432	6.30[b,f]
dibenz[a,c]anthracene	81.897	6.641 ± 0.496	
dibenz[a,h]anthracene	70.438	6.547 ± 0.478	
dibenz[a,j]anthracene	56.194	6.406 ± 0.453	
pyrene		6.153[g]	
Substituted PAH			
anthraquinone	2.057	4.342 ± 0.256	
9-anthracenecarboxamide	1.279	4.045 ± 0.278	
9-acetylanthracene	1.705	4.225 ± 0.263	
9-methoxyanthracene	2.358	4.427 ± 0.252	
9-anthracenecarboxylicacidmethylester	2.412	4.441 ± 0.251	
9-anthracenecarbonitrile	3.829	4.730 ± 0.248	
9-nitroanthracene	4.056	4.765 ± 0.249	
9-methylanthracene	4.555	4.838 ± 0.251	
9-chloroanthracene	6.627	5.072 ± 0.265	
9-bromoanthracene	8.981	5.262 ± 0.281	
1-bromopyrene	18.065	5.698 ± 0.336	
2-bromopyrene	18.803	5.723 ± 0.340	
4-bromopyrene	19.435	5.743 ± 0.343	
1,3-dibromopyrene	42.501	6.232 ± 0.422	
1,6-dibromopyrene	34.136	6.095 ± 0.399	
1,8-dibromopyrene	41.018	6.210 ± 0.418	
Quinoline derivatives			
2-hydroxyquinoline	0.248	2.7	
N-methylquinoliniumiodid	0.222	2.0	
quinoline-N-oxide	0.245	3.1	

Table 8.1. *Measured capacity coefficients on the chemically bonded humic acid column together with predicted log K_{oc} values of this study and literature log K_{oc} values for*

Aldrich humic acid. [a] Nielsen et al. (1997), [b] McCarthy and Jimenez (1985), [c] Gauthier et al. (1986), [d] Kumke et al. (1994), [e] Sojitra et al. (1986), [f] McCarthy et al. (1989). [g] The K_{oc} value for pyrene is predicted from the theoretical model developed in chapter 8.31.

A simple linear model $\log K_{oc} = a \cdot \log k' + b$ was fitted with the data from Table 8.1 and the fitted equation was

$$\log K_{oc} = (1.437 \pm 0.133) \cdot \log k' + (3.891 \pm 0.125),\ r^2 = 0.97 \qquad \text{eq 8.1}$$

The model is shown with data on Fig 8.1.

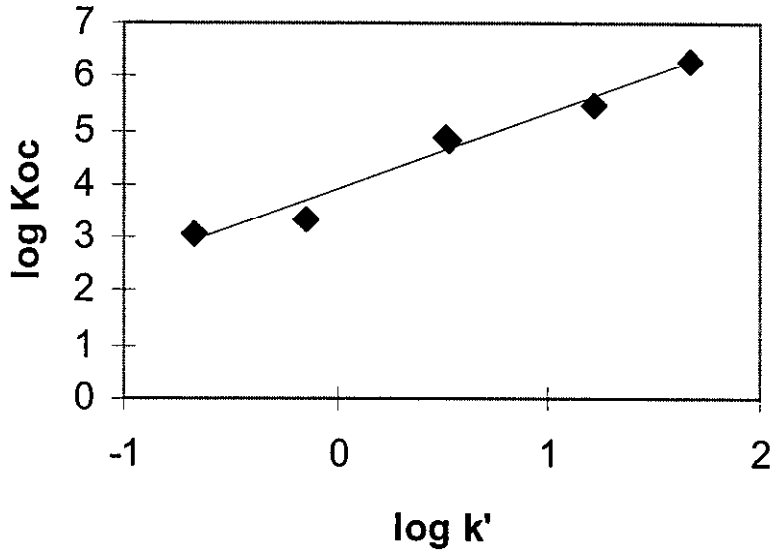


Fig 8.1. *The relationship between capacity coefficient on the humic acid column and literature K_{oc} values from Table 8.1.*

As can be noted, the fitted relationship is only based on 6 corresponding sets of capacity coefficients and literature log K_{oc}s of which 5 is from PAHs and one is from an azaarene (quinoline). This dataset is obviously not representative of all test compounds, but as the retention mechanisms in the column is supposed to be very similar or almost identical to the mechanisms of the sorption process in nonbonded humic acid, the fitted equation are used for prediction of K_{oc} for all the test compounds except the very polar quinoline derivatives. These were shown to deviate from the behavior of azaarenes and PAH by Nielsen et al. (1997), and the values suggested by Nielsen et al. for these compounds have been given in Table 8.1 in stead of predictions. The fact that the relationship between log k' and log K_{oc} are the same for the polar quinoline and the nonpolar PAHs, supports the general applicability of equation 8.1. The calculated log K_{oc} values are given in Table 8.1.

8.3.1 Azaarenes, PAH and two S,O-heterocycles

For the azaarenes, the PAHs and the two S,O-heterocycles it can be seen that K_{oc} like K_{ow} seems to be dependent of size and hydrogen bonding ability and a model like the best simple specific model, with the parameters second order connectivity index (2nd

CI), nitrogen occurrence index (NI) and shielding index (SI), used for prediction of log K_{ow} in chapter 7, can be fitted with good results. The resulting model is

$$\log K_{oc} = (0.620 \pm 0.022)\cdot 2nd\ CI - (0.525 \pm 0.078)\cdot NI + (0.032 \pm 0.08)\cdot SI + (1.348 \pm 0.140) \quad \text{eq 8.2}$$
$$r^2 = 0.99$$

As in the case of the octanol water partition coefficient, the shielding parameter does not contribute significantly to the model, at a 95 % level, while the other two parameters do according to a stepwise regression test (Montgomery 1991). The predicted log K_{oc} values are given in Table 8.2 together with HPLC determined K_{oc} values. A cross validation test showed that the model was quite robust and the average of errors only increased from 2 % to 4 % when predictions were made from models calibrated with datasets not including the compounds for which predictions were made. This means that good predictions can be made from equation 8.2.

	HPLC log K_{oc}	Simple model log K_{oc}	Error log K_{oc}	Error in %	Cross validation error	Cross validation error in %
naphtalene	3.69	3.56	0.13	3%	0.22	6%
anthracene	4.63	4.65	0.01	0%	0.02	0%
phenanthrene	4.64	4.58	0.06	1%	0.10	2%
9-methylanthracene	4.84	4.94	0.10	2%	0.08	2%
benz[a]anthracene	5.65	5.71	0.06	1%	0.04	1%
benzo[a]pyrene	6.29	6.23	0.06	1%	0.10	2%
dibenz[a,c]anthracene	6.64	6.67	0.03	0%	0.02	0%
dibenz[a,h]anthracene	6.55	6.74	0.20	3%	0.23	4%
dibenzofuran	4.14	4.41	0.27	7%	0.28	7%
dibenzothiophene	4.56	4.41	0.15	3%	0.21	5%
quinoline	2.93	3.03	0.10	3%	0.24	8%
isoquinoline	2.94	3.03	0.09	3%	0.23	8%
9H-carbazole	4.68	4.41	0.28	6%	0.80	17%
acridine	3.99	4.12	0.13	3%	0.28	7%
phenanthridine	4.05	4.05	0.01	0%	0.14	3%
benzo[f]quinoline	4.07	4.09	0.02	0%	0.06	1%
benzo[h]quinoline	4.12	4.05	0.06	2%	0.06	1%
10-azabenzo[a]pyrene	5.97	5.71	0.26	4%	0.20	3%
dibenz[a,c]acridine	6.20	6.18	0.02	0%	0.04	1%
dibenz[c,h]acridine	6.28	6.28	0.00	0%	0.12	2%
			0.10	2%	0.17	4%

Table 8.2. *Test compounds together with HPLC determined log K_{oc} values, simple model log K_{oc} predictions (log K_{oc} = (0.620±0.022)·2nd CI -(0.525 ± 0.078) ·NI ± (0.032 ± 0.08)·SI + (1.348 ± 0.140)), simple model log K_{oc} prediction errors and cross validation log K_{oc} errors.*

The importance of endocyclic nitrogen for log K_{oc} can be gathered from the fitted model in that an exchange of an endocylic C-H with a N atom causes a decrease in log K_{oc} of 0.525 log K_{oc} units. This can also be observed in Fig 8.2 that furthermore shows that the

influence of nitrogen on log K_{oc} is constant and independent of size (molecular weight), as assumed in the simple specific model.

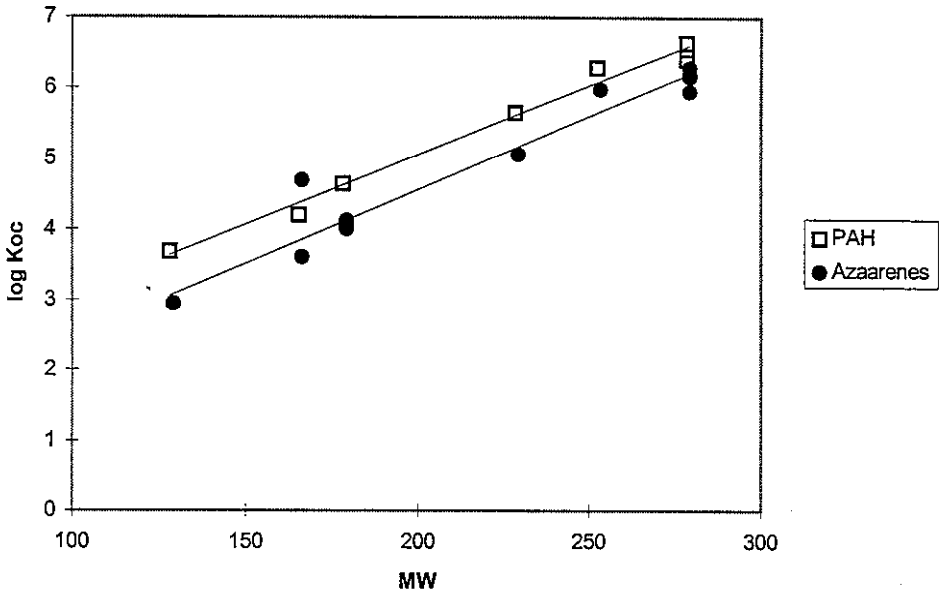


Fig 8.2. *log K_{oc} as a function of molecular weight (MW) for azaarenes and PAHs. The largely deviating azaarene is 9H-carbazole.*

Even though the shielding index was insignificant in the K_{oc} model, shielding of the nitrogen atom lonepairs seems to have an effect if the dibenzacridines are considered in that K_{oc} diminishes through dibenz[c,h]acridine >, dibenz[a,c]acridine = dibenz[a,h]acridine > dibenz[a,j]acridine as number of shielding rings also do. Also for the benzoquinolines, the benzo[h]quinoline, that has one shielding benzene ring, has a higher K_{oc} than benzo[f]quinoline that has no shielding benzene rings. From Table 8.1 it can also be seen that there was quite a large variation between the dibenzanthracenes which could suggest that the geometric structure itself may have some importance for K_{oc}, but the second order connectivity index, that correlates well with suface area, is larger for the dibenz[a,j]anthracene and dibenz[a,h]anthracene (8.70) than for dibenz[a,c]anthracene (8.59) which has the largest K_{oc} according to the HPLC measurements. This means that the larger molecule has a lower K_{oc} which is opposite to what would be expected from general theory. On the other hand, anthracene and phenanthrene, though structurally different, have almost equal K_{oc} values, and it seems likely that the reason for the variation in log K_{oc} values of the dibenzanthracenes mainly should be found in the general inaccuracy of determinations.

8.3.2 The relationship between K_{oc} and K_{ow}

As K_{oc}s are organic material specific, difficult to measure and only have been measured directly for very few compounds (Sablijc et al. 1995) prediction from a more standardized and extensively measured coefficient like K_{ow} is interesting. If a simple relationship exists between K_{oc} and K_{ow}, this relationship could be established for the different organic materials of interest and predictions could be made from K_{ow} values. Today, such predictions from linear relationships between K_{oc} and K_{ow} are standard procedure (Schwarzenbach et al. 1993), but some have found that one K_{oc}-K_{ow} LFER only can be applied for one group of compounds (Schwarzenbach et al. 1993, Sablijc et

al. 1995). As explained in chapter 3 the same mechanisms have to be responsible for partitioning in the two systems for a LFER to exist and this may not be the case for structurally different compounds.

As the azaarenes, the PAH, and the two S,O-heterocycles that are included in the calibration of the log K_{oc} -simple specific model are exactly the same as those used for calibration of the simple specific log K_{ow} model, the models can well be compared, either directly from coefficients or from the relative importance of the coefficients. The relative importance has been calculated as the relative reduction in error that each parameter causes (Montgomery 1991). This has been done in connection with the stepwise regression significance test that was used to decide whether the individual parameters were significantly contributing to the model or not. For all three parameters, the regression sums of squares for the reduced model (the full model minus the parameter of choice) was subtracted from the regression sums of squares for the full model and the relative size of these numbers were used as indicators of relative parameter importance. In this way scaling problems are avoided. However it should be noted that the percentages, given in Table 8.3, only concern the variation accounted for by the models, and not the total variance in the data.

	Model coefficients			Relative importance	
Parameter	K_{oc} model	K_{ow} model	Normalized K_{oc} model	K_{oc} model	K_{ow} model
2nd CI	0.620	0.654	0.448	94.62%	65.95%
NI	-0.525	-0.974	-0.379	5.36%	15.67%
SI	0.032	0.257	0.023	0.02%	0.95%
Const.	1.348	0.974	0.974		

Table 8.3. *Comparison of simple specific model coefficients for the three parameters. In the normalized K_{oc} model, coefficients have been adjusted(scaled) so that the constant are equal to the constant of the K_{ow} model.*

A comparison of the K_{oc} and the K_{ow} models, as shown in Table 8.3 shows that the nitrogen occurrence in a molecule has a larger relative effect on K_{ow} than on K_{oc} and followingly shielding is also more important for K_{ow} than for K_{oc}. The absolute model coefficients for size (second order connectivity index) are comparable which means that K_{oc} and K_{ow} varies equally with molecular size. The relative importance of size for K_{ow} is naturally smaller than for K_{oc} because of the larger importance of nitrogen occurrence. The difference between the model parameters indicates that prediction of K_{oc} from K_{ow} should apply different relationships between K_{ow} and K_{oc} for polar and non polar compounds which is confirmed when log K_{oc} is plotted against log K_{ow} for azaarenes and PAH + two S,O-heterocycles as done in Fig 8.3.

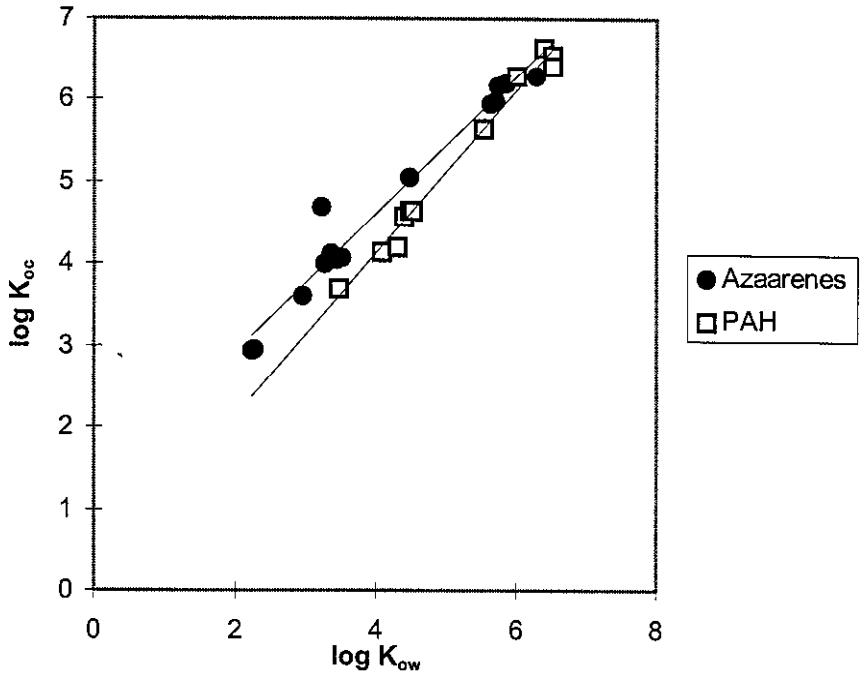


Fig 8.3. *The relationship between log K_{ow} and log K_{oc} for azaarenes, PAH and two S,O-heterocycles. The linear relationships are log K_{oc} = (0.837 ± 0.049)·log K_{ow} + (1.250 ± 0.208), r^2 = 0.96 for azaarenes and log K_{oc} = (0.992 ± 0.040)·log K_{ow} + (0.153 ± 0.220), r^2 = 0.99 for PAH + two S,O heterocycles. The azaarene that deviate noticeable from the relationship is 9H-carbazol.*

From Fig 8.3 it is clear that azaarenes and PAHs follow different relationships between K_{oc} and K_{ow}. Gerstl (1990) also found that log K_{oc} of compounds from different structural groups could not be predicted from log K_{ow} using one log K_{ow}-log K_{oc} relationship.

8.3.3 The polar substituted PACs

K_{oc} for the polar substituted PAHs was predicted from HPLC measurements in exactly the same way as for the azaarenes and PAH, using the same predictive expression, and the resulting log K_{oc} values are given in Table 8.1. The substituent effects, calculated as the difference between log K_{oc} for the substituted PAC and log K_{oc} for the mother PAC, are shown on Fig 8.4.

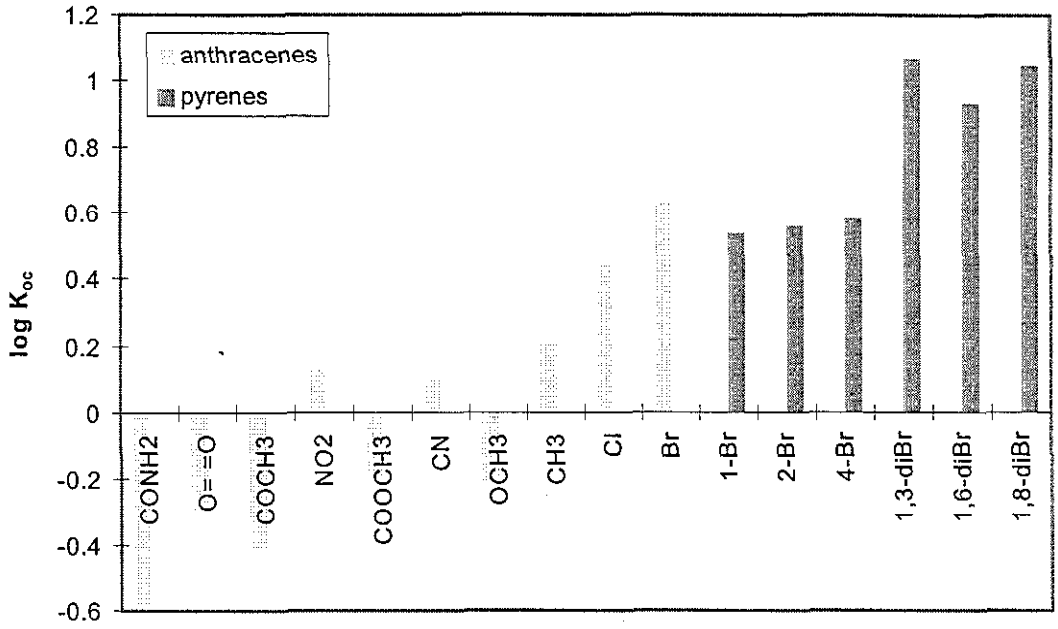


Fig 8.4. *The log K_{oc} substituent effects calculated as the difference between log K_{oc} of the substituted PAC and the mother PAC (anthracene or pyrene).*

From Fig 8.4 it can be seen that addition of the hydrogen bonding substituents, $COONH_2$, $COCH_3$, OCH_3 and $COOCH_3$ causes a decrease in K_{oc} as would be expected, but the two hydrogen bonding substituents NO_2 and CN cause an increase in K_{oc}. This was also found by Nielsen et al. (1997), even though to a lesser degree, using the same method so experimental error seems not to be the reason. The non hydrogen bonding substituents increase K_{oc} when added to PACs in accordance with the increase in size they cause. The effect of Bromine is very similar whether bound to anthracene or pyrene and two bromines cause approximately the double increase of one bromine, wherefore the effect of bromine seems to be additive and independent of aromatic system.

Nielsen et al. (1997) studied the highly polar quinoline derivatives: N-methylquinolinium iodide, quinoline N-oxide and 2-hydroxyquinoline using the same method and column type and found that K_{oc} can not be calculated from the same linear relationship as that used for the other PACs. Instead capacity coefficients were determined with mobile phases of 95 % water and from these K_{oc} was determined. The values from Nielsen at al. are given in Table 8.1.

8.3.4 The relationship between K_{oc} and K_{ow}

The substituted anthracenes, though roughly of the same size and shape, constitute a group with diverse electronic properties and it was therefore interesting to examine the log K_{ow}-log K_{oc} relationship for these compounds.

The substituent effects on log K_{oc} and log K_{ow} when attached to anthracene in the 9-position (anthraquinone in the 9,10 positions) have been compared in Fig 8.5.

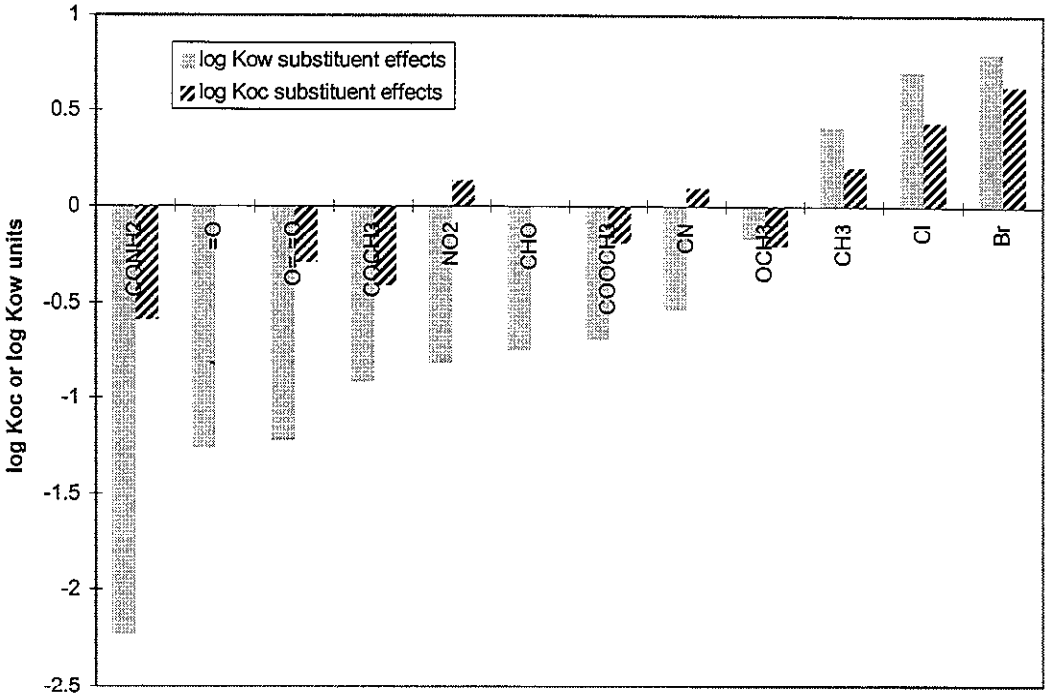


Fig 8.5. *Substituent effects for log K_{oc} and log K_{ow}*

From Fig 8.5 it can be seen that the overall trend is similar in effect of substituents on K_{oc} and K_{ow}. In both the octanol-water system and the humic acid-water system the CHO, $COCH_3$, $COOCH_3$ and $CONH_2$ substituents cause an increased partitioning into the water phase when added to anthracene, while addition of the halogen substituents or CH_3 to anthracene causes an increased partitioning into the organic phase. In both systems the largest increase in partitioning into the water phase is caused by the $CONH_2$ substituent. But, at a more detailed level there are noticeable differences between the effects on K_{oc} and K_{ow}. Especially NO_2 and CN do not follow the general trend.

From the comparison it is clear that the effect of substituents are smaller on log K_{oc} than on log K_{ow}, but interestingly the difference is much larger for the polar hydrogen bonding substituents than for the nonpolar substituents. The explanation seems to be that while octanol, as the nonpolar phase in the octanol water system mainly excerts lipophilic or dispersive interactions with the solutes, the organic humic acid phase can interact strongly with polar compounds because of the carbonylic and phenolic groups present in the humic acid material (Senesi and Testini 1983). This means that the polar compounds will be relatively stronger bound to the humic acid phase than nonpolar and the effect of adding a polar substituent will be relatively smaller than the effect of adding a nonpolar substituent. The range of log K_{oc} values is consequently smaller than the range of K_{ow} values.

The different sorption/partitioning mechanisms are probably also responsible for the deviation of the NO_2 and CN substituents as these two are the most strongly electron donating (electron rich) of the substituents, according to σ_p (Chapman and Shorter 1978) and could make especially strong bonds with humic acid. However, there was no general relationship between σ_p and log K_{oc} effect/log K_{ow} effect.

From the above, it is not surprising that the correlation between log K_{oc} and log K_{ow} for substituted anthracenes is rather low as can be seen on Fig 8.6.

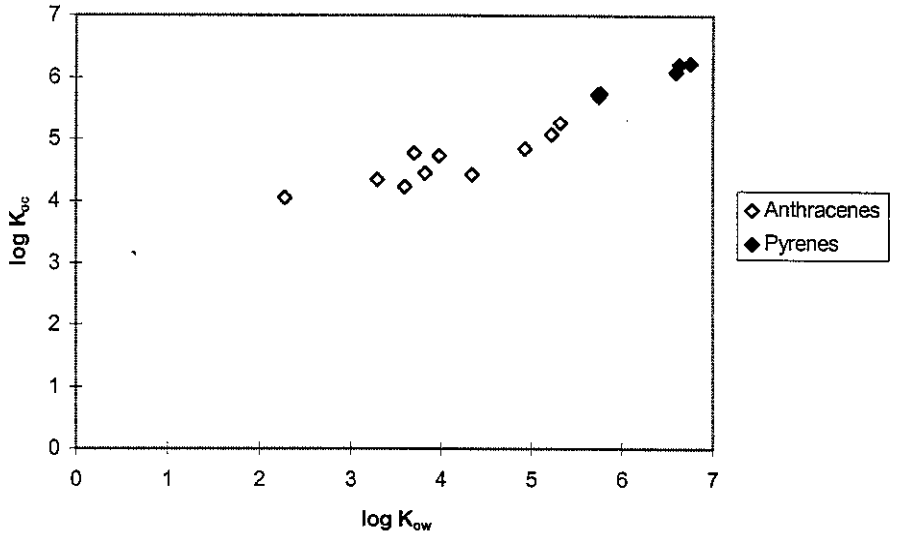


Fig 8.6. *Log K_{oc} against log K_{ow} for substituted anthracenes and pyrenes.*

The correlation coefficient r^2 for a linear relationship (log K_{oc} = (0.36 ± 0.07) · log K_{ow} + (3.15 ± 0.28)) is as low as 0.78. The 95 % prediction intervals for the HPLC determined log K_{ow}s are rather large, approx. ±0.4 log K_{ow} units and likewise for K_{oc}, approx. ±0.28 log K_{oc} units, but even when this is taken into consideration, a linear relationship does not seem reasonable as K_{oc}s in some cases are nearly identical for compounds with quite different K_{ow}. This shows that when specific electronic or steric interactions occur, the mechanisms of partitioning can be quite different in the two systems and accurate predictions of log K_{oc} from log K_{ow} values are not possible.

If the substituted quinolines are examined, 2-hydroxyquinoline and quinoline-N-oxide behave more or less identical in the two systems, having reduced K_{ow} and K_{oc} compared to quinoline, but this is not the case with the N-methylquinolinium ion. K_{oc} for the N-methylquinolinium ion is as large as that of quinoline, but K_{ow} is almost three magnitudes lower. The reason for this seems to be that while octanol does not facilitate ionic bonding, humic acids do (Senesi and Testini 1983).

8.4 Summary

The measurements of K_{oc} for azaarenes, PAHs, two S,O-heterocycles and polar substituted PAHs have shown that K_{oc} is dependent on molecular size and hydrogen bonding ability in that K_{oc} increases with size and decreases with increasing hydrogen bonding ability, as was found for K_{ow}. The effect of nitrogen in azaarenes was in average 0.53 log K_{oc} units. For the polar substituted anthracenes and pyrenes it was found that in general hydrogen bonding substituent decreases K_{oc} while non hydrogen bonding substituents increases K_{oc}. The two substituents NO_2 and CN behaves opposite to what would be expected in that they cause an increase in K_{oc} when added to anthracene. When K_{oc} are compared to K_{ow} it is found that the effect of the hydrogen bonding caused by nitrogen, relative to the effect of molecular size, is smaller for K_{oc} than for K_{ow}. Likewise it is found that for K_{oc} the effect of polar substituents is smaller

than the effect of the nonpolar substituents when compared to the effects of polar and nonpolar substituents on K_{ow}. From these findings it is clear that polar and nonpolar compounds have different relationships between K_{ow} and K_{oc}.

9. Determination of K_d for azaarenes

9.1 Introduction

To investigate the relationship between K_{oc}, K_d and K_{ow} for azaarenes and to provide some practically applicable soil sorption coefficients (K_ds), K_d was determined for quinoline, acridine and benz[a]acridine. For quinoline and acridine the results were to be used in plant toxicity experiments conducted in connection with this study (Gissel Nielsen and Nielsen 1996). K_d was determined with simple batch experiments where soil, water and solute were mixed and shaken until equilibrium whereafter the solute was quantified in the aqueous phase using HPLC or GC-MS.

Before sorption experiments were initiated, kinetic experiments were performed to determine the time necessary for equilibration.

9.2 Kinetic experiment

To determine the time necessary for the distribution of the solute between soil and water to reach equilibrium, a kinetic experiment was conducted. With acridine as test compound a number of batches with soil, water and solute was prepared and shaken for different periods of time.

9.2.1 Experimental

Materials

The soil was a natural agricultural sandy loam consisting of 28.2 % w/w Clay, 69.6 % silt and 2.2 % organic material. The pH of the soil was 7.5. All water used was laboratory grade ion exchanged water, extra purified on a Millipore-Q water purification system. Lab Scan HPLC grade methanol was used for the HPLC eluent. Benz[a]acridine was obtained from Community bureau of reference materials, Commission of the European communities and quinoline and acridine were obtained from Fluka. The shaking bath used was a Hetofrig CB60VS with a Heto thermostat, The filters used were Anotop 25, 0.02 μm ceramic filters from Frisenette. A test with acridine showed that this filter removed approximately 10 % of the acridine from an aqueous solution. (A Minisart filter removed 95 % of the acridine).

Quantification of quinoline and acridine was done on a low pressure gradient Shimadzu LC10-HPLC system with PDA detector, thermostated column oven and autoinjector. The quantification of benz[a]acridine was done on a Varian star 3400/Saturn 4DGC-MS-MS. (Conditions are described under the individual experiments below)

Procedure

As a first approach the soil was sieved to remove particles larger than 2 mm, but the sieved soil was quite inhomogenic and as small amounts of soil (5 g) was used with each flask, we experienced that after shaking, the amount of suspended particles was very different in different flasks. Therefore, another approach was used. First the soil particle size distribution was determined by sieving and weighing and followingly, small portions of the soil, one for each flask, was constructed according to the size distribution by weighing small amounts of soil from each size fraction and adding them

to the flasks. Only the soil particles smaller than 2 mm in diameter was used in the experiments. The result of the sieving are given in Table 9.1 and shown on Fig 9.1.

ASTM sieve	Net size mm	Weight %	g soil fraction used for 5 g samples
10	2 <	13	0
40-10	0.42-2	37	2.144
70-40	0.21-0.42	30	1.724
120-70	0.125-0.21	14	0.786
200-120	0.074-0.125	3	0.177
200	> 0.074	3	0.161

Table 9.1. *Sieving fractions found for the soil.*

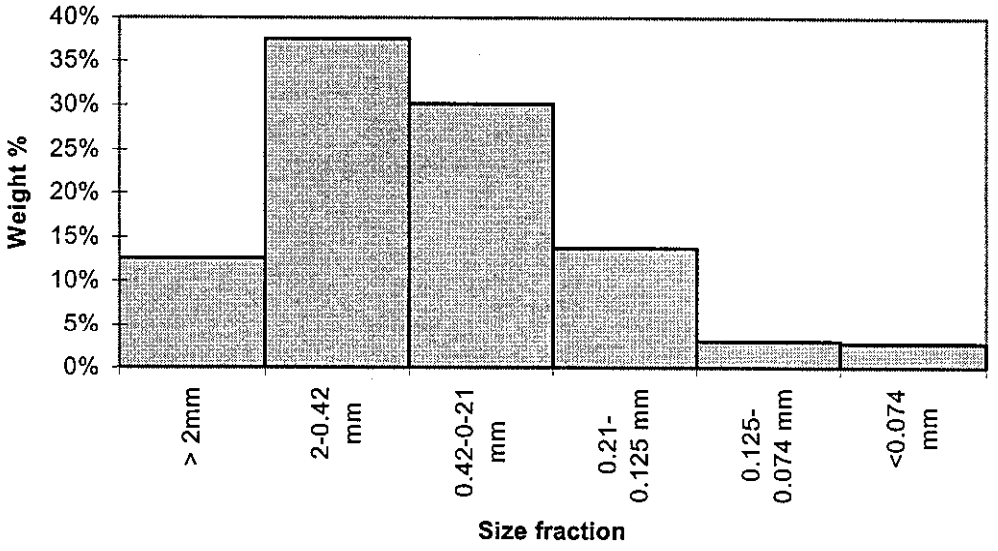


Fig 9.1. *Size fractions of the soil used in sorption experiments.*

21 Erlenmeyer flasks with glass lids were divided into 14 test flasks and 7 control flasks. Small samples of 5 g soil was constructed according to the established sieving fractions, see Table 9.1, and added to the test flasks only. The soil and flasks were partly sterilized by heating under nitrogen (to avoid oxidation) at 120 C for 12 h. Other sterilization procedures were considered (addition of poison, irradiation, autoclavation) but these all seemed to be more prone to changing the soil properties than the simple heating, and as the experiment would be rather short and the solutes were not easily degradable, soil heating under nitrogen was considered the best choice. After heating, the soil and flasks were left to cool before use. An acridine solution was made by solubilizing 0.02013 g of acridine in 10 mL HPLC grade methanol, transferring the methanol to a 1000 mL measuring flask and adding HPLC grade water to the 1000 mL mark. The resulting concentration of 0.02013 g acridine/L is less than half of the solubility of acridine in water ($300*10^{-6}$ mol/L or 0.054 g/L)(Pearlman et al.1984).

To each flask, (both test and control flasks), 20.00 mL of acridine solution was added and the flasks were closed and placed in the dark thermostated (25°C) shaking bath. The time was recorded and the shaking was started with approx. 230 shakes /min. This rate caused most of the soil particles to be suspended. At intervals, two test flasks and one

control flask were removed from the shaking bath. The test flasks were left to precipitate for 10 min where after the aqueous phase of each flask (also the control flask) was transferred to a glass tube and centrifuged at 3500 rev/min for 1 min. The supernatant was poured into a glass beaker and filtrated through an anotop 25 disposable syringe filter (0.02 μm) with a glass syringe. The filtration was done as follows: First 3 times 10 mL of clean millipore water was drawn through the filter and here after 1 ml of sample from the beaker. This 1 mL was discarded and the following 4 mL was filtrated into two autosampler vials of 2 mL each. The vials were capped (teflon septum) and stored dark in a refrigerator until analysis.

The amount of acridine in the aqueous phase samples was quantified on HPLC (UV PDA detection at 249 nm, Phenomenex 5 μm ODS II column 50 mm*4.6 mm ID, 65/35 MeOH/Water, using a 1-point calibration curve. All samples were analyzed three times and standard deviations between determinations was in average 5 %, but ranged from 0 to 23 %. The samples from controls and from the test flasks were injected alternately.

9.2.2 Results

From the amount of solute in the aqueous phase, K_d can be calculated. The difference between the amount of solute found in the aqueous phase of the control flask and in the aqueous phase of the test flask is considered to be sorbed to the soil as expressed in equation 9.1.

$$C_{soil}^{Test} = \frac{V_{water}^{Control} \cdot C_{water}^{Control} - V_{water}^{Test} \cdot C_{,water}^{Test})}{m_{soil}^{Test}} \qquad \text{eq. 9.1}$$

C_{soil}^{Test} is the solute soil concentration in the test flask, $V_{water}^{Control}$ is the volume of the aqueous phase in the control flask and m_{soil}^{Test} is the mass of the soil in the test flask. K_d was calculated from the measured water concentration and the calculated soil concentration, and plotted as a function of time in Fig 9.2 below. The results from the equilibrium experiment show that an equilibration time of 48 hours seems sufficient for attaining a reasonable equilibrium (see Fig 9.2).

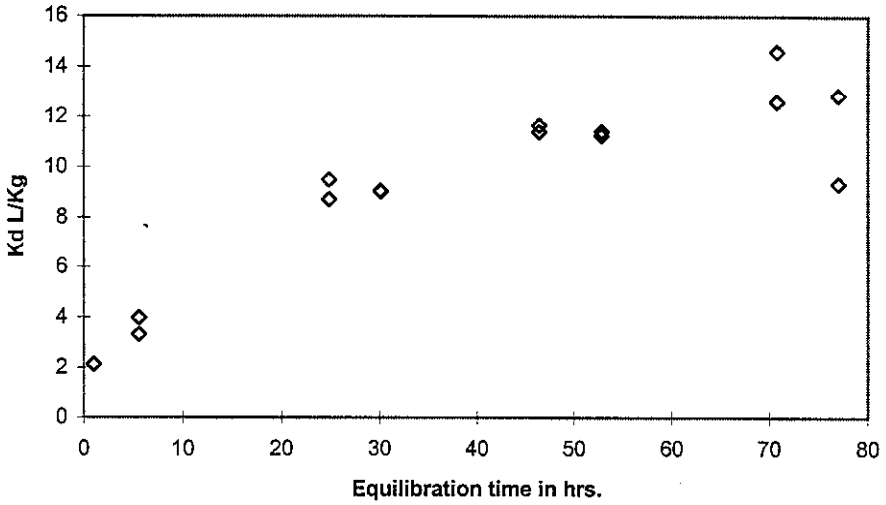


Fig 9.2. *Kinetic experiment with acridine.*

9.3 Experiments to determine soil sorption K_d

To determine K_d for the three selected azaarenes, quinoline, acridine and benz[a]acridine sorption experiments were conducted. As the sorption to soil may be non-linear (Schwarzenbach et al. 1993) it is necessary to determine sorption at different solute concentrations and this was done by batch experiments where solute concentration was varied between batches.

9.3.2 Quinoline

Procedure

The soil sorption experiment was conducted the same way as the kinetic experiment except that aqueous solutions (20 mL) of different concentrations were added to the flasks. 5 g of soil was added to the testflasks while no soil was added to the control flasks. All flasks were shaken for 48 h. For quinoline the concentrations used were 10.50, 8.750, 4.375, 1.750, 0.8750 and 0.4375mg/L and two test flasks and one control flask was made for each concentration. The aqueous solubility of quinoline is 6000 mg/L (Zachara et al. 1987) Analysis was done on HPLC at 226 nm and a multiple point calibration curve was used for quantification of quinoline in the aqueous phase. The soil concentrations were calculated from the measured aqueous concentration using equation 9.1.

Results

The sobed concentration (C_s) versus the aqueous phase solute concentration (C_w) results were fitted nonlinearly with the Freundlich isotherm model. and gave the following result: Kd = 3.575 ± 0.169, n = 0.661 ± 0.029. The 95 % confidence intervals were for K_d [3.236;3.913] and for n [0.604;0.718]. It can be noted that linarisation of the

Freundlich isotherm gave less precise estimation of K_d (MSE = 223.13) than the non-linear procedure (MSE = 41.73).

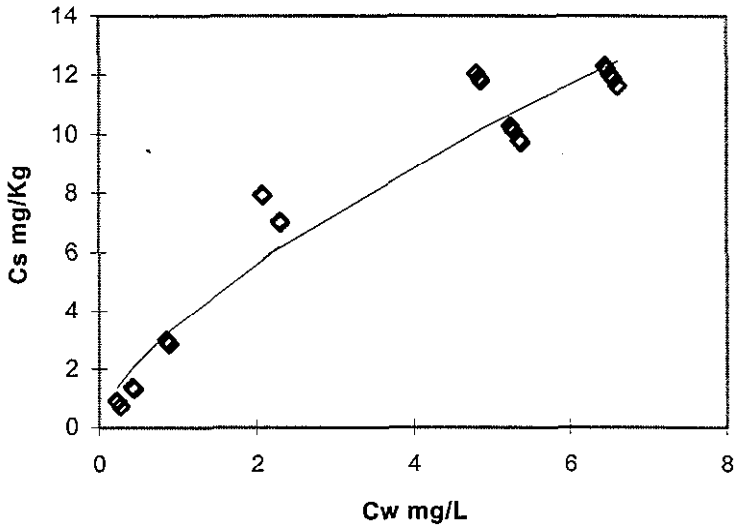


Fig 9.3. *Sorption experiment with quinoline. Each diamond corresponds to one determination of C_w. The line corresponds to the Freundlich isotherm model* $C_s = 3.575 \cdot C_w^{0.661}$

9.3.3 Acridine

Procedure

The sorption experiment with acridine was conducted the same way as the quinoline sorption experiment, but 249 nm was used for UV detection. The concentrations used were 18.735, 12.49, 6.245, 2.498, 1.249 and 0.6245 mg/L and two test flasks and one control flask was made with each concentration.

Results

The C_s/C_w results were fitted nonlinearly with the Freundlich isotherm model and gave the following result: $K_d = 877.356 \pm 174.051$, $n = 2.717 \pm 0.197$. The 95 % confidence intervals were for K_d [528.158;1226.553] and for n [2.322;3.112].

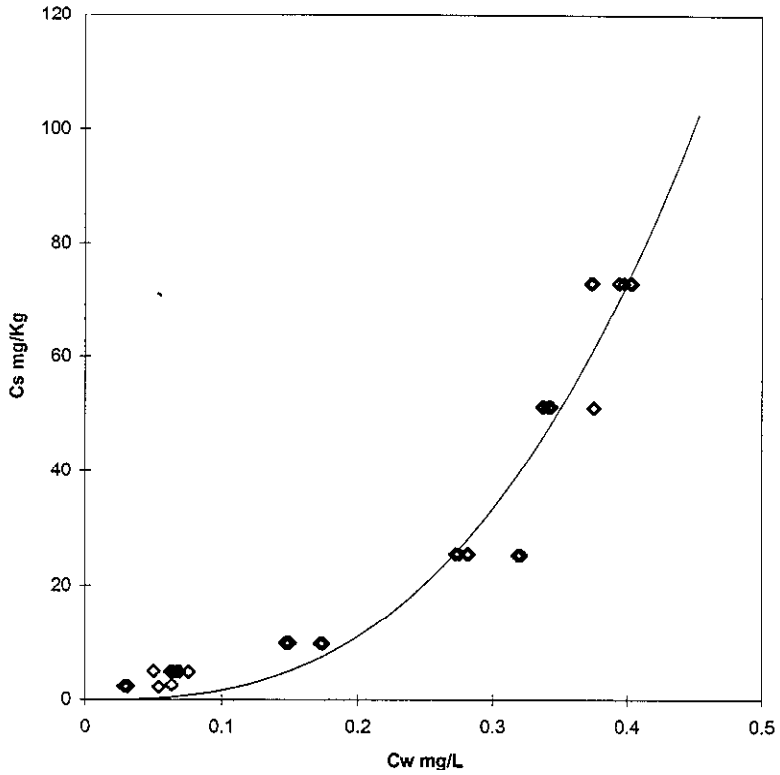


Fig 9.4. *Sorption experiment with acridine. Each diamond corresponds to one determination of C_w. The line corresponds to the Freundlich isotherm model* $C_s = 877.356 \cdot C_w^{2.717}$

9.3.4 **Benz[a]acridine**

Procedure

The sorption experiment with benz[a]acridine was conducted slightly different than the two other experiments because the resulting aqueous concentrations after equilibration was expected to be very low. To get a maximum amount of benz[a]acridine in the aqueous phase after equilibration, only 0.5 g of soil was used with each test flask. It was not possible to prepare these small amounts individually so instead approx. 10 g was made up according to the size fraction distribution and from this 0.5 g was added to each test flask. 20 mL of aqueous benz[a]acridine solution was added to each flask. Two test flasks and one control flask was made for each concentration. The concentrations used were 0.191, 0.230, 0.383, 0.459, 0.574 and 0.765 mg/L. The aqueous solubility of benz[a]acridine was estimated to be $5.6 \cdot 10^{-6}$ mol/L or 1.289 mg/L by extrapolation of a linear relationship between K_{ow} and S for quinoline and acridine. After the 48 hours of shaking, the aqueous phase was isolated by centrifugation and filtering, as described for the kinetic experiment, but followingly benz[a]acridine was extracted into an organic phase for quantification on GC-MS, as HPLC measurements were not sensitive enough.

The volume of the isolated aqueous phases were adjusted to 10 mL and 2 mL of 0.1 M NaOH, 5 M NaCl solution was added to increase ion strength and pH. Then 5 mL of Cyclohexane with an internal standard of acridine (0.5 mg/1000 mL) was added and the flasks were shaken for 30 min. The cyclohexane was isolated and the water phase left in

the flask for a second extraction. Another 5 mL of cyclohexane with internal standard was added to the waterphase and the flasks were again shaken for 30 min. The cyclohexane was isolated and added to the cyclohexane from the first extraction. The combined extract was evaporated to 1 mL under nitrogen and analysed on GC-MS. The loss of the full procedure, including sample handling, centrifugation, filtering and extraction, was approximately 10 % for benz[a]acridine according to the control flasks.

Sample analysis was performed on a Varian Saturn III GC/MS equipped with an ion trap mass spectrometer. The column temperature was held at 76 ^{0}C for 3.5 min., then ballistically increased to 280 ^{0}C with a rate of 20 ^{0}C/min and held at 280 ^{0}C for 5 min. Samples were injected in the temperature programmable mode with the injection port directly
connected to the column entrance. The injector was held at 76 ^{0}C for 1.5 min, then heated rapidly (200 ^{0}C/min) to 300 ^{0}C and held at 300 ^{0}C during the run. The gas chromatograph was equipped with a Rtx-1 column (Restek Inc.). The ion trap temperature was 250 ^{0}C.

Results

Unfortunately the results of the benz[a]acridine experiment are less convincing than the results obtained for the other two azaarenes. There is a large variation in the measured aqueous concentration for the test flask replications as can be seen in fig 9.5.

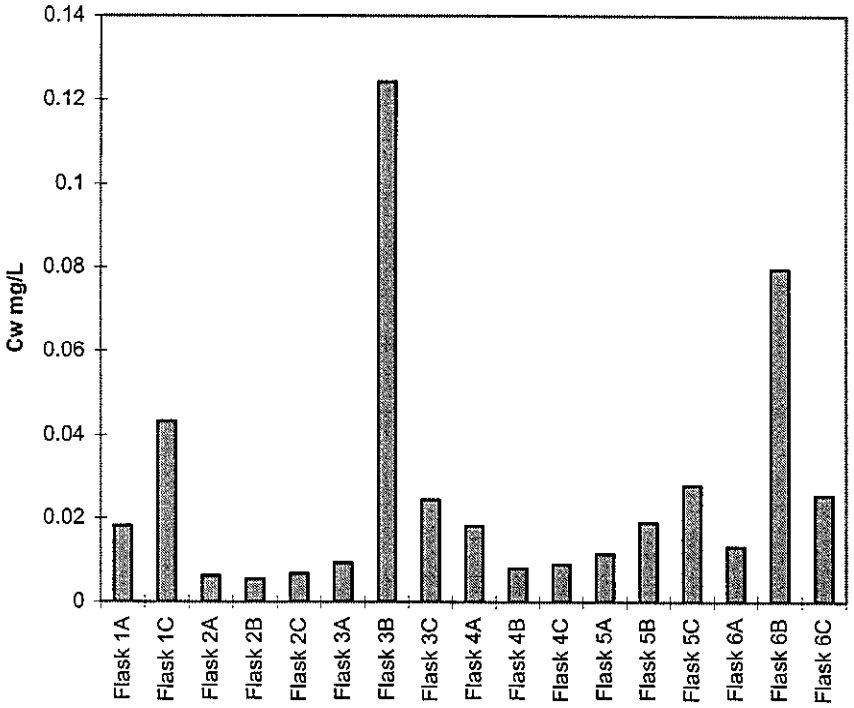


Fig 9.5. *The aqueous concentration of benz[a]acridine in the different test flasks. The start concentrations were 0.191, 0.230, 0.383, 0.459, 0.574 and 0.765 mg/L for flask 1-6 respectively.*

The fault seems not to be in the procedure as controls, that had undergone exactly the same procedure as test flasks, gave reasonable results. Biotic degradation seems unlikely as benz[a]acridine most likely is less degradable than the smaller azaarenes investigated. The only difference between this experiment and the two previous is that

the soil was not individually prepared for the benz[a]acridine experiment and this may be the cause for the scattering of the results.

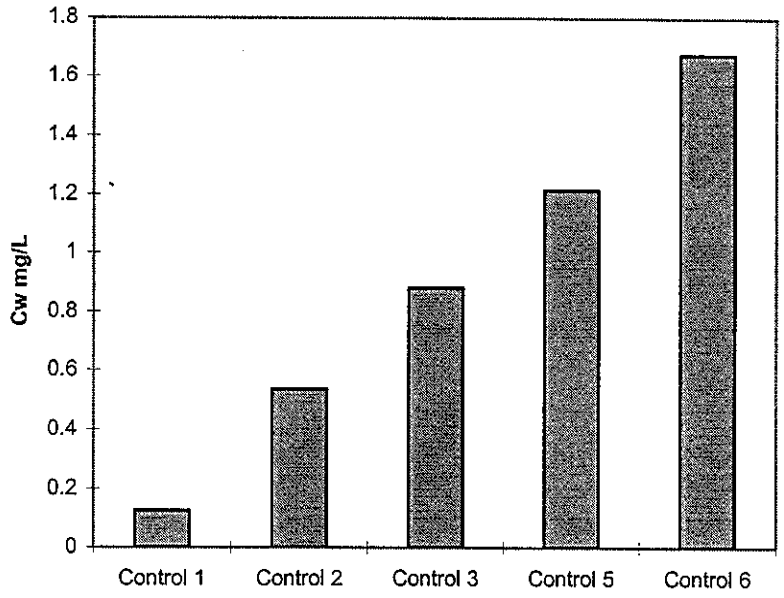


Fig 9.6. *Measured concentration of benz[a]acridine in control flasks at the end of the sorption experiment. Control flask 4 was lost.*

In spite of the scattered results an attempt to estimate K_d has been made. A non-linear datafit did not provide any useful information with these data as n was estimated to be very close to 0, which means that the sorbed concentration would be independent of the aqueous phase concentration which is unlikely. Instead K_d for n = 1, was calculated for all flasks which gave $K_d = 1448 \pm 642$ with a maximum of 2501 and a minimum of 382. Flask 1A, 1B and 3B were not included in these considerations as they deviate from the rest of the data. This result seems resonable when compared to K_d for acridine and quinoline.

9.4 Summary and discussion

The K_d results have been gathered in Table 9.2 below.

Compound	K_d	n
quinoline	3.58 ± 0.169	0.661 ± 0.029
acridine	877 ± 174	2.72 ± 0.179
benz[a]acridine	1450 ± 642	1 (set)

Table 9.2. *Determined Freundlich isotherm K_d and n values.*

As no previous measurements of K_d values for this soil and these solutes have been made, it is not possible to evaluate the results by comparison. However, the results are considered reliable, within the intervals given, because the compounds are relatively persistent and the experiment relatively short so that degradation is small. Slow sorption

may occur but this would only change K_d very little. The reversibility of the sorption was not investigated. As also could be noted from Fig 9.3 and 9.4, n is smaller than 1 for quinoline whereas it is larger for 1 for acridine. This could lead to the idea that different sorption mechanisms are responsible for the sorption of the two compounds in that sorption increases at higher concentrations for acridine whereas it decreases at higher concentrations for quinoline as competition limits sorption. However, the structures of the two compounds are quite similar and the explanation should rather be found in the different concentrations applied. The concentrations applied in the experiment was limited by the water solubility of the compounds and therefore the concentrations used for quinoline was much higher than for acridine and sufficient concentrations for competition to occur may have been reached. If carefully examined, the first part of the quinoline sorption isotherm actually resembles the shape of the acridine sorption isotherm, indicating that mechanisms well could be similar.

With a soil content of 2.2 % w/w organic matter and a organic material composition of 58 % C there is 0.58*0.022 = 0.0128 kg C/kg soil and the sorption coefficients would correspond to organic matter partition coefficients as given in Table 9.3

Compound	log K_{oc} (sorption)	n	log K_{oc} (sorption) n=1	log K_{oc} (Aldrich)
quinoline	2.61	0.661 ± 0.029	2.30	2.93
acridine	5.00	2.72 ± 0.179	4.31	3.99
benz[a]acridine	5.22	1 (set)	5.22	5.05

Table 9.3. *Calculated soil sorption coefficients and n-values. The K_{oc} values given in column three are calculated from the best linear fit to the experimental data. K_{oc} values for Alrich humic acid are taken from Tabe 8.1.*

The K_{oc} values calculated in the linear fashion can be compared with Aldrich humic acid K_{oc} values (from Table 8.1) and it can be seen that values are similar in size. This tells that the sorption of azaarenes to the examined soil well could be caused entirely by the organic material of the soil.

The results found showed that the larger molecules have higher sorption coefficients than the smaller molecules as could be expected thermodynamically.

10. Summary and conclusion

Both experimental and theoretical methods have been developed/tested and applied for determination of the environmentally important physical chemical properties, the octanol-water partition coefficient, the organic matter-water partition coefficient and the soil sorption coefficient for a range of azaarenes and polar substituted PAHs.

The HPLC method was found to be an accurate method for determination of log K_{ow} for polar compounds if calibration data sets of similar polar compounds was used for calibration. A Diol column proved to be advantageous over the traditionally applied ODS column for determination of log K_{ow} for polar compounds. For a group of compounds with different polarity a two system model applying both a ODS column and a Diol column gave the most accurate predictions. Within the eluent compositions tested, the largest amount of water in the eluent gave the most precise predictions for all types of compounds on both columns.

The theoretical group contribution methods, Rekker and Mannholds *f*-fragment and ClogP, was found not to be suitable for prediction of K_{ow} for azaarenes as none of these methods take shielding into account. Furthermore, the *f*-fragment method was found to lead to erroneous predictions if aromatic fragment values were used for aromatic compounds where peri hydrogens forces the substituents out of plane. In these cases better results are obtained by using the aliphatic fragment values. A simple specific model utilizing the parameters of size, nitrogen occurrence and shielding gave almost as accurate predictions of log K_{ow} for azaarenes as the HPLC method.

It was found that the effect of nitrogen on log K_{ow} was about 1.1-1.3 log K_{ow} units independent of molecular size. The effect of nitrogen on K_{ow} was found to be dependent on the steric structure of the azaarene as neighbouring benzene rings could reduce the nitrogen lonepair availability. For the substituted PAHs the hydrogen bonding substituents caused a decrease in K_{ow} when added to PAHs and it was found that the substituent effect was dependent on position in the PAH due to obstruction by peri hydrogens.

A chemically immobilized humic acid HPLC column was used for prediction of K_{oc} and retention parameters gave good correlations with literature values. K_{oc} Increased with molecular size and decreased with increasing hydrogen bonding ability of the solute. The azaarenes had log K_{oc}s 0.5 lower than the homologue PAHs but shielding of the nitrogen lonepairs by neighbouring benzene rings diminished the reducing effect of nitrogen on K_{oc}. The K_{oc}s of substituted PACs showed that specific interactions may occur between substituents and humic material.

When K_{oc} are compared to K_{ow} for polar PACs it is found that the effect of nitrogen is smaller on K_{oc} than on K_{ow} and that the effect of the polar substituents on K_{oc} is relatively smaller than on K_{ow} because of the polar accommodating nature of the humic acid. Consequently it was shown that different relationships exist between K_{oc} and K_{ow} for polar and nonpolar compounds.

11. Dansk sammenfatning

Både eksperimentelle og teoretiske metoder er blevet udviklet/testet og anvendt til bestemmelse af de miljømæssigt vigtige fysisk kemiske egenskaber, oktanol-vand fordelingskoefficienten, organisk stof-vand fordelingskoefficienten og jord sorptions koefficienten, for azaarener og polære substituerede PAHer.

HPLC metoden viste sig at være en præcis metode til bestemmelse af log K_{ow} for polære stoffer, hvis datasæt af lignende polære stoffer bliver anvendt til kalibrering. En Diol kolonne viste sig at give mere nøjagtige forudsigelser for polære stoffer end den traditionelle ODS kolonne. For grupper af stoffer med forskellig polaritet, var en to system model, hvor retentions resultaterne fra både Diol og ODS kolonnen anvendes samtidig, fordelagtig. Blandt de testede eluent kompositioner, gav det højeste vandindhold de mest nøjagtige forudsigelser for alle stofgrupper og på begge typer kolonne.

De teoretiske group contribution metoder, Rekker and Mannholds *f*-fragment metode og ClogP, blev fundet uegnede til forudsigelse af K_{ow} for azaarener idet ingen af disse metoder tager hensyn til eventuel shielding af nitrogen atomet. Ydermere blev det fundet at *f*-fragment metoden gav fejlagtige forudsigelser hvis aromatiske fragment værdier blev anvendt for substituerede aromatiske stoffer hvor perihydrogener tvinger substituenten ud af plan. I disse tilfælde kan mere nøjagtige forudsigelser opnås ved at anvende de alifatiske fragment værdier. En simpel specifik model der tog hensyn til størrelse, nitrogen tilstedeværelse og shielding gav næsten lige så præcise forudsigelser af K_{ow} for azaarener som HPLC metoden.

Det blev fundet at effekten af nitrogen på log K_{ow} er omkring 1.1-1.3 log K_{ow} enheder uafhængig af molekyle størrelsen. Effekten af nitrogen viste sig at være afhængig af den steriske struktur idet nabo benzen ringe kan skygge for nitrogens frie lone pair. For de substituerede PAHer medførte addition af hydrogen bindings dannende substituenter et fald i K_{ow} og effekten viste sig at være afhængig af placeringen på grund af peri hydrogener.

En kemisk immobiliseret humus syre HPLC kolonne blev anvendt til at forudsige K_{oc} og retentionsparametre gav en god korrelation med litteratur værdier. K_{oc} øgede med stigende molekyle størrelse og mindskedes med stigende hydrogen bindings evne. Azaarenerne havde log K_{oc} 0.5 lavere end de homologe PAHer, men shielding af nitrogens lonepair af nabo benzen ringe mindskede den K_{oc} reducerende effekt af nitrogen atomet. K_{oc} af polære substituerede PACer viste at specifikke interaktioner kan forekomme mellem substituenter og humussyrer.

Ved at sammenligne K_{ow} med K_{oc} blev det fundet at effekten af nitrogen er mindre på K_{oc} end på K_{ow} og at effekten af polære substituenter på K_{oc} er relativt mindre end effekten på K_{ow} på grund af den polære natur af humussyre. Det blev derfor vist at der eksisterer forskellige forhold mellem K_{oc} og K_{ow} for polære og upolære stoffer

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Appendix

Compound	Source	Purity
quinoline	Fluka chemische fabrik, CH-9470 Buchs SG	
isoquinoline	Aldrich, I-2,820-8	0.98
Acridine	Fluka chemische fabrik, CH-9470 Buchs SG	0.97
phenanthridine	Fluka chemische fabrik, CH-9470 Buchs SG	
4-azaflourene	Fluka chemische fabrik, CH-9470 Buchs SG	
benzo[f]quinoline	Aldrich, B1018-8, Gold label	0.99
benzo[h]quinoline	Aldrich	0.97
benz[a]acridine	Commision of the european communities, Community bureau of reference materials	
dibenz[a,c]acridine	Commision of the european communities, Community bureau of reference materials	
dibenz[a,h]acridine	Promochem GMBH, D46485 Wesel, Lot. W520 E	0.993
dibenz[a,h]acridine	Aldrich, 13797-9	
dibenz[a,j]acridine	Commision of the european communities, Community bureau of reference materials	
dibenz[a,j]acridine	Ferak, Berlin	
dibenz[c,h]acridine	Commision of the european communities, Community bureau of reference materials	
10-azabenzo[a]pyrene	Commision of the european communities, Community bureau of reference materials	
9H-Carbazol	BDH, Laboratory chemicals division, The British drug house.	0.98
flourene	Mikrolab AArhus	
Anthracene	Fluka chemische fabrik, CH-9470 Buchs SG	
phenanthrene	Commision of the european communities, Community bureau of reference materials	
benz[a]anthracene	Commision of the european communities, Community bureau of reference materials	
dibenz[a,c]anthracene	Commision of the european communities, Community bureau of reference materials	
dibenz[a,c]anthracene	Commision of the european communities, Community bureau of reference materials	
dibenz[a,h]anthracene	Dr. Ehrenstorfer GMBH, lot 40330	0.973
dibenz[a,j]anthracene	Commision of the european communities, Community bureau of reference materials	
Pyrene	Mikrolab AArhus	
benzo[a]pyrene	Commision of the european communities, Community bureau of reference materials	
dibenzofuran	Merk-schuchardt, Art. 820408	
dibenzothiophene	Aldrich	
anthraquinone	Aldrich	
9-anthrone	Ole Hammerich and Tove Thomsen, University of Copenhagen	
9-anthracenecarbonitrile	Ole Hammerich and Tove Thomsen, University of Copenhagen	
9-anthracenecarboxaldehyde	Ole Hammerich and Tove Thomsen, University of Copenhagen	
9-antracenecarboxylicacidmethylester	Ole Hammerich and Tove Thomsen, University of Copenhagen	
9-acetylanthracene	Aldrich, A1,260-7	0.95
9-methoxyanthracene	Ole Hammerich and Tove Thomsen, University of Copenhagen	
9-methylanthracene	Aldrich	0.99
9-nitroanthracene	Ole Hammerich and Tove Thomsen, University of Copenhagen	
9-bromoanthracene	Ole Hammerich and Tove Thomsen, University of Copenhagen	
9-chloroanthracene	Aldrich	0.75
9,10-dibromoanthracene	Aldrich	0.98
2-Hydroxyquinoline	Fluka chemische fabrik, CH-9470 Buchs SG	
quinoline-N-oxide-hydrate	Janssen Chimica, JAS 1613-37-2	
N-methylquinolinium iodid	Prepared at Risø	
1-bromopyrene	Prepared according to Vollman et al (1937)	
2-bromopyrene	Prepared according to Vollman et al (1937)	
4-bromopyrene	Prepared according to Vollman et al (1937)	
1,3-dibromopyrene	Prepared as described by Nielsen et al (1997)	
1,6-dibromopyrene	Prepared according to Vollman et al (1937)	
1,8-dibromopyrene	Prepared according to Vollman et al (1937)	
1-acetylpyrene	Prepared according to Vollman et al (1937)	
2-acetylpyrene	Prepared as described by helweg et al (1997 a)	
4-acetylpyrene	Prepared as described by helweg et al (1997 a)	
1-pyrenealdehyde	Prepared according to Vollman et al (1937)	
1-pyrenecarboxylicacidmetylester	Prepared according to Vollman et al (1937)	
2-pyrenecarboxylicacidmetylester	Prepared according to Vollman et al (1937)	
4-pyrenecarboxylicacidmetylester	Prepared from corresponding acid	

Table A1. *Sources of test compounds*

Compound	Water solubility	Method	Temp	Reference
quinoline	6000 10-6 g/mL			Zachara et al (1987)
isoquinoline	35000 10-6 mol/L		20	Pearlman et al (1984)
Acridine	300 10-6 mol/L		20	Pearlman et al (1984)
Acridine	38.4 10-6 g/mL			Zachara et al (1987)
benzo[f]quinoline	425 10-6 mol/L		25	Pearlman et al (1984)
Naphtalene	29.9 ppm	dialysis tubing		Etzweiler et al (1994)
Naphtalene	31.3 +/- 0.5 g/m3	generator column		Billington et al (1988)
Anthracene	0.0488 ppm	dialysis tubing	25	Etzweiler et al (1994)
Anthracene	0.420-0.450 10-6 mol/L		25	Pearlman et al (1984)
Anthracene	0.039 g/m3	generator column		Billington et al (1988)
phenanthrene	1.002 mg/kg	generator column	25	May et al (1978)
phenanthrene	1.08 +/- 0.06 g/m3	generator column		Billington et al (1988)
phenanthrene	1.03 ppm	dialysis tubing	25	Etzweiler et al (1994)
benz[a]anthracene	0.0094 +/- 0.0001 g/m3	generator column	25	May et al (1978)
dibenz[a,c]anthracene	0.0016 +/-0.0004 g/m3	generator column		Billington et al (1988)
dibenz[a,h]anthracene	0.0022 +/- 0.00002 g/m3	generator column		Billington et al (1988)
Pyrene	0.118 +/- 0.002 g/m3	generator column		Billington et al (1988)
Pyrene	0.132 +/- 0.001 mg/kg	generator column	25	May et al (1978)
benzo[a]pyrene	0.006-0.019 10-6 mol/L		25	Pearlman et al (1984)
benzo[a]pyrene	0.0016+/-0.0002 g/m3	generator column		Billington et al (1988)
9-nitroanthracene	0.114 mg/L	generator column	25	Gang and Xiaobai (1992)

Table A2. *Solubility of test compounds .*

Compound	Indirect/measured	Log Kow	uncertainty	temp	equil. method	Primary reference
quinoline	i	1.99				Southworth and Keller (1984)
quinoline	mes	2.03	0.10		Shake flask	Iwasa et al (1965)
quinoline		2.03				HSDB
quinoline	mes	2.10			Shake flask	de Voogt (1990)
isoquinoline		2.03				HSDB
isoquinoline		2.08				Hansch and Anderson (1967)
isoquinoline	mes	2.08	0.20		Shake flask	Sangster (1989)
Acridine	mes	3.29			Shake flask	Unger and Chiang (1983)
Acridine	mes	3.40				Hansch and Fujita (1964)
Acridine		3.40	0.25			Hansch and Fujita (1964)
Acridine	mes	3.45			Shake flask	de Voogt (1990)
phenanthridine	mes	3.47				Debnath and Hansch, Pomona College,unpublished analysis
benzo[f]quinoline	mes	3.40			Shake flask	De Voogt et al (1988)
benzo[h]quinoline	mes	3.60			Shake flask	De Voogt et al (1988)
benz[a]acridine	i	4.45				Southworth and Keller (1984)
dibenz[a,h]acridine	i	5.60				Southworth and Keller (1984)
dibenz[a,h]acridine	i	5.60				Southworth and Keller (1984)
9H-Carbazol	mes	3.29		25	Shake flask	Rogers and Cammarata (1969)
9H-Carbazol	mes	3.72				Nikaitani, and Hansch, Pomona College, unpublished analysis
Naphtalene	mes	3.01				Rogers and Cammarata (1969)
Naphtalene	mes	3.35		25	Several different	Sangster, J. [1989]
Naphtalene	mes	3.45				Leo et al (1971)
Naphtalene	mes	3.30				Kim and Hansch, Pomona College, unpublished analysis
Naphtalene	mes	3.37				Hansch and Fujita (1964)
Anthracene	mes	4.45				Hansch and Fujita (1964)
Anthracene	mes	4.50		25	Shake flask	Sangster (1989)
phenanthrene	mes	4.46				Hansch and Fujita (1964)
benz[a]anthracene	mes	5.70			Shake flask	Eadsforth (1986)
benz[a]anthracene	i	5.75		25		Sangster (1989)
benz[a]anthracene	mes	5.79				Wang et al (1986)
dibenz[a,c]anthracene	mes	6.17				Gould, and Hansch, Pomona College, unpublished analysis
dibenz[a,c]anthracene	i	7.19		25		Miller et al (1985)
dibenz[a,h]anthracene	mes	6.50		AMB	Shake flask	Means et al (1980)
dibenz[a,h]anthracene	mes/i	6.75			Different	Sangster (1989)
Pyrene	mes	4.88				Nikaitani, and Hansch, Pomona College, unpublished analysis
Pyrene		4.88				Rekker and Mannhold (1992)
benzo[a]pyrene		6.04				HSDB
benzo[a]pyrene	mes	5.97				Mallon and Harrison (1984)
dibenzofuran	mes	4.12				Leo et al (1971)
dibenzothiophene	mes	4.38				Means, J. Et al. EPA-600/3-80-041, NTIS
dibenzothiophene		4.38	0.40			Sangster (1989)
dibenzothiophene	mes	4.49			Shake flask	De Voogt (1990)
anthraquinon	mes	3.39				Pratesi et al (1979)
9-anthrone	mes	3.66				Debnath and Hansch, Pomona College unpublished results
9-anthracenecarbonitrile	mes	4.26				Debnath and Hansch, Pomona College unpublished results
9-methylanthracene	mes	5.07				Kenaga and Goring (1978)
9-methylanthracene	mes	5.07		25	Shake flask	Karickhoff et al (1979)
9-nitroanthracene	mes	4.76				Debnath and Hansch, Pomona college unpublished results

Table A3. *Literature log K_{ow} values for test compounds*

	Capacity coefficient				
Compound	**Diol-35**	**Diol-50**	**ODS-65**	**ODS-75**	**ODS-85**
quinoline	0.396	0.282	0.859	0.605	0.353
isoquinoline	0.407	0.210	0.904	0.543	0.307
acridine	0.891	0.484	2.847	1.271	0.621
benzo[f]quinoline	1.069	0.537	3.964	1.718	0.846
phenanthridine	1.014	0.432	2.816	1.584	0.699
9H-carbazole	0.856	0.559	4.024	1.895	0.681
benzo[h]quinoline	0.959	0.525	5.174	1.723	0.824
10-azabenzo[a]pyrene	5.854	2.042	24.508	7.808	3.419
dibenz[a,c]acridine	6.586	2.204	74.187	19.107	6.433
dibenz[c,h]acridine	9.191	2.620	154.716	33.619	12.449
2-hydroxyquinoline	0.196	0.168	0.419	0.212	0.167
4-azaflourene	0.698	0.426	1.409	1.038	0.389
benz[a]acridine	2.282	1.000	7.965	3.045	1.249
dibenz[a,j]acridine	5.554	1.195	24.118	8.299	2.525
dibenz[a,h]acridine	6.019	2.023	73.462	19.004	5.626
naphtalene	0.418	0.276	5.291	2.706	1.110
dibenzofuran	0.754	0.437	10.981	4.191	1.585
dibenzothiophene	1.191	0.719	15.932	6.709	2.157
phenanthrene	1.374	0.774	17.456	5.570	1.865
anthracene	1.377	0.736	18.498	6.646	2.423
pyrene	2.862		26.475		
benz[a]anthracene	4.431	1.677	60.326	14.951	4.352
benzo[a]pyrene	9.707	2.872	105.130	27.667	7.246
dibenz[a,c]anthracene	14.859	3.839	165.882	35.852	9.159
dibenz[a,h]anthracene	17.121	4.225	193.757	39.891	11.235
flourene	0.763	0.499	14.328	5.921	2.316
dibenz[a,j]anthracene	14.131	3.650	193.180	39.214	10.947
anthraquinone	0.448	0.346	4.210	1.963	1.087
9-anthrone	0.723	0.225	3.234	2.087	0.598
9-anthracenecarbonitrile	1.297	0.660	8.021	3.297	1.444
9-nitroanthracene	1.559	0.612	4.820	2.277	1.101
9-methylanthracene	1.808	0.934	31.820	11.527	3.937
9-anthracenecarboxylicacid			0.012	5.262	0.026
9-anthracenecarboxamide	0.556		0.763		
9-acetylanthracene	0.920		5.106		
9-anthracenecarboxylicacidmethylester	1.350		6.205		
9-anthracenecarboxaldehyde	0.802	0.325	7.090	2.863	1.292
9-methoxyanthracene	1.229	0.654	14.781	5.455	1.958
9-chloroanthracene	2.324	1.054	46.299	16.247	5.409
9-bromoanthracene	2.359	1.187	53.256	17.796	5.840
9,10-dibromanthracene	5.517		155.048		
4-acetylpyrene	1.809		9.219		
1-pyrenealdehyd	1.844		10.215		
2-acetylpyrene	1.876		10.839		
1-acetylpyrene	1.887		9.250		
2-pyrenecarboxylicacidmethylester	2.245		24.331		
1-pyrenecarboxylicacidmethylester	2.278		19.989		
4-pyrenecarboxylicacidmethylester	2.291		20.057		
2-bromopyrene	4.606		78.481		
1-bromopyrene	5.006		77.239		
4-bromopyrene	5.160		79.232		
1,6-dibromopyrene	8.603		237.414		
1,8-dibromopyrene	9.087		246.326		
1,3-dibromopyrene	9.623		293.238		
n-methyl quinolinium iodid	0.005		0.110		
Quinoline-n-oxide	0.262		0.190		

Table A4. *Measured capacity coefficients.*

	log Kow				
Compound	**Diol-35-1**	**Diol-50-1**	**ODS-65-1**	**ODS-75-1**	**ODS-85-1**
quinoline	2.229 ± 0.503	2.433 ± 0.609	2.208 ± 0.636	2.347 ± 0.661	2.479 ± 0.721
isoquinoline	2.263 ± 0.501	1.942 ± 0.629	2.251 ± 0.635	2.234 ± 0.664	2.316 ± 0.729
acridine	3.271 ± 0.477	3.332 ± 0.587	3.213 ± 0.620	3.129 ± 0.641	3.140 ± 0.696
benzo[f]quinoline	3.506 ± 0.475	3.505 ± 0.584	3.490 ± 0.617	3.446 ± 0.637	3.502 ± 0.689
phenanthridine	3.438 ± 0.475	3.145 ± 0.590	3.204 ± 0.620	3.360 ± 0.638	3.279 ± 0.693
9H-carbazole	3.220 ± 0.478	3.571 ± 0.584	3.503 ± 0.617	3.549 ± 0.636	3.249 ± 0.694
benzo[h]quinoline	3.366 ± 0.476	3.468 ± 0.585	3.714 ± 0.616	3.449 ± 0.637	3.471 ± 0.689
10-azabenzo[a]pyrene	5.694 ± 0.506	5.724 ± 0.622	5.017 ± 0.625	5.039 ± 0.648	5.139 ± 0.712
dibenz[a,c]acridine	5.846 ± 0.512	5.850 ± 0.628	5.946 ± 0.645	5.980 ± 0.679	5.880 ± 0.751
dibenz[c,h]acridine	6.275 ± 0.530	6.138 ± 0.642	6.562 ± 0.664	6.575 ± 0.707	6.653 ± 0.807
2-hydroxyquinoline	1.322 ± 0.541	1.575 ± 0.647	1.606 ± 0.652	1.247 ± 0.707	1.602 ± 0.774
4-azaflourene	2.958 ± 0.483	3.120 ± 0.590	2.624 ± 0.628	2.915 ± 0.645	2.591 ± 0.716
benz[a]acridine	4.482 ± 0.476	4.538 ± 0.587	4.075 ± 0.616	4.048 ± 0.635	3.959 ± 0.686
dibenz[a,j]acridine	5.627 ± 0.504	4.834 ± 0.593	5.004 ± 0.625	5.103 ± 0.650	4.784 ± 0.699
dibenz[a,h]acridine	5.730 ± 0.507	5.708 ± 0.622	5.937 ± 0.644	5.975 ± 0.679	5.723 ± 0.741
naphtalene	2.296 ± 0.500	2.398 ± 0.610	3.732 ± 0.616	3.924 ± 0.635	3.821 ± 0.686
dibenzofuran	3.056 ± 0.481	3.163 ± 0.589	4.344 ± 0.618	4.384 ± 0.637	4.239 ± 0.688
dibenzothiophene	3.645 ± 0.474	3.990 ± 0.582	4.656 ± 0.620	4.879 ± 0.645	4.599 ± 0.694
phenanthrene	3.829 ± 0.473	4.111 ± 0.583	4.733 ± 0.621	4.683 ± 0.641	4.429 ± 0.691
anthracene	3.832 ± 0.473	4.029 ± 0.583	4.782 ± 0.621	4.869 ± 0.645	4.736 ± 0.698
pyrene	4.774 ± 0.481	0.000 ± 0.000	5.082 ± 0.626	0.000 ± 0.000	0.000 ± 0.000
benz[a]anthracene	5.336 ± 0.494	5.397 ± 0.609	5.772 ± 0.640	5.722 ± 0.669	5.422 ± 0.725
benzo[a]pyrene	6.345 ± 0.533	6.291 ± 0.650	6.238 ± 0.653	6.370 ± 0.696	6.019 ± 0.760
dibenz[a,c]anthracene	6.893 ± 0.561	6.773 ± 0.677	6.620 ± 0.666	6.642 ± 0.710	6.294 ± 0.779
dibenz[a,h]anthracene	7.075 ± 0.571	6.932 ± 0.688	6.750 ± 0.671	6.755 ± 0.716	6.533 ± 0.798
flourene	3.073 ± 0.480	3.382 ± 0.586	4.567 ± 0.619	4.748 ± 0.642	4.682 ± 0.696
dibenz[a,j]anthracene	6.828 ± 0.558	6.689 ± 0.672	6.748 ± 0.670	6.737 ± 0.715	6.503 ± 0.795
anthraquinone	2.388 ± 0.497	2.775 ± 0.598	3.541 ± 0.617	3.586 ± 0.636	3.796 ± 0.686
9-anthrone	3.003 ± 0.482	2.061 ± 0.624	3.320 ± 0.619	3.651 ± 0.636	3.096 ± 0.698
9-anthracenecarbonitrile	3.755 ± 0.473	3.847 ± 0.582	4.081 ± 0.616	4.132 ± 0.635	4.129 ± 0.687
9-nitroanthracene	3.991 ± 0.473	3.722 ± 0.583	3.654 ± 0.617	3.742 ± 0.635	3.811 ± 0.686
9-methylanthracene	4.182 ± 0.474	4.425 ± 0.586	5.236 ± 0.628	5.449 ± 0.660	5.304 ± 0.719
9-anthracenecarboxylicacid			-1.365 ± 0.788	4.624 ± 0.640	-0.594 ± 0.979
9-anthracenecarboxamide	2.665 ± 0.489		2.110 ± 0.639		
9-acetylanthracene	3.312 ± 0.477		3.703 ± 0.616		
9-anthracenecarboxylicacidmethylester	3.807 ± 0.473		3.866 ± 0.616		
9-anthracenecarboxaldehyde	3.136 ± 0.479	2.672 ± 0.601	3.978 ± 0.616	3.983 ± 0.635	3.999 ± 0.686
9-methoxyanthracene	3.685 ± 0.473	3.832 ± 0.582	4.593 ± 0.619	4.661 ± 0.641	4.486 ± 0.692
9-chloroanthracene	4.506 ± 0.477	4.625 ± 0.589	5.551 ± 0.635	5.810 ± 0.672	5.677 ± 0.739
9-bromoanthracene	4.524 ± 0.477	4.823 ± 0.593	5.668 ± 0.638	5.905 ± 0.676	5.766 ± 0.744
9,10-dibromanthracene	5.618 ± 0.503		6.564 ± 0.664		
4-acetylpyrene	4.183 ± 0.474		4.198 ± 0.617		
1-pyrenealdehyd	4.207 ± 0.474		4.284 ± 0.617		
2-acetylpyrene	4.230 ± 0.474		4.333 ± 0.617		
1-acetylpyrene	4.237 ± 0.474		4.201 ± 0.617		
2-pyrenecarboxylicacidmethylester	4.461 ± 0.476		5.011 ± 0.625		
1-pyrenecarboxylicacidmethylester	4.480 ± 0.476		4.846 ± 0.622		
4-pyrenecarboxylicacidmethylester	4.487 ± 0.476		4.849 ± 0.622		
2-bromopyrene	5.386 ± 0.496		5.993 ± 0.646		
1-bromopyrene	5.493 ± 0.499		5.980 ± 0.646		
4-bromopyrene	5.532 ± 0.501		6.001 ± 0.646		
1,6-dibromopyrene	6.190 ± 0.526		6.921 ± 0.677		
1,8-dibromopyrene	6.260 ± 0.529		6.952 ± 0.678		
1,3-dibromopyrene	6.334 ± 0.533		7.098 ± 0.684		
n-methyl quinolinium iodid	-3.361 ± 0.875		0.489 ± 0.694		
Quinoline-n-oxide	1.699 ± 0.523		0.944 ± 0.675		

Table A5. *Predicted log Kow values with prediction intervals. Models calibrated with polar compounds*

	log Kow				
Compound	**Diol-35-2**	**Diol-50-2**	**ODS-65-2**	**ODS-75-2**	**ODS-85-2**
quinoline	3.527 ± 0.552	3.486 ± 0.531	1.917 ± 0.329	1.879 ± 0.495	2.089 ± 0.595
isoquinoline	3.548 ± 0.551	3.159 ± 0.555	1.960 ± 0.328	1.761 ± 0.497	1.909 ± 0.603
acridine	4.165 ± 0.529	4.084 ± 0.497	2.934 ± 0.321	2.694 ± 0.482	2.819 ± 0.569
benzo[f]quinoline	4.309 ± 0.525	4.200 ± 0.492	3.215 ± 0.320	3.025 ± 0.478	3.220 ± 0.557
phenanthridine	4.267 ± 0.526	3.960 ± 0.503	2.925 ± 0.321	2.935 ± 0.479	2.973 ± 0.564
9H-carbazole	4.133 ± 0.530	4.243 ± 0.490	3.228 ± 0.319	3.132 ± 0.476	2.939 ± 0.565
benzo[h]quinoline	4.223 ± 0.527	4.175 ± 0.493	3.441 ± 0.318	3.028 ± 0.478	3.186 ± 0.558
10-azabenzo[a]pyrene	5.648 ± 0.523	5.676 ± 0.485	4.761 ± 0.315	4.686 ± 0.466	5.028 ± 0.532
dibenz[a,c]acridine	5.741 ± 0.525	5.760 ± 0.488	5.702 ± 0.315	5.667 ± 0.467	5.846 ± 0.537
dibenz[c,h]acridine	6.003 ± 0.533	5.952 ± 0.496	6.326 ± 0.317	6.287 ± 0.470	6.701 ± 0.553
2-hydroxyquinoline	2.972 ± 0.582	2.915 ± 0.576	1.307 ± 0.335	0.732 ± 0.519	1.120 ± 0.639
4-azaflourene	3.973 ± 0.535	3.943 ± 0.504	2.337 ± 0.325	2.472 ± 0.485	2.213 ± 0.590
benz[a]acridine	4.906 ± 0.517	4.887 ± 0.476	3.807 ± 0.317	3.653 ± 0.471	3.724 ± 0.545
dibenz[a,j]acridine	5.606 ± 0.523	5.084 ± 0.475	4.748 ± 0.315	4.752 ± 0.466	4.636 ± 0.533
dibenz[a,h]acridine	5.670 ± 0.524	5.666 ± 0.485	5.693 ± 0.315	5.661 ± 0.467	5.673 ± 0.535
naphtalene	3.568 ± 0.550	3.463 ± 0.533	3.460 ± 0.318	3.523 ± 0.472	3.572 ± 0.548
dibenzofuran	4.033 ± 0.533	3.972 ± 0.502	4.080 ± 0.316	4.003 ± 0.469	4.033 ± 0.540
dibenzothiophene	4.394 ± 0.523	4.522 ± 0.481	4.396 ± 0.315	4.519 ± 0.466	4.431 ± 0.535
phenanthrene	4.507 ± 0.521	4.603 ± 0.480	4.473 ± 0.315	4.315 ± 0.467	4.244 ± 0.537
anthracene	4.508 ± 0.521	4.548 ± 0.481	4.523 ± 0.315	4.509 ± 0.466	4.582 ± 0.534
pyrene	5.084 ± 0.517	0.000 ± 0.000	4.827 ± 0.315	0.000 ± 0.000	0.000 ± 0.000
benz[a]anthracene	5.428 ± 0.520	5.459 ± 0.480	5.526 ± 0.315	5.398 ± 0.466	5.341 ± 0.533
benzo[a]pyrene	6.046 ± 0.534	6.053 ± 0.501	5.998 ± 0.316	6.073 ± 0.469	6.000 ± 0.539
dibenz[a,c]anthracene	6.381 ± 0.547	6.374 ± 0.518	6.385 ± 0.318	6.358 ± 0.471	6.304 ± 0.544
dibenz[a,h]anthracene	6.493 ± 0.552	6.480 ± 0.525	6.517 ± 0.318	6.475 ± 0.472	6.568 ± 0.550
flourene	4.043 ± 0.532	4.118 ± 0.495	4.306 ± 0.315	4.382 ± 0.467	4.524 ± 0.534
dibenz[a,j]anthracene	6.342 ± 0.545	6.318 ± 0.515	6.514 ± 0.318	6.456 ± 0.472	6.535 ± 0.549
anthraquinone	3.624 ± 0.548	3.713 ± 0.516	3.266 ± 0.319	3.171 ± 0.476	3.544 ± 0.549
9-anthrone	4.001 ± 0.534	3.238 ± 0.549	3.042 ± 0.320	3.238 ± 0.475	2.771 ± 0.570
9-anthracenecarbonitrile	4.461 ± 0.522	4.427 ± 0.484	3.813 ± 0.317	3.740 ± 0.471	3.912 ± 0.542
9-nitroanthracene	4.606 ± 0.520	4.344 ± 0.487	3.381 ± 0.319	3.334 ± 0.474	3.561 ± 0.548
9-methylanthracene	4.722 ± 0.518	4.812 ± 0.476	4.983 ± 0.315	5.113 ± 0.466	5.211 ± 0.532
9-anthracenecarboxylicacid			-1.703 ± 0.376	4.253 ± 0.467	-1.306 ± 0.783
9-anthracenecarboxamide	3.794 ± 0.541		1.817 ± 0.330		
9-acetylanthracene	4.190 ± 0.528		3.430 ± 0.318		
9-anthracenecarboxylicacidmethylester	4.493 ± 0.521		3.595 ± 0.318		
9-anthracenecarboxaldehyde	4.082 ± 0.531	3.645 ± 0.521	3.708 ± 0.317	3.585 ± 0.472	3.769 ± 0.544
9-methoxyanthracene	4.418 ± 0.523	4.417 ± 0.484	4.332 ± 0.315	4.292 ± 0.467	4.307 ± 0.536
9-chloroanthracene	4.920 ± 0.517	4.945 ± 0.475	5.301 ± 0.315	5.489 ± 0.466	5.622 ± 0.535
9-bromoanthracene	4.932 ± 0.517	5.076 ± 0.475	5.420 ± 0.315	5.589 ± 0.466	5.721 ± 0.536
9,10-dibromanthracene	5.601 ± 0.522		6.327 ± 0.317		
4-acetylpyrene	4.723 ± 0.518		3.931 ± 0.316		
1-pyrenealdehyd	4.738 ± 0.518		4.018 ± 0.316		
2-acetylpyrene	4.752 ± 0.518		4.069 ± 0.316		
1-acetylpyrene	4.756 ± 0.518		3.934 ± 0.316		
2-pyrenecarboxylicacidmethylester	4.893 ± 0.517		4.755 ± 0.315		
1-pyrenecarboxylicacidmethylester	4.904 ± 0.517		4.588 ± 0.315		
4-pyrenecarboxylicacidmethylester	4.909 ± 0.517		4.591 ± 0.315		
2-bromopyrene	5.459 ± 0.520		5.749 ± 0.316		
1-bromopyrene	5.525 ± 0.521		5.736 ± 0.315		
4-bromopyrene	5.549 ± 0.521		5.757 ± 0.316		
1,6-dibromopyrene	5.951 ± 0.531		6.689 ± 0.319		
1,8-dibromopyrene	5.994 ± 0.532		6.720 ± 0.319		
1,3-dibromopyrene	6.039 ± 0.534		6.868 ± 0.320		
n-methyl quinolinium iodid	0.106 ± 0.825		0.175 ± 0.348		
Quinoline-n-oxide	3.203 ± 0.569		0.636 ± 0.342		

Table A6. *Predicted log K_{ow} values with prediction intervals. Models calibrated with nonpolar compounds*

	log Kow							
Compound	Diol-35-3	Diol-50-3	ODS-65-3	ODS-75-3	ODS-95-3	Diol-35,ODS-65-3	Diol-50,ODS-65-3	Diol-35,ODS-85-3
quinoline	2.778 ± 0.977	2.826 ± 0.873	2.181 ± 0.486	2.302 ± 0.599	2.417 ± 0.574	2.265 ± 0.452	2.314 ± 0.463	2.453 ± 0.503
isoquinoline	2.805 ± 0.978	2.402 ± 0.892	2.222 ± 0.486	2.195 ± 0.601	2.253 ± 0.578	2.303 ± 0.451	2.222 ± 0.451	2.338 ± 0.516
acridine	3.637 ± 0.954	3.605 ± 0.849	3.142 ± 0.480	3.041 ± 0.591	3.079 ± 0.561	3.227 ± 0.434	3.245 ± 0.440	3.192 ± 0.495
benzo[f]quinoline	3.831 ± 0.951	3.755 ± 0.846	3.407 ± 0.479	3.341 ± 0.588	3.443 ± 0.556	3.462 ± 0.429	3.484 ± 0.433	3.520 ± 0.485
phenanthridine	3.775 ± 0.952	3.443 ± 0.853	3.133 ± 0.480	3.260 ± 0.589	3.219 ± 0.559	3.253 ± 0.439	3.192 ± 0.435	3.335 ± 0.493
9H-carbazole	3.594 ± 0.955	3.812 ± 0.845	3.419 ± 0.479	3.439 ± 0.587	3.189 ± 0.560	3.433 ± 0.425	3.509 ± 0.434	3.262 ± 0.489
benzo[h]quinoline	3.715 ± 0.953	3.723 ± 0.847	3.621 ± 0.478	3.344 ± 0.588	3.412 ± 0.557	3.620 ± 0.423	3.632 ± 0.427	3.464 ± 0.484
10-azabenzo[a]pyrene	5.638 ± 0.960	5.676 ± 0.858	4.868 ± 0.477	4.847 ± 0.585	5.086 ± 0.552	5.056 ± 0.447	5.110 ± 0.461	5.259 ± 0.496
dibenz[a,c]acridine	5.763 ± 0.964	5.765 ± 0.861	5.756 ± 0.480	5.738 ± 0.589	5.629 ± 0.561	5.792 ± 0.428	5.796 ± 0.433	5.853 ± 0.487
dibenz[c,h]acridine	6.117 ± 0.974	6.034 ± 0.870	6.345 ± 0.483	6.300 ± 0.595	6.605 ± 0.576	6.339 ± 0.437	6.302 ± 0.444	6.535 ± 0.507
2-hydroxyquinoline	2.028 ± 1.010	2.064 ± 0.908	1.606 ± 0.491	1.261 ± 0.617	1.536 ± 0.597	1.631 ± 0.464	1.676 ± 0.471	1.583 ± 0.529
4-azafluorene	3.379 ± 0.959	3.421 ± 0.854	2.578 ± 0.483	2.839 ± 0.593	2.529 ± 0.571	2.722 ± 0.453	2.777 ± 0.467	2.706 ± 0.517
benz[a]acridine	4.637 ± 0.948	4.649 ± 0.840	3.967 ± 0.477	3.911 ± 0.585	3.902 ± 0.552	4.116 ± 0.436	4.152 ± 0.446	4.089 ± 0.498
dibenz[a,j]acridine	5.582 ± 0.959	4.905 ± 0.842	4.855 ± 0.477	4.908 ± 0.585	4.729 ± 0.551	5.042 ± 0.444	4.880 ± 0.424	4.976 ± 0.511
dibenz[a,h]acridine	5.667 ± 0.961	5.662 ± 0.856	5.746 ± 0.480	5.733 ± 0.589	5.671 ± 0.556	5.762 ± 0.427	5.755 ± 0.432	5.708 ± 0.485
naphtalene	2.832 ± 0.975	2.797 ± 0.874	3.639 ± 0.478	3.793 ± 0.586	3.783 ± 0.553	3.419 ± 0.456	3.379 ± 0.470	3.481 ± 0.524
dibenzofuran	3.459 ± 0.957	3.459 ± 0.853	4.224 ± 0.477	4.226 ± 0.584	4.182 ± 0.551	4.030 ± 0.445	4.001 ± 0.456	3.971 ± 0.502
dibenzothiophene	3.946 ± 0.949	4.175 ± 0.841	4.522 ± 0.477	4.697 ± 0.584	4.544 ± 0.550	4.382 ± 0.432	4.426 ± 0.430	4.379 ± 0.491
phenanthrene	4.098 ± 0.948	4.260 ± 0.840	4.598 ± 0.477	4.511 ± 0.584	4.373 ± 0.550	4.477 ± 0.429	4.510 ± 0.428	4.293 ± 0.479
anthracene	4.100 ± 0.948	4.209 ± 0.841	4.642 ± 0.477	4.687 ± 0.584	4.681 ± 0.551	4.514 ± 0.430	4.524 ± 0.433	4.525 ± 0.490
pyrene	4.877 ± 0.948	0.000 ± 0.000	4.930 ± 0.477	0.000 ± 0.000	0.000 ± 0.000	4.929 ± 0.419	0.000 ± 0.000	0.000 ± 0.000
benz[a]anthracene	5.342 ± 0.954	5.392 ± 0.851	5.590 ± 0.479	5.494 ± 0.587	5.370 ± 0.555	5.559 ± 0.428	5.562 ± 0.431	5.390 ± 0.481
benzo[a]pyrene	6.175 ± 0.976	6.166 ± 0.875	6.035 ± 0.481	6.106 ± 0.593	5.969 ± 0.563	6.111 ± 0.436	6.112 ± 0.440	6.073 ± 0.496
dibenz[a,c]anthracene	6.627 ± 0.995	6.583 ± 0.894	6.401 ± 0.483	6.364 ± 0.596	6.245 ± 0.568	6.508 ± 0.448	6.501 ± 0.450	6.407 ± 0.510
dibenz[a,h]anthracene	6.778 ± 1.001	6.721 ± 0.901	6.525 ± 0.484	6.470 ± 0.597	6.485 ± 0.573	6.642 ± 0.451	6.632 ± 0.453	6.629 ± 0.513
fluorene	3.473 ± 0.957	3.648 ± 0.848	4.437 ± 0.477	4.572 ± 0.584	4.628 ± 0.550	4.200 ± 0.458	4.212 ± 0.456	4.310 ± 0.533
dibenz[a,j]anthracene	6.574 ± 0.992	6.510 ± 0.890	6.523 ± 0.484	6.453 ± 0.597	6.454 ± 0.572	6.590 ± 0.444	6.570 ± 0.448	6.549 ± 0.505
anthraquinone	2.907 ± 0.973	3.123 ± 0.862	3.456 ± 0.479	3.474 ± 0.587	3.738 ± 0.553	3.294 ± 0.443	3.338 ± 0.438	3.483 ± 0.516
9-anthrone	3.416 ± 0.958	2.505 ± 0.887	3.244 ± 0.480	3.535 ± 0.587	3.036 ± 0.562	3.252 ± 0.427	3.005 ± 0.466	3.037 ± 0.491
9-anthracenecarbonitrile	4.037 ± 0.948	4.051 ± 0.842	3.972 ± 0.477	3.990 ± 0.585	4.072 ± 0.551	3.974 ± 0.420	3.985 ± 0.424	4.050 ± 0.477
9-nitroanthracene	4.231 ± 0.947	3.943 ± 0.843	3.564 ± 0.479	3.621 ± 0.586	3.753 ± 0.553	3.702 ± 0.437	3.653 ± 0.433	3.864 ± 0.488
9-methylanthracene	4.389 ± 0.946	4.551 ± 0.840	5.077 ± 0.478	5.235 ± 0.586	5.252 ± 0.554	4.925 ± 0.437	4.943 ± 0.437	5.035 ± 0.507
9-anthracenecarboxylicacid			-1.236 ± 0.532	4.455 ± 0.584	-0.667 ± 0.684			
9-anthracenecarboxamide	3.138 ± 0.966		2.087 ± 0.487			2.279 ± 0.474		
9-acetylanthracene	3.671 ± 0.953		3.610 ± 0.478			3.601 ± 0.423		
9-anthracenecarboxylicacidmethylester	4.079 ± 0.948		3.787 ± 0.478			3.823 ± 0.424		
9-anthracenecarboxaldehyde	3.525 ± 0.956	3.034 ± 0.865	3.873 ± 0.478	3.849 ± 0.585	3.942 ± 0.552	3.772 ± 0.428	3.620 ± 0.456	3.809 ± 0.488
9-methoxyanthracene	3.979 ± 0.949	4.038 ± 0.842	4.462 ± 0.477	4.491 ± 0.584	4.430 ± 0.550	4.343 ± 0.429	4.342 ± 0.433	4.303 ± 0.485
9-chloroanthracene	4.656 ± 0.946	4.725 ± 0.841	5.378 ± 0.478	5.577 ± 0.588	5.625 ± 0.558	5.225 ± 0.439	5.214 ± 0.446	5.390 ± 0.518
9-bromoanthracene	4.672 ± 0.946	4.695 ± 0.842	5.490 ± 0.479	5.667 ± 0.589	5.715 ± 0.559	5.317 ± 0.445	5.346 ± 0.443	5.462 ± 0.522
9,10-dibromanthracene	5.575 ± 0.959		6.346 ± 0.483			6.208 ± 0.451		
4-acetylpyrene	4.390 ± 0.946		4.084 ± 0.477			4.146 ± 0.422		
1-pyrenealdehyd	4.410 ± 0.946		4.166 ± 0.477			4.217 ± 0.421		
2-acetylpyrene	4.429 ± 0.946		4.214 ± 0.477			4.259 ± 0.420		
1-acetylpyrene	4.435 ± 0.946		4.087 ± 0.477			4.161 ± 0.423		
2-pyrenecarboxylicacidmethylester	4.619 ± 0.946		4.862 ± 0.477			4.813 ± 0.421		
1-pyrenecarboxylicacidmethylester	4.635 ± 0.946		4.704 ± 0.477			4.693 ± 0.419		
4-pyrenecarboxylicacidmethylester	4.641 ± 0.946		4.707 ± 0.477			4.697 ± 0.419		
2-bromopyrene	5.383 ± 0.955		5.601 ± 0.480			5.734 ± 0.431		
1-bromopyrene	5.471 ± 0.956		5.788 ± 0.480			5.746 ± 0.429		
4-bromopyrene	5.504 ± 0.957		5.808 ± 0.480			5.769 ± 0.429		
1,6-dibromopyrene	6.047 ± 0.972		6.688 ± 0.485			6.591 ± 0.451		
1,8-dibromopyrene	6.105 ± 0.974		6.717 ± 0.486			6.628 ± 0.451		
1,3-dibromopyrene	6.186 ± 0.976		6.857 ± 0.487			6.752 ± 0.456		
n-methyl quinolinium iodid	-1.840 ± 1.311		0.537 ± 0.504			-0.151 ± 0.734		
Quinoline-n-oxide	2.338 ± 0.995		0.972 ± 0.498			1.211 ± 0.518		

Table A7. *Predicted log K_{ow} values with prediction intervals. Models calibrated with both polar and nonpolar compounds*

Models based only on data from polar compounds

Explanation

for the equation
Y=m1 x1 + m2 x2 + b

m2	m1	b
stdev m1	stdev m2	stdev b
r2	stdev y	
F	Degrees of freedom	
regression SS	residual SS	

Diol-35-1

2.963	3.420
0.131	0.066
0.985	0.196
513.466	8
19.628	0.306
	0.038

X mean	0.166
Sx	1.656
SSx	2.510
Sxx	2.259
alpha	0.050
n	10
t,alpha/2, n-2=	2.306

ODS-65-1

1.930	2.336
0.112	0.122
0.974	0.255
299.113	8
19.415	0.519
	0.065

X mean	0.816
Sx	8.160
SSx	11.870
Sxx	10.683
alpha	0.050
n	10
t,alpha/2, n-2=	2.306

ODS-75-1

2.423	2.877
0.144	0.103
0.972	0.263
281.203	8
19.383	0.551
	0.069

X mean	0.427
Sx	4.269
SSx	5.125
Sxx	4.613
alpha	0.050
n	10
t,alpha/2, n-2=	2.306

Diol-50,ODS-65-1

0.919	2.050	3.497
0.390	0.772	0.447
0.987	0.192	#N/A
266.195	7	#N/A
19.675	0.259	#N/A
	0.037	

Diol-35,ODS-85-1

0.564	2.358	3.476
0.721	0.785	0.098
0.986	0.200	#N/A
244.602	7	#N/A
19.653	0.281	#N/A
	0.040	

Diol-35, ODS-65-1

0.723	1.882	3.009
0.360	0.549	0.212
0.990	0.166	#N/A
356.360	7	#N/A
19.740	0.194	#N/A
	0.028	

$$\left[\hat{y}_0 \pm t_{\alpha/2,n-2}\sqrt{MS_E\left(\frac{1}{n}+\frac{1}{k}+\frac{\left(x_0-\bar{x}\right)^2}{S_{xx}}\right)}\right]$$

Prediction interval

Table A8. *Statistics for the HPLC models.*

Models based only on data from nonpolar compounds

Diol-50-1

3.826	4.538	1.813	4.256
0.209	0.083	0.130	0.085
0.977	0.241	0.956	0.219
335.851	8	195.136	9
19.470	0.464	9.335	0.431
	0.058	0.000	0.048

X mean	-0.164	X mean	0.419
Sx	-1.639	Sx	4.604
SSx	1.599	SSx	4.765
Sxx	1.439	Sxx	4.332
alpha	0.050	alpha	0.050
n	10	n	11
t,alpha/2, n-2=	2.306	t,alpha/2, n-2=	2.262

Diol-50-2

2.546	4.887
0.163	0.063
0.968	0.196
244.520	8
9.436	0.309
	0.039

X mean	0.056
Sx	0.557
SSx	1.487
Sxx	1.338
alpha	0.050
n	10
t,alpha/2, n-2=	2.306

ODS-85-1

2.698	3.699	2.526	2.431
0.174	0.091	0.158	0.174
0.968	0.284	0.970	0.192
239.784	8	255.036	8
19.291	0.644	9.449	0.296
	0.080		0.037

X mean	0.079	X mean	1.028
Sx	0.787	Sx	10.284
SSx	2.712	SSx	12.056
Sxx	2.441	Sxx	10.851
alpha	0.050	alpha	0.050
n	10	n	10
t,alpha/2, n-2=	2.306	t,alpha/2, n-2=	2.306

ODS-75-2

ODS-85-2

2.981	3.437
0.214	0.134
0.960	0.220
193.383	8
9.358	0.387
	0.048

X mean	0.534
Sx	5.341
SSx	3.905
Sxx	3.515
alpha	0.050
n	10
t,alpha/2, n-2=	2.306

Diol-50,ODS-65-2

2.764	-1.063	0.862
0.966	1.266	1.407
0.985	0.143	#N/A
236.269	7	#N/A
9.603	0.142	#N/A
	0.020	

Diol-35,ODS-85-2

1.492	0.923	3.849
1.079	0.657	0.319
0.969	0.208	#N/A
109.451	7	#N/A
9.443	0.302	#N/A
	0.043	

Diol-35, ODS-65-2

2.617	-0.629	1.302
0.645	0.607	0.730
0.986	0.133	#N/A
273.440	8	#N/A
9.624	0.141	#N/A
	0.018	

Models based on data from both polar and nonpolar compounds

ODS-65-2

1.955	2.046
0.084	0.134
0.984	0.133
541.369	9
9.605	0.160
0.000	0.018

X mean	1.519
Sx	16.710
SSx	27.899
Sxx	25.363
alpha	0.050
n	11
t,alpha/2, n-2=	2.262

Diol-35-3

2.447	3.760
0.190	0.112
0.897	0.442
166.061	19
32.382	3.705
	0.195

X mean	0.298
Sx	6.260
SSx	7.275
Sxx	6.928
alpha	0.050
n	21
t,alpha/2, n-2=	2.093

Diol-50-3

3.311	4.649
0.224	0.088
0.924	0.390
217.968	18
33.186	2.741
	0.152

X mean	-0.054
Sx	-1.082
SSx	3.086
Sxx	2.932
alpha	0.050
n	20
t,alpha/2, n-2=	2.101

ODS-65-2 Without pyrene

1.957	2.037
0.088	0.142
0.984	0.140
489.929	8
9.589	0.157
	0.020

ODS-75-3

2.291	2.803
0.106	0.098
0.963	0.271
470.142	18
34.602	1.325
	0.074

ODS-85-3

2.708	3.640
0.117	0.068
0.967	0.256
531.841	18
34.751	1.176
	0.065

Diol-35-2 Without pyrene

1.817	4.275
0.130	0.088
0.961	0.219
194.730	8
9.361	0.385
	0.048

X mean	0.728
Sx	14.553
SSx	17.182
Sxx	16.323
alpha	0.050
n	20
t,alpha/2, n-2=	2.101

X mean	0.306
Sx	6.127
SSx	6.617
Sxx	6.286
alpha	0.050
n	20
t,alpha/2, n-2=	2.101

Diol-35, ODS-65-3

1.444	0.598	2.600
0.160	0.221	0.137
0.981	0.193	#N/A
476.498	18	#N/A
35.418	0.669	#N/A
	0.037	

Table A8

ODS-65-3

1.846	2.303
0.069	0.095
0.974	0.223
708.893	19
35.145	0.942
	0.050

X mean	1.184
Sx	24.870
SSx	39.769
Sxx	37.876
alpha	0.050
n	21
t,alpha/2, n-2=	2.093

Diol-35-3 Without pyrene

2.447	3.760
0.196	0.116
0.897	0.454
156.542	18.000
32.222	3.705
	0.206

Diol-50,ODS-65-3

1.361	0.949
0.182	0.335
0.982	0.194
470.393	17
35.289	0.638
0.000	0.038

ODS-65-3 Without pyrene

1.847	2.304
0.071	0.098
0.974	0.228
670.412	18.000
34.987	0.939
	0.052

Compound	Wiener	1st CI	2nd CI	NI	SI
quinoline	218	4.966326	3.564696	1	0
isoquinoline	218	4.966326	3.564696	1	0
2-hydroxyquinoline	288	5.360173	4.198463	1	0
4-azaflourene	438	6.44949	4.935657	1	0
9H-carbazole	438	6.44949	4.935657	0	0
acridine	558	6.932653	5.320558	1	0
benzo[f]quinoline	542	6.94949	5.212427	1	1
benzo[h]quinoline	542	6.94949	5.212427	1	0
phenanthridine	542	6.94949	5.212427	1	0
benz[a]acridine	1106	8.915816	7.033167	1	0
10-azabenzo[a]pyrene	1360	9.915816	7.877566	1	0
dibenz[a,c]acridine	1814	10.91582	8.586858	1	1
dibenz[a,h]acridine	1942	10.89898	8.702524	1	1
dibenz[a,j]acridine	1942	10.89898	8.702524	1	0
dibenz[c,h]acridine	1942	10.89898	8.702524	1	2
Naphtalene	218	4.966326	3.564696	0	0
flourene	438	6.44949	4.935657	0	0
phenanthrene	542	6.94949	5.212427	0	0
anthracene	558	6.932653	5.320558	0	0
pyrene	724	7.9326	6.153052	0	0
benz[a]anthracene	1106	8.915816	7.033167	0	0
benzo[a]pyrene	1360	9.915816	7.877566	0	0
dibenz[a,c]anthracene	1814	10.91582	8.586858	0	0
dibenz[a,h]anthracene	1942	10.89898	8.702524	0	0
dibenz[a,j]anthracene	1942	10.89898	8.702524	0	0
anthraquinone	756	7.787694	6.225739	0	0
9-anthracenecarbonitrile	776	7.898178	6.00776	0	0
9-anthracenecarboxaldel	776	7.898178	6.00776	0	0
9-bromoanthracene	652	7.360173	5.794774	0	0
9-chloroanthracene	652	7.360173	5.794774	0	0
9-cyanoanthracene	776	7.898178	6.00776	0	0
9-hydroxyanthracene	652	7.360173	5.794774	0	0
9-methoxyanthracene	776	7.898178	6.00776	0	0
9-methylanthracene	652	7.360173	5.794774	0	0
9-nitroanthracene	904	8.270857	6.757025	0	0
dibenzofuran	438	6.44949	4.935657	0	0
dibenzothiophene	438	6.44949	4.935657	0	0

Table A9. *Connectivity indices calculated by computer program and other descriptive indices used in models.*

Compound	Parent	log Kow Parent	Heterocyclic	Ring condensation	Phenyl ring	f(C)	f(H)	f(N)	f(O)	f(S)	log Kow
isoquinoline		0	0	2	1	9	7	1	0	0	2.096
quinoline		0	0	2	1	9	7	1	0	0	2.096
4-azaflourene	flourene	4.251	0	0	0	0	-1	1	0	0	3.068
benzo[f]quinoline	quinoline	2.03	0	1	1	4	2	0	0	0	3.316
benzo[h]quinoline	quinoline	2.03	0	1	1	4	2	0	0	0	3.316
phenanthridine	phenanthrene	4.53	0	0	0	0	-1	1	0	0	3.347
acridine	quinoline	2.03	0	2	3	13	9	1	0	0	3.316
9H-carbazole		0	2	2	3	12	9	1	0	0	3.71
benz[a]acridine	acridine	3.4	0	1	1	4	2	0	0	0	4.686
10-azabenzo[a]pyrene	pyren	4.88	0	1	1	4	1	1	0	0	4.983
dibenz[a,c]acridine	acridine	3.4	0	2	2	8	4	0	0	0	5.972
dibenz[a,h]acridine	acridine	3.4	0	2	2	8	4	0	0	0	5.972
dibenz[a,j]acridine	acridine	3.4	0	2	2	8	4	0	0	0	5.972
dibenz[c,h]acridine	acridine	3.4	0	2	2	8	4	0	0	0	5.972
naphtalene		0	0	1	2	10	8	0	0	0	3.389
flourene		0	0	3	2	12	9	0	0	0	4.251
anthracene	naphthalene	3.38	0	2	3	14	10	0	0	0	4.666
phenanthrene	naphthalene	3.38	0	2	3	14	10	0	0	0	4.666
pyrene		0	0	1	4	16	10	0	0	0	4.895
benz[a]anthracene	anthracene	4.63	0	1	1	4	2	0	0	0	5.916
benzo[a]pyrene	pyrene	4.88	0	1	1	4	2	0	0	0	6.166
dibenz[a,c]anthracene	anthracene	4.63	0	2	2	8	4	0	0	0	7.202
dibenz[a,h]anthracene	anthracene	4.63	0	2	2	8	4	0	0	0	7.202
dibenz[a,j]anthracene	anthracene	4.63	0	2	2	8	4	0	0	0	7.202
dibenzofurane		0	2	2	3	12	8	0	1	0	4.035
dibenzothiophene		0	2	3	2	12	8	0	0	1	4.584

	parent	heterocyclic	ring condensation	phenyl ring	f(C)	f(H)	f(N)	f(O)	f(S)
f-fragments	1	0.219	0.219	0.219	0.11	0.204	-0.98	-0.45	0.099

Table A10. *Scheme used in calculation of log K_{ow} with Rekker and Mannholds f-fragment method*

Nonpolar	
MW, nonpolar	
0.020305	0.830203
0.0014	0.29707
0.963365	0.211249
210.3712	8
9.388076	0.35701
	0.044626

Polar	
MW, polar	
0.026276	-1.22185
0.00152	0.307754
0.973911	0.254965
298.6467	8
19.41414	0.520056
	0.065007

Pooled	
MW	
0.024544	-0.46495
0.002293	0.475417
0.864259	0.520509
114.6054	18
31.04994	4.876726
	0.270929

Nonpolar	
WI, nonpolar	
0.001608	3.570409
0.000195	0.209557
0.895128	0.357418
68.28371	8
8.723103	1.021984
	0.127748

Polar	
WI, polar	
0.002246	2.075085
0.000203	0.207021
0.938596	0.391157
122.2856	8
18.71017	1.224031
	0.153004

Pooled	
WI	
0.002001	2.744361
0.00024	0.251842
0.79411	0.641047
69.42543	18
28.52974	7.396933
	0.410941

Nonpolar	
1st CI, nonpolar	
0.494885	1.081693
0.037164	0.305143
0.956831	0.229316
177.3183	8
9.324401	0.420685
	0.052586

Polar	
1st CI, polar	
0.663231	-1.12271
0.038125	0.300229
0.974246	0.253324
302.6313	8
19.42081	0.513385
	0.064173

Pooled	
1st CI	
0.606028	-0.24665
0.060437	0.486184
0.848166	0.5505
100.5501	18
30.47176	5.454907
	0.30305

Nonpolar	
2nd CI, nonpolar	
0.585588	1.400071
0.037349	0.23958
0.968482	0.195941
245.8261	8
9.437944	0.307142
	0.038393

Polar	
2nd CI, polar	
0.775065	-0.59929
0.039449	0.24032
0.979696	0.224927
386.0169	8
19.52946	0.404738
	0.050592

Pooled	
2nd CI	
0.714852	0.175092
0.066843	0.418124
0.864022	0.520963
114.3739	18
31.04142	4.885253
	0.271403

Polar		
MW and SI, polar		
0.049011	0.025865	-1.16115
0.174189	0.002179	0.391898
0.974203	0.27104	
132.1752	7	
19.41996	0.514241	
	0.073463	

Polar		
1st CI and SI, polar		
0.650701	0.059446	-1.0514
0.054285	0.171959	0.379261
0.974678	0.268532	
134.7214	7	
19.42943	0.504768	
	0.07211	

Explanation

m2	m1	b
stdev m1	stdev m2	stdev b
r2	stdev y	
F	Degrees of freedom	
regression SS	residual SS	

Polar		
2nd CI and SI		
0.650701	0.059446	-1.0514
0.054285	0.171959	0.379261
0.974678	0.268532	
134.7214	7	
19.42943	0.504768	
	0.07211	

Table A11. *Statistics for simple specific models*

Pooled
MW and NI

0.023604	-0.84814	0.148097
0.001238	0.125676	0.27074
0.963105	0.279235	
221.8806	17	
34.60114	1.325531	
	0.077972	

Pooled
MW, N-index and SI

0.226456	0.022555	-0.95072	0.365136
0.147816	0.001374	0.138267	0.296629
0.967824	0.268789		
160.4237	16		
34.77071	1.155962		
	0.072248		

Pooled
WI og NI

-0.94418	0.001942	3.267531
0.187281	0.000157	0.194122
0.917483	0.417596	
94.50876	17	
32.9621	2.96457	
	0.174386	

Pooled
1st CI, SI and NI

-1.00725	0.555848	0.260054	0.595486
0.144798	0.035864	0.155036	0.299763
0.964174	0.283627		
143.5345	16		
34.63956	1.287108		
MSE	0.080444		

Pooled
1st CI og NI

-0.89187	0.58528	0.360755
0.134039	0.032905	0.278877
0.957874	0.298373	
193.2758	17	
34.41322	1.513446	
	0.089026	

Pooled
2nd CI, SI and NI

0.256644	0.654302	-0.97352	0.974291
0.138303	0.037442	0.129806	0.245441
0.971438	0.253246		
181.3948	16		
34.90053	1.026138		
	0.064134		

Pooled
2nd order og NI

-0.85807	0.688196	0.764268
0.121838	0.034955	0.232914
0.965291	0.270835	
236.3927	17	
34.67969	1.246981	
	0.073352	

Pooled
Number of C and NI

-0.66519	0.28288	0.530804
0.146172	0.017184	0.291469
0.951237	0.321016	
165.814	17	
34.17479	1.751877	
	0.103052	

Compound	smiles	Calculated log Kow
quinoline	c1cnc2ccccc2c1	2.03
isoquinoline	c1ncc2ccccc2c1	1.82
2-hydroxyquinoline	c1ccc2nc(OH)ccc2c1	2.32
9H-carbazole	c1ccc2n(H)c3ccccc3c2c1	3.52
4-azafluorene	c1ccc2cc3cccnc3c2c1	2.85
acridine	c1ccc2nc3ccccc3cc2c1	3.41
benzo[f]quinoline	c1nc2ccc3ccccc3c2cc1	3.2
benzo[h]quinoline	c1cnc2c3ccccc3ccc2c1	3.2
phenanthridine	c1ccc2c3ccccc3cnc2c1	3.2
benz[a]acridine	c1ccc2ccc3nc4ccccc4cc3c2c1	4.59
10-azabenzo[a]pyrene	n1ccc2ccc3c4c2c1ccc4c5ccccc5c3	4.84
dibenz[a,c]acridine	c1ccc2c3ccccc3c4nc5ccccc5cc4c2c1	5.76
dibenz[a,h]acridine	c1ccc2ccc3nc4c5ccccc5ccc4cc3c2c1	5.76
dibenz[a,j]acridine	c1ccc2ccc3nc4ccc5ccccc5c4cc3c2c1	5.76
dibenz[c,h]acridine	c1cc2c3nc4c5ccccc5ccc4cc3ccc2cc1	5.76
naphtalene	c1ccc2ccccc2c1	3.32
flourene	c1ccc2cc3ccccc3c2c1	4.13
phenanthrene	c1ccc2c3ccccc3ccc2c1	4.49
anthracene	c1ccc2cc3ccccc3cc2c1	4.49
pyrene	c1ccc2ccc3cccc4c3c2c1cc4	4.95
benz[a]anthracene	c1ccc2ccc3cc4ccccc4cc3c2c1	5.66
dibenz[a,c]anthracene	c1ccc2c3ccccc3c4cc5ccccc5cc4c2c1	6.84
dibenz[a,h]anthracene	c1ccc2ccc3cc4c5ccccc5ccc4cc3c2c1	6.84
dibenz[a,j]anthracene	c1ccc2ccc3cc4ccc5ccccc5c4cc3c2c1	6.84
dibenzofuran	c1ccc2oc3ccccc3c2c1	4.09
dibenzothiophene	c1ccc2Sc3ccccc3c2c1	4.56
9-anthrone	O=C2c1ccccc1Cc3ccccc23	2.65
anthraquinone	c1ccc2c(=O)c3ccccc3c(=O)c2c1	2.62
9-methylanthracene	c1ccc2cc3ccccc3c(C)c2c1	4.99
9-anthracenecarbonitrile	c1ccc2cc3ccccc3c(C#N)c2c1	3.92
9-nitroanthracene	c1ccc2cc3ccccc3c(N(=O)=O)c2c1	4.23
9-acetylanthracene	c1ccc2cc3ccccc3c(C(=O)C)c2c1	3.93
9-anthracene carboxylicacid-methyl ester	c1ccc2cc3ccccc3c(C(=O)OC)c2c1	4.46
9-anthracenecarboxaldehyde	c1ccc2cc3ccccc3c(CH=O)c2c1	3.84
9-anthracenecarboxamide	c1ccc2cc3ccccc3c(C(=O)ON)c2c1	
9-bromoanthracene	c1ccc2c(Br)c3ccccc3cc2c1	5.35
9-chloroanthracene	c1ccc2c(Cl)c3ccccc3cc2c1	5.2
9-methoxyanthracene	c1ccc2cc3ccccc3c(OC)c2c1	4.41
1,3-dibromopyrene	c1(Br)cc(Br)c2ccc3cccc4c3c2c1cc4	6.68
1,6-dibromopyrene	c1cc(Br)c2ccc3ccc(Br)c4c3c2c1cc4	6.68
1,8-dibromopyrene	c1(Br)ccc2ccc3ccc(Br)c4c3c2c1cc4	6.68
1-bromopyrene	c1ccc2ccc3ccc(Br)c4c3c2c1cc4	5.81
2-bromopyrene	c1c(Br)cc2ccc3cccc4c3c2c1cc4	5.81
4-bromopyrene	c1ccc2c(Br)cc3cccc4c3c2c1cc4	5.81

Table A12. *Smiles notation and log K_{ow} calculated with the ClogP program.*