Persistent organic pollutants - sample analysis

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1. Environmental analytical chemistry
Specific features, general scheme

2. Sampling
Sampling plan, strategy, sampling protocol, sample size and quality, transport, storage

3. Sample preparation
Extraction of solid (Soxhlet, automatic extraction, MAE, ASE, SFE) and liquid (L-L, SPE, SPME, head-space) samples, fractionation and clean-up (column chromatography, gel permeation)

4. Analytical techniques
Chromatographic techniques, principals, instrumentation, HPLC, GC, GC-MS

5. Persistent organic pollutants
Priority pollutants (PCBs, PCDDs/Fs, PAHs, pesticides), emerging pollutants (SCCPs/MCCPs, antibiotics, degradation products)

6. QA/QC
Calibration, limit of detection and quantification, internal and recovery standards, blanks, certified reference materials, interlaboratory calibration tests, method validation and verification, GLP

Environmental science brings together scientists from many fields to perform complex studies of various environmental compartments, processes, and interactions.

They may include:

- water and food quality monitoring
- level of contamination of environmental compartments
- ozone depletition as a result of the presence of certain chemicals in the atmosphere
- regional contamination studies
- evaluation of the impact of local sources of pollution
- toxicity of chemical compounds as a function of their chemical structure
- impact of chemical substances on living organisms
- bioavailability
- bioaccumulation
- biotic and abiotic transformations
- transport of pollutants in the environment
- global fate of pollutants
- international directives and their impact on the global contamination
- remediation actions and their quality control
- sustainable development

Most of them involve the chemical analysis as one of necessary steps.

Persistent Organic Pollutants

Resistent to chemical, biochemical, photochemical degradation

Long life-time in the environment (years)

Physical properties supporting a high degree of mobility in the environment

Can be bioaccumulated in food chains

Toxic properties at low levels (ng-mg.kg^{-1}) or toxic metabolites

Main groups: technical chemicals, pesticides and industrial by-products
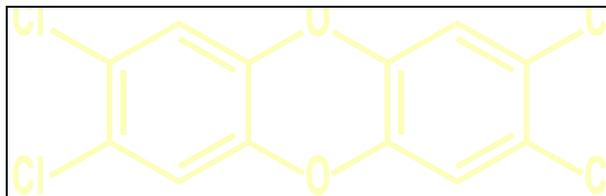
Fate of the pollutants in the environment

Transport

Persistent toxic substances (based on their octanol/water K_{ow} and octanol/air K_{oa} partitioning coefficients) are often a subject of the transport in the environment (within or between compartments), including a long-range transport

Transformation

Bioconcentration bioconcentration factor)
Bioaccumulation bioaccumulation factor)
Biomagnification between the trophic levels of the foodchain)

Environmental analytical chemistry chalenges:

- international conventions focus attention on the new groups of pollutants
- old contamination brings the problem of residue analyses
- lowering limits as well as environmental levels require low detection limits
- large-scale monitoring is crutial for the studies of the long-range transport
- development of new sampling techniques is encouraged
- increasing number of samples stresses the need for automatization
- fate studies require understanding of distribution processes and equilibria
- photochemical reaction complicate the sampling and data interpretation
- consideration of both, analytical and toxicological data is important
  for successful risk assessment
- methods of biochemistry and molecular-biology are often implemented in
  toxicological studies
- international studies require standardization of all procedures

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There are several steps necessary for environmental contamination control:

   problem definition
- screening of the situation, data interpretation
- evaluation of the extent of the problem
- selection of the best procedure to monitor the situation
- evaluation of the present state and future development
- exposure evaluation and risk assessment
- suggestion of correcting measures or remediation activities
- new directives to control the situation
- monitoring designed to evaluate effectiveness of measures

Košetice

alfa-HCH
beta-HCH
gama-HCH
delta-HCH

Košetice

	16.2.-15.3.2004
	15.3.-13.4.2004
	13.4.-11.5.2004
	11.5.-8.6.2004
	8.6.-7.7.2004
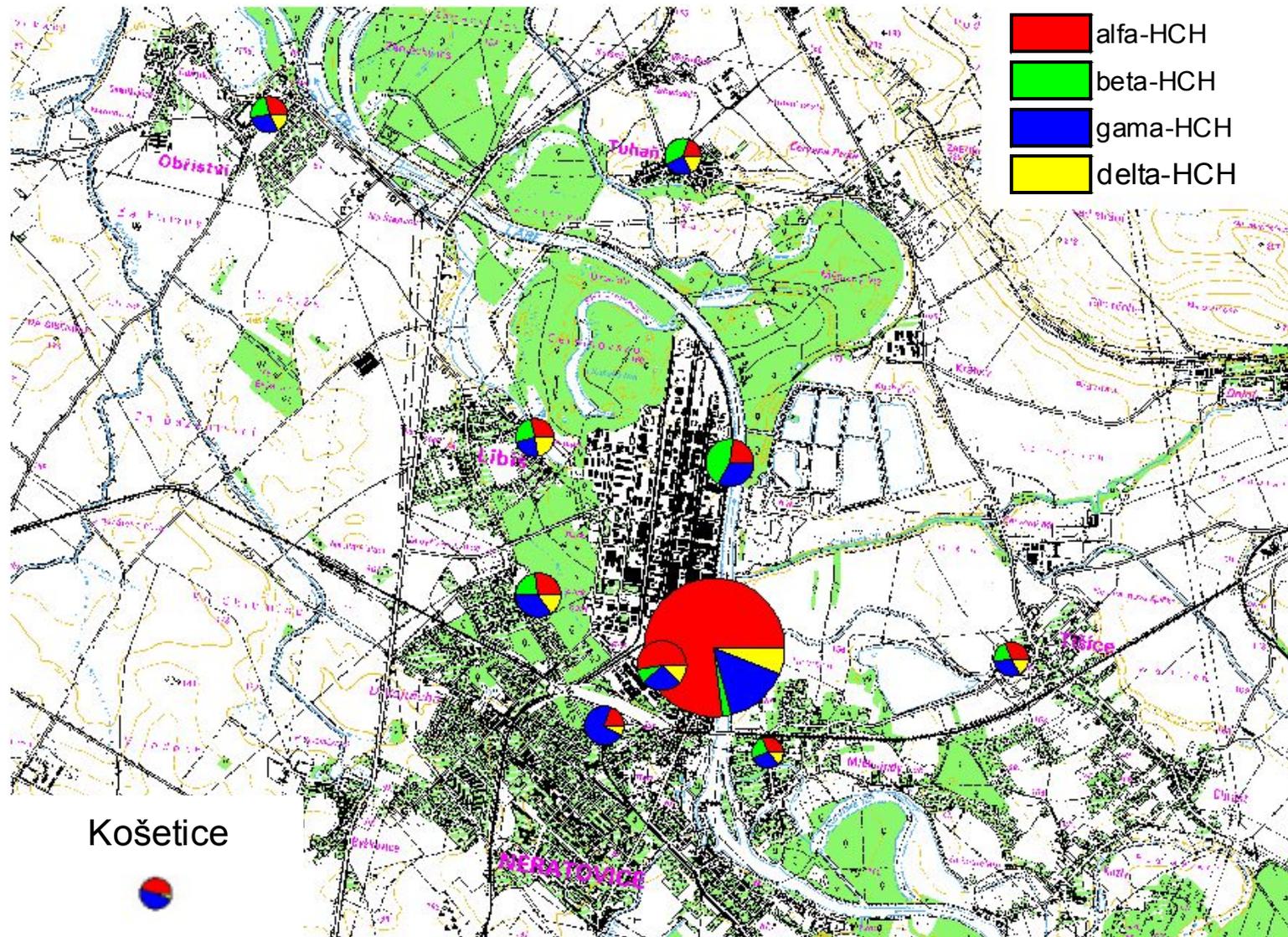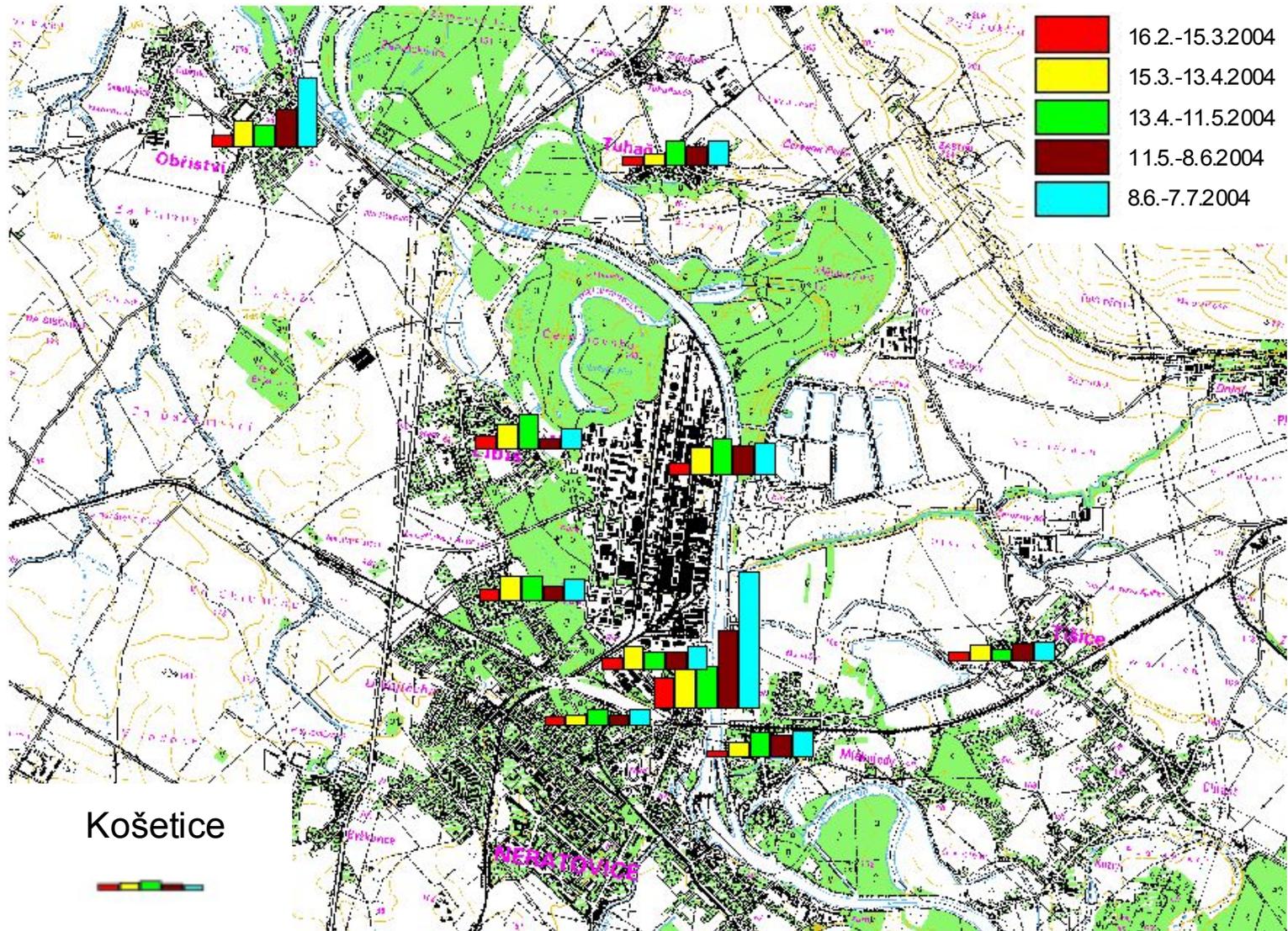
Specific problems of environmental analysis

- low homogenity of samples (soil)
- low stability of samples (biota)
- various matrices (methods for extraction of analytes from matrices)
- wide range of analytes (method development)
- wide range of concentration (robust methods)
- monitoring on the levels close to the detection limits (high deviations)
- risk of secondary contamination
- price of ultra-trace analysis (instrumentation, chemicals, standards)

General scheme of environmental analysis

Sampling   - homogenization
        - conservation
        - transport
        - storage

- Sample preparation - extraction
          - clean-up
          - selective elution
          - concentration
          - derivatization

- Sample analysis
- Data interpretation

Sampling – documentation required

sampling plan (a goal, selection of sampling sites, analytes, sampling method, number of samples, sampling period and frequency, safety procedures), seeks the balance between the value of data and its price

- **standard operational procedure** for sampling various matrices (sampling devices, steps involved in collecting of representative sample -homogenous, of reasonable size and stability, quality of transport and storage)

-**sampling protocols** (name and number of the sample, sampling site, matrix, date of sampling, local conditions and measurements, methods, sample size, responsible person)

Sampling site 1. DEZA
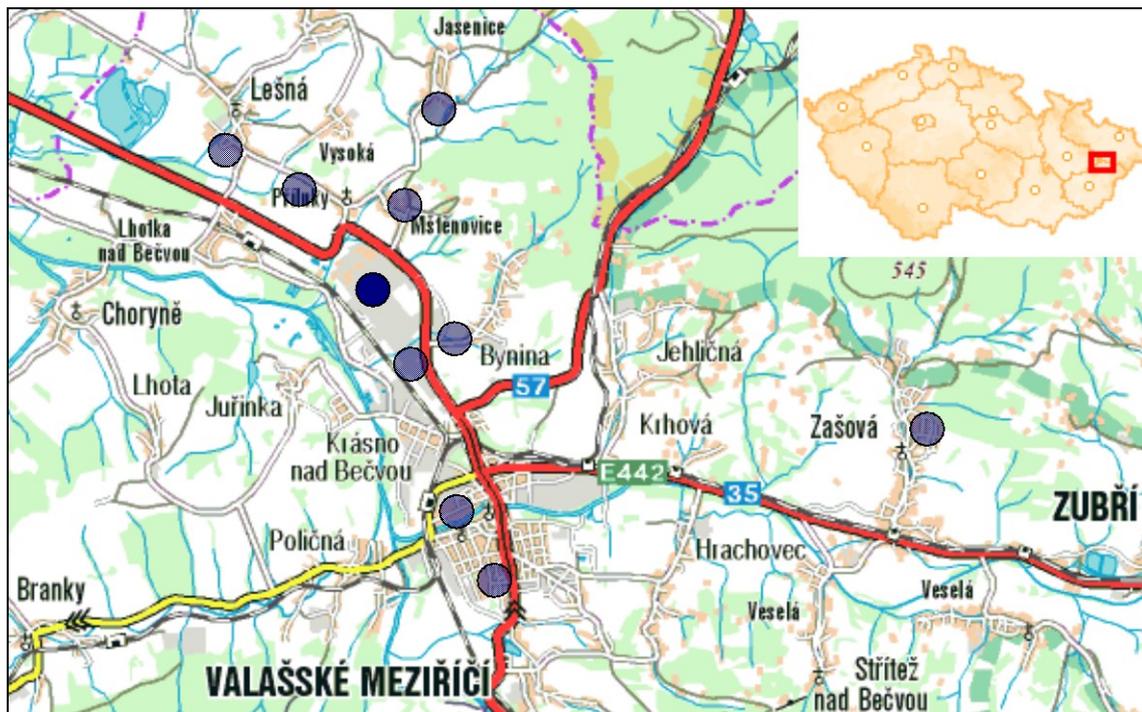
GPS:

49 29'48''
17 57'14''
245 m

Local conditions:

Sampling Techniques
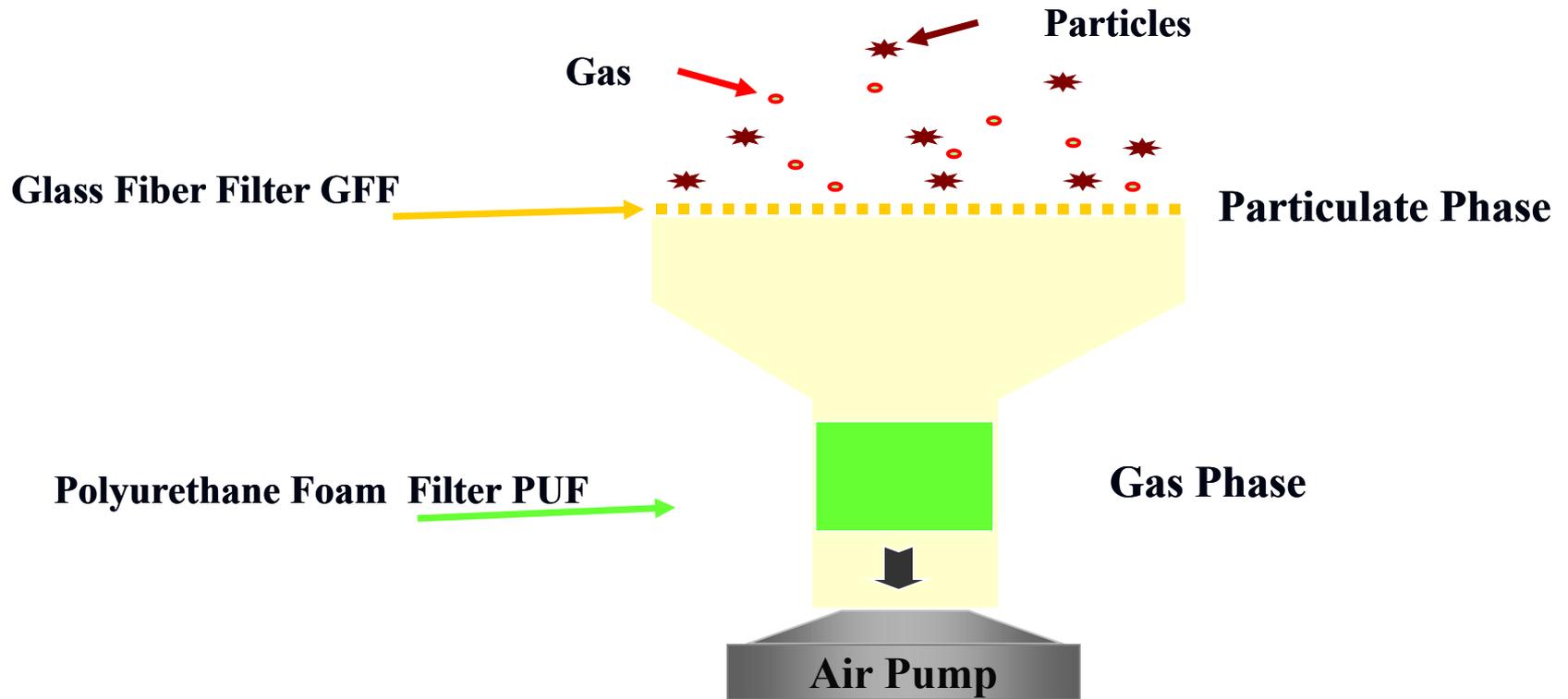
High-Volume sampler

Passive sampling

Can environmental concentrations of pollutants be calculated from the analyte levels accumulated in an integrative passive sampler?

- Calibration conditions should approximate field conditions

- Performance Reference Compounds

Calibration of a passive sampler in a flow-through system

Chemicals in MeOH

overhead stirrer

Water reservoir

Samplers

waste water

Analytes In MeOH

Water

Peristaltic pump 100 μl/min

Exposure tank

Peristaltic pump 30 ml/min

B. Vrana, R. Greenwood, G. Mills

Sampling rates of PAHs

B. Vrana, R. Greenwood, G. Mills
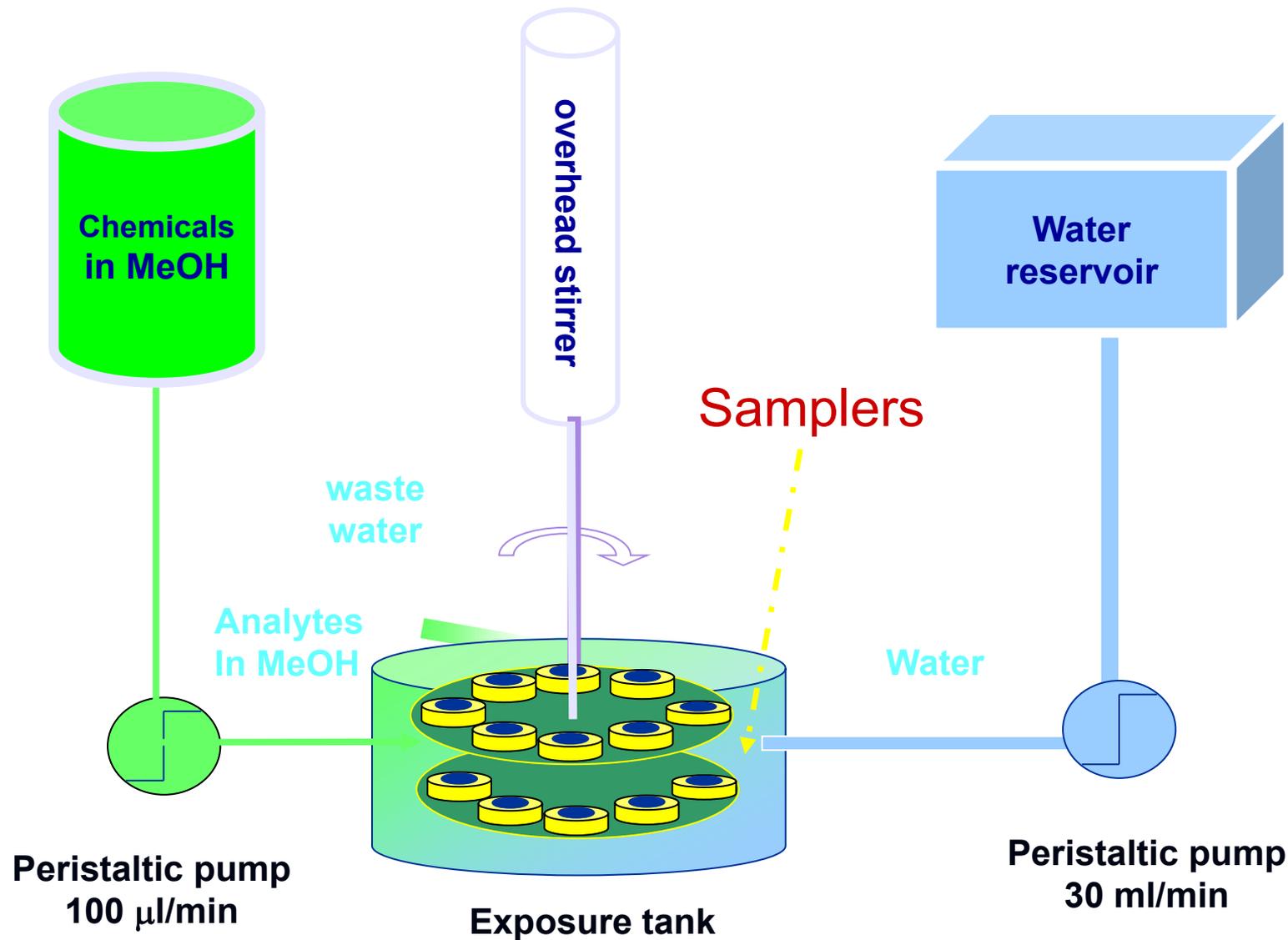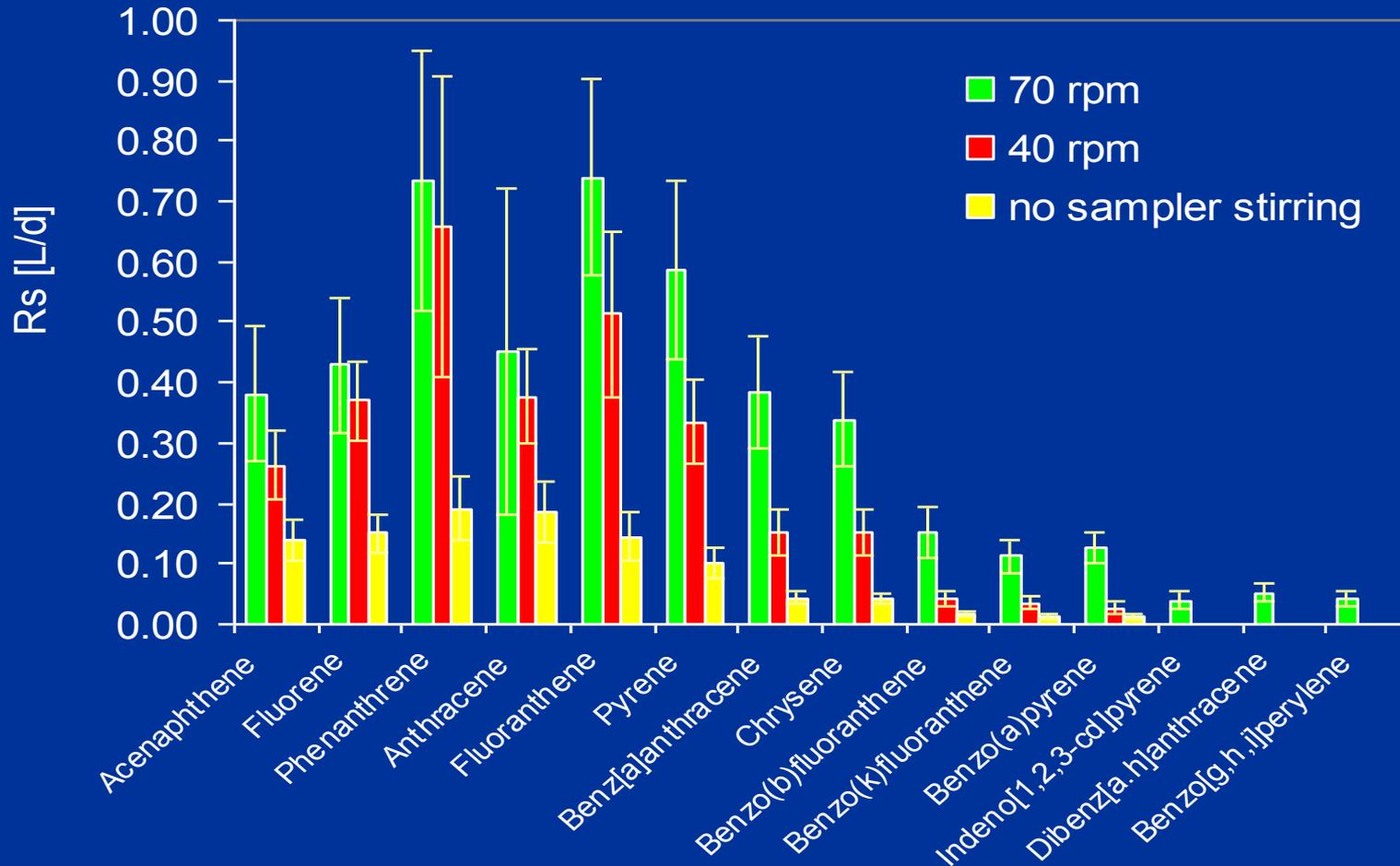
Performance reference compounds

PRCs are non-interfering compounds added to the sampler prior to exposure.

They are used for in situ calibration approach, where the rate
of PRC loss during an exposure is related to the target compound uptake.

This is accomplished by measuring PRC loss rates during calibration studies and
field exposures.

Use of performance reference compounds

B. Vrana, R. Greenwood, G. Mills
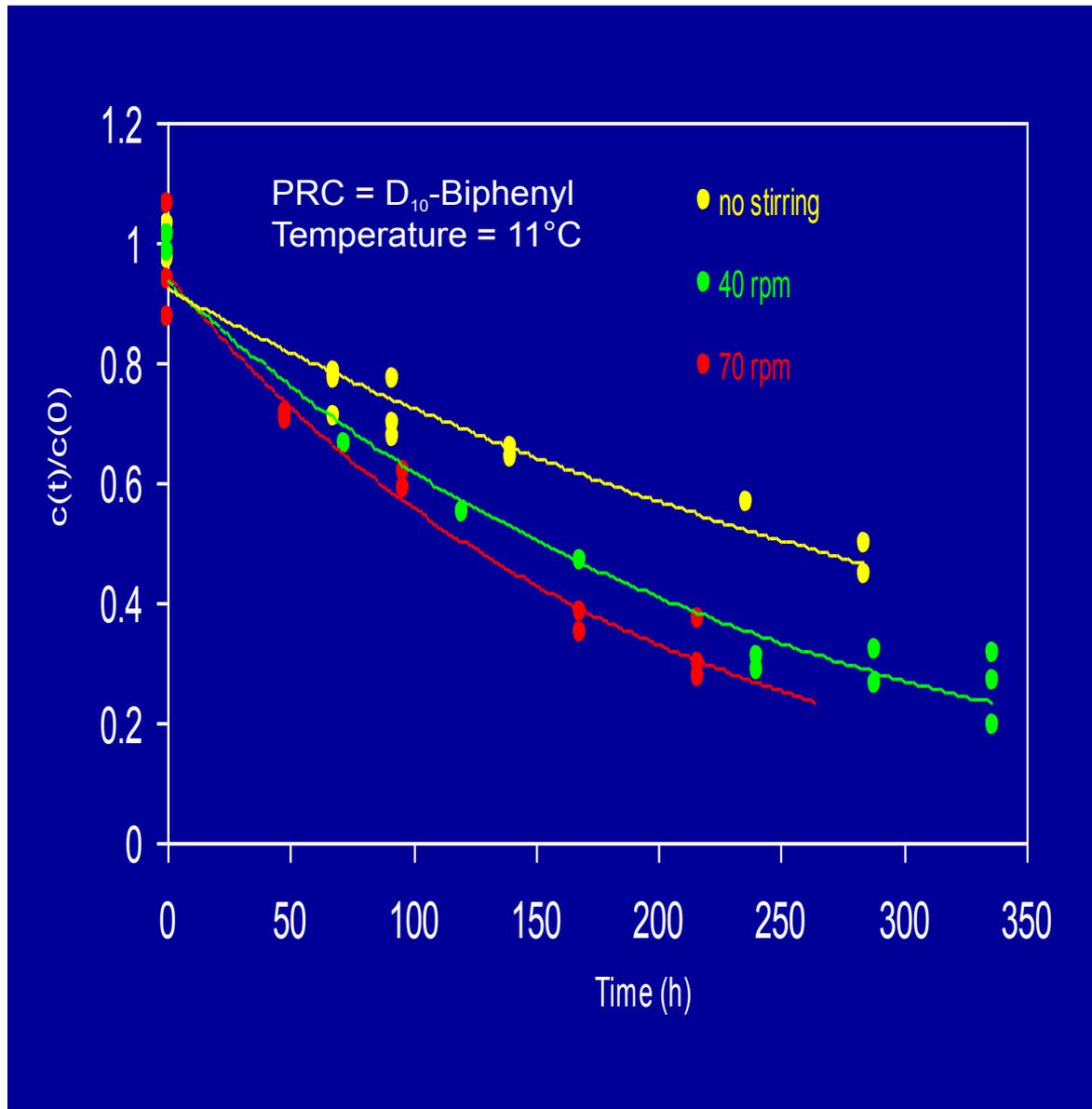
Preparation of the sample before extraction

Soil samples
- lyofilization or air-drying
- sieving (< 2mm) and homogenization
- appropriate storage (protected from sunlight, heat and humidity)

Sediment samples
- stone and water removal, lyofilization or air-drying
- grating and sieving (<63um), homogenization
- powder copper treatment for sulphur removal

Plant samples
- lyofilization or air-drying
- grating, homogenization

Animal samples
- lyofilization or
- homogenization of a wet sample with sodium sulphate

Extraction and clean-up

The goal: transfer of analytes to the chemical phase suitable for analysis, removal of interferences and pre-concentration of the sample.

Extraction techniques:
- solvent extraction (Soxhlet, automatic Soxtec, MAE, ASE, SFE)
- liquid-liquid extraction
- solid phase extraction and microextraction (SPE, SPME)
- semipermeable membrane separation
- head space analysis

Clean-up techniques
- sulphuric acid treatment
- column liquid chromatography (silica gel, alumina, florisil)
- gel permeation chromatography

Solid sample extraction

Extraction thimble
containing sample

Heating mantle

Extraction solvent
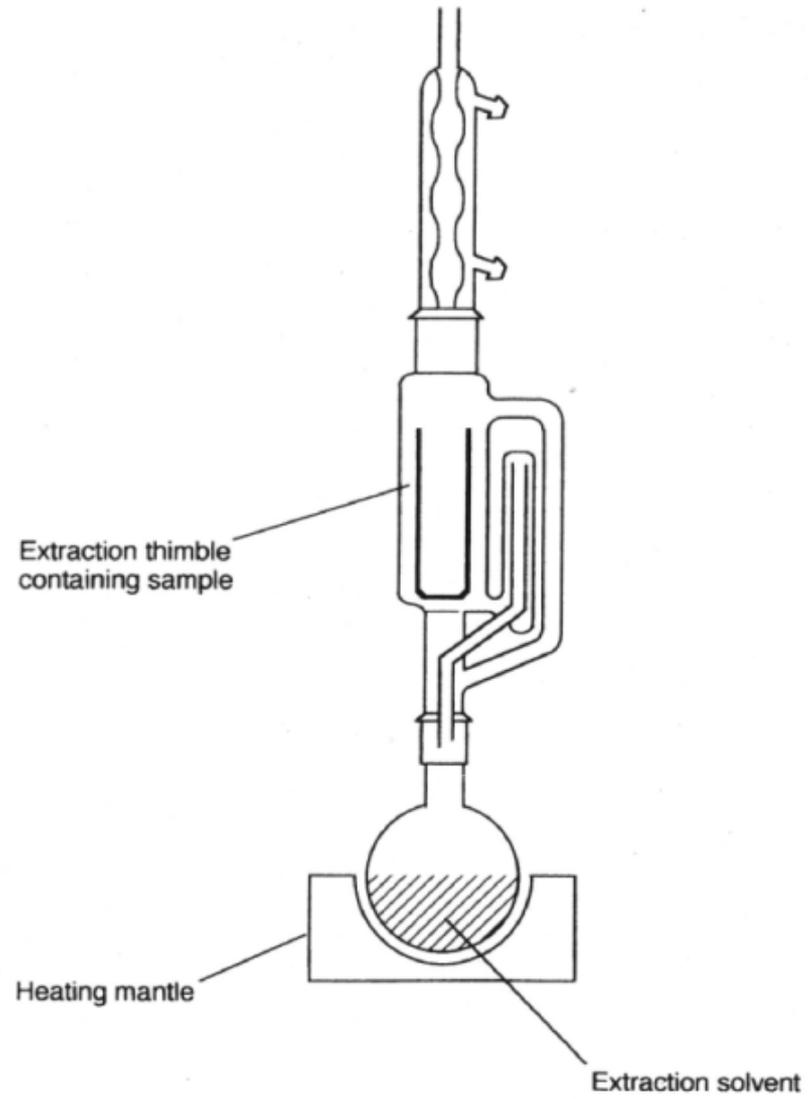
Schematic of a Soxhlet extraction system.

Liquid sample extraction
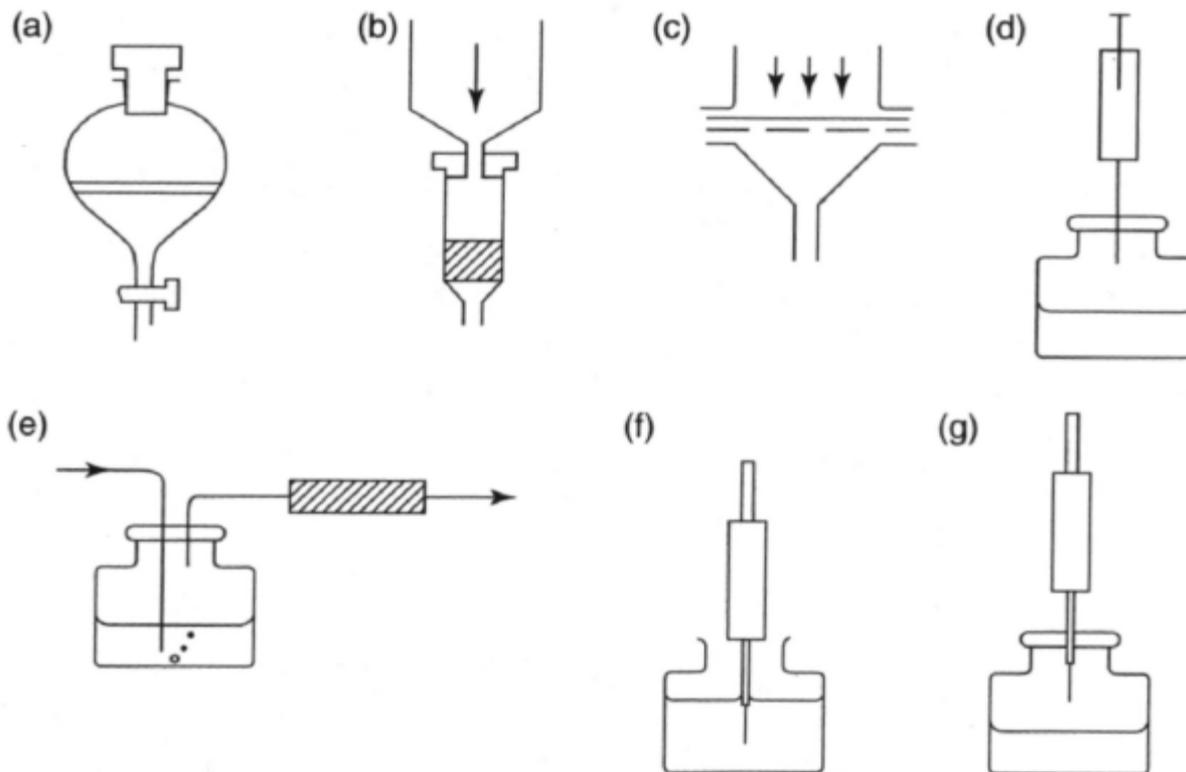
Summary of extraction methods: (a) solvent extraction; (b) solid-phase extraction – cartridge; (c) solid-phase extraction – disc; (d) head-space analysis; (e) purge and trap; (f) solid-phase microextraction – direct; (g) solid-phase microextraction – head-space.

Air samples
- filters from high volume samplers or passive samplers are extracted as solid samples
  (Soxhlet, MAE, ASE, SFE)

Water samples
  direct analysis of the samples with high concentration of pollutants
- head space, SPE, L-L

Soil and sediment samples
  Soxhlet, MAE, ASE, SFE
- powder copper treatment for the sulphur removal in sediment samples

Biotic samples
- high molecular compounds removal by gel permeation chromatography and column
  chromatography

Presence	Availability	Activity
Total mass	Fraction of total mass	Measure that drives diffusion and partitioning
How much is there?	How much is available for ... ?	How high is the diffusive pressure into other media?
Exhaustive Extraction	Depletive Extraction/ Sampling	Equilibrium Sampling Devices

P. Mayer, F. Reichenberg

Supercritical Fluid Extraction (SFE)

High pressure CO_2 (100 to 400 bar, 40 to 150 ^{o}C) is pumped through a sample, and extracted analytes are collected in a suitable solvent for GC analysis.

Why to use supercritical carbon dioxide?

- CO_2 is a lipophilic solvent much like biological lipids in polarity

- PAH solubilities in CO_2 are proportional to those in water, but ca. 10^4 higher

- pressure and temperature gradients enable the extraction of both, non-polar and polar compounds

- mild SFE can be used to predict bioavailability of compounds

Earthworm Mortality Depends on Available PAHs (measured by SFE), not on Total PAH Concentrations

Soil Mortality	Total PAH (ug/g soil)	Available Fraction (SFE)	Available Total PAH (ug/g C)	%
CG15	1020	0.25	1040	0
OG14	168	0.46	2720	0
CG11	15600	0.06	3280	0
CG12	3790	0.16	7880	0
OG17	17200	0.27	9720	0
OG5	1870	0.41	11100	0
OG10	42100	0.33	16300	0
CG3	4100	0.83	45700	100
OG18	17300	0.74	50100	100

S. B. Hawthorne, C. B. Grabanski, D. J. Miller

SFE rates mimic bioremediation
(1 min SFE=10 days bioremediation)

Bioremediation (1 year)

SFE (40 min)

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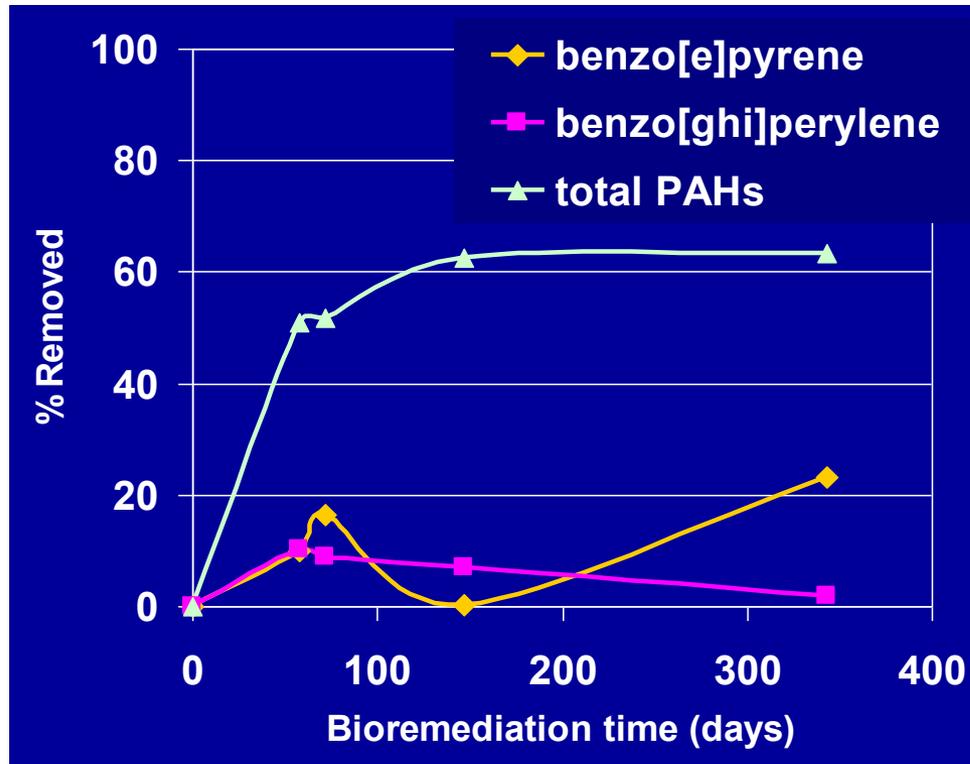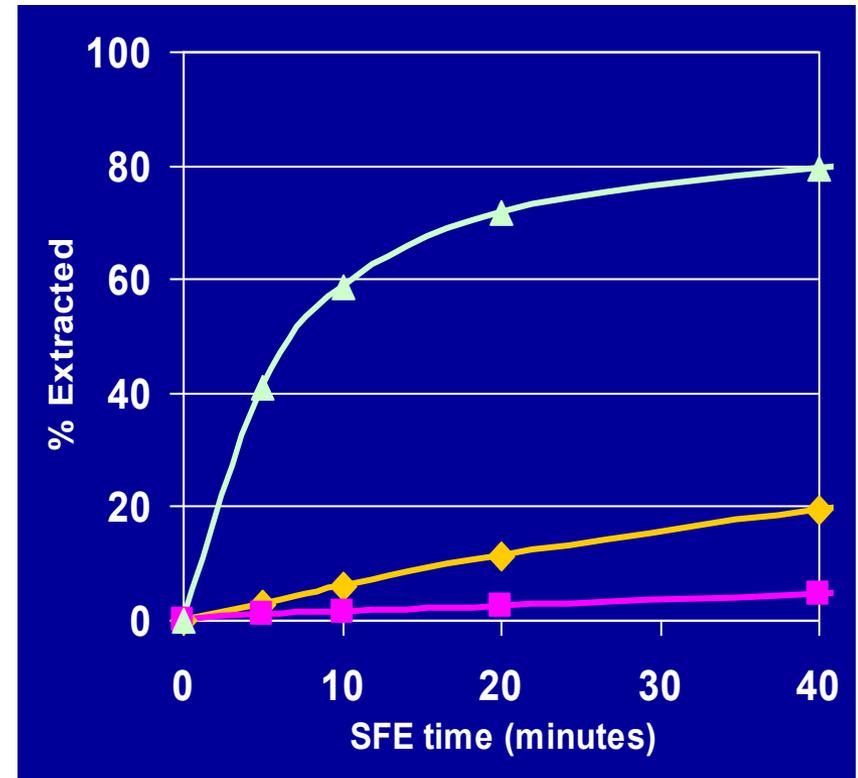
Priority pollutants

- polychlorinated biphenyls
- polychlorinated dibenzo-*p*-dioxins and furans
- organochlorinated pesticides and their metabolites
- polyaromatic hydrocarbons
    - aromatics and nitro-aromatics
- chlorinated benzenes
- fenol and chlorinated fenols
- halogenated alkans

Polychlorinated biphenyls
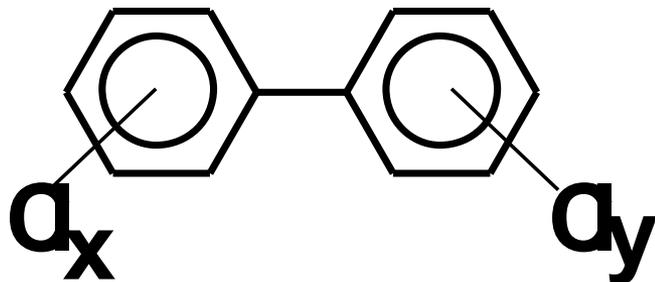
- sulphuric acid treatment
- silica gel column chromatography
- activated carbon for non-ortho PCBs
- GC-ECD, GC-MS, GC- HRMS

IUPAC numbering and substitution pattern of PCB congeners

No.	Structure	No.	Structure	No.	Structure	No.	Structure
	Monochlorobiphenyls		Tetrachlorobiphenyls		Pentachlorobiphenyls		Hexachlorobiphenyls
1	2	52	2,2',5,5'	105	2,3,3',4,4'	161	2,3,3',4,5',6
2	3	53	2,2',5,6'	106	2,3,3',4,5	162	2,3,3',4',5,5'
3	4	54	2,2',6,6'	107	2,3,3',4',5	163	2,3,3',4',5,6
		55	2,3,3',4	108	2,3,3',4,5'	164	2,3,3',4',5',6
	Dichlorobiphenyls	56	2,3,3',4'	109	2,3,3',4,6	165	2,3,3',5,5',6
4	2,2'	57	2,3,3',5	110	2,3,3',4',6	166	2,3,4,4',5,6
5	2,3	58	2,3,3',5'	111	2,3,3',5,5'	167	2,3',4,4',5,5'
6	2,3'	59	2,3,3',6	112	2,3,3',5,6	168	2,3',4,4',5',6
7	2,4	60	2,3,4,4'	113	2,3,3',5',6	169	3,3',4,4',5,5'
8	2,4'	61	2,3,4,5	114	2,3,4,4',5		
9	2,5	62	2,3,4,6	115	2,3,4,4',6		Heptachlorobiphenyls
10	2,6	63	2,3,4',5	116	2,3,4,5,6	170	2,2',3,3',4,4',5
11	3,3'	64	2,3,4',6	117	2,3,4',5,6	171	2,2',3,3',4,4',6
12	3,4	65	2,3,5,6	118	2,3',4,4',5	172	2,2',3,3',4,5,5'
13	3,4'	66	2,3',4,4'	119	2,3',4,4',6	173	2,2',3,3',4,5,6
14	3,5	67	2,3',4,5	120	2,3',4,5,5'	174	2,2',3,3',4,5,6'
15	4,4'	68	2,3',4,5'	121	2,3',4,5',6	175	2,2',3,3',4,5',6
		69	2,3',4,6	122	2',3,3',4,5	176	2,2',3,3',4,6,6'
	Trichlorobiphenyls	70	2,3',4',5	123	2',3,4,4',5	177	2,2',3,3',4',5,6
16	2,2',3	71	2,3',4',6	124	2',3,4,5,5'	178	2,2',3,3',5,5',6
17	2,2',4	72	2,3',5,5'	125	2',3,4,5,6'	179	2,2',3,3',5,6,6'
18	2,2',5	73	2,3',5',6	126	3,3',4,4',5	180	2,2',3,4,4',5,5'
19	2,2',6	74	2,4,4',5	127	3,3',4,5,5'	181	2,2',3,4,4',5,6
20	2,3,3'	75	2,4,4',6			182	2,2',3,4,4',5,6'
21	2,3,4	76	2',3,4,5		Hexachlorobiphenyls	183	2,2',3,4,4',5',6
22	2,3,4'	77	3,3',4,4'	128	2,2',3,3',4,4'	184	2,2',3,4,4',6,6'
23	2,3,5	78	3,3',4,5	129	2,2',3,3',4,5	185	2,2',3,4,5,5',6
24	2,3,6	79	3,3',4,5'	130	2,2',3,3',4,5'	186	2,2',3,4,5,6,6'
25	2,3',4	80	3,3',5,5'	131	2,2',3,3',4,6	187	2,2',3,4',5,5',6
26	2,3',5	81	3,4,4',5	132	2,2',3,3',4,6'	188	2,2',3,4',5,6,6'
27	2,3',6			133	2,2',3,3',5,5'	189	2,3,3',4,4',5,5'
28	2,4,4'		Pentachlorobiphenyls	134	2,2',3,3',5,6	190	2,3,3',4,4',5,6
29	2,4,5	82	2,2',3,3',4	135	2,2',3,3',5,6'	191	2,3,3',4,4',5',6
30	2,4,6	83	2,2',3,3',5	136	2,2',3,3',6,6'	192	2,3,3',4,5,5',6
31	2,4',5	84	2,2',3,3',6	137	2,2',3,4,4',5	193	2,3,3',4',5,5',6
32	2,4',6	85	2,2',3,4,4'	138	2,2',3,4,4',5'		
33	2',3,4	86	2,2',3,4,5	139	2,2',3,4,4',6		Octachlorobiphenyls
34	2',3,5	87	2,2',3,4,5'	140	2,2',3,4,4',6'	194	2,2',3,3',4,4',5,5'
35	3,3',4	88	2,2',3,4,6	141	2,2',3,4,5,5'	195	2,2',3,3',4,4',5,6
36	3,3',5	89	2,2',3,4,6'	142	2,2',3,4,5,6	196	2,2',3,3',4,4',5',6
37	3,4,4'	90	2,2',3,4',5	143	2,2',3,4,5,6'	197	2,2',3,3',4,4',6,6'
38	3,4,5'	91	2,2',3,4',6	144	2,2',3,4,5',6	198	2,2',3,3',4,5,5',6
39	3,4',5	92	2,2',3,5,5'	145	2,2',3,4,6,6'	199	2,2',3,3',4,5,6,6'
		93	2,2',3,5,6	146	2,2',3,4',5,5'	200	2,2',3,3',4,5',6,6'
	Tetrachlorobiphenyls	94	2,2',3,5,6'	147	2,2',3,4',5,6	201	2,2',3,3',4,5',6'
40	2,2',3,3'	95	2,2',3,5',6	148	2,2',3,4',5,6'	202	2,2',3,3',5,5',6,6'
41	2,2',3,4	96	2,2',3,6,6'	149	2,2',3,4',5',6	203	2,2',3,4,4',5,5',6
42	2,2',3,4'	97	2,2',3',4,5	150	2,2',3,4',6,6'	204	2,2',3,4,4',5,6,6'
43	2,2',3,5	98	2,2',3',4,6	151	2,2',3,5,5',6	205	2,3,3',4,4',5,5',6
44	2,2',3,5'	99	2,2',4,4',5	152	2,2',3,5,6,6'		
45	2,2',3,6	100	2,2',4,4',6	153	2,2',4,4',5,5'		Nonachlorobiphenyls
46	2,2',3,6'	101	2,2',4,5,5'	154	2,2',4,4',5,6'	206	2,2',3,3',4,4',5,5',6
47	2,2',4,4'	102	2,2',4,5,6'	155	2,2',4,4',6,6'	207	2,2',3,3',4,4',5,6,6'
48	2,2',4,5	103	2,2',4,5',6	156	2,3,3',4,4',5	208	2,2',3,3',4,5,5',6,6'
49	2,2',4,5'	104	2,2',4,6,6'	157	2,3,3',4,4',5'		
50	2,2',4,6			158	2,3,3',4,4',6		Decachlorobiphenyl
51	2,2',4,6'			159	2,3,3',4,5,5'	209	2,2',3,3',4,4',5,5',6,6'
				160	2,3,3',4,5,6		

Organochlorinated pesticides

(DDT, HCH, hexachlorobenzene, toxaphene, aldrin, dieldrin, endrin, endosulfane, chlordane)

HCH
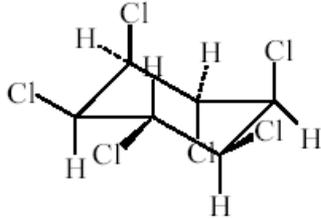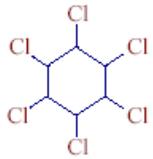
- for HCHs and DDTs analytical procedures similar to PCBs
- GC-ECD, GC-MS, NCI-MS, HRMS
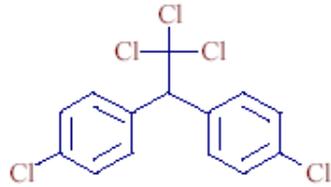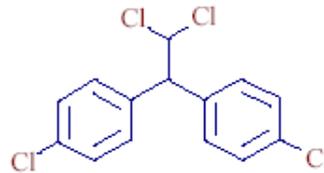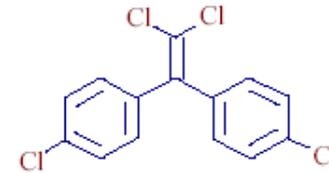
p,p'-DDT   *p,p'*-DDD   *p,p'*-DDE

- analytical procedures similar to PCBs for toxaphene,
- sulphuric acid has to be omitted for aldrin or endosulfane
- GC-MS, NCI-MS, HRMS

Polychlorinated dibenzodioxins and dibenzofurans

  combined modified silica gel clean-up
- fractionation on alumina/florisil column
- non-ortho PCBs separation on activated carbon column
- HRGC-HRMS
- kapilary columns 50-60m (DB-5, DB-17, DB-DIOXIN)
- EI, NCI
- SIM
- MS-MS

Polyaromatic hydrocarbons

- silica gel column chromatography
- GC-MS, FLD-HPLC
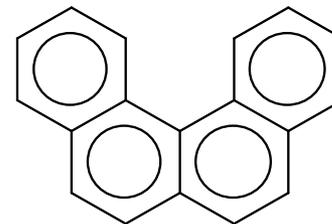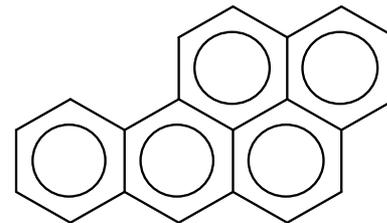
# Sample analysis

Chromatographic separation (GC, HPLC) is the most common technique for the analysis of environmental samples.
It is a physical method based on the distribution of compounds between two phases (stationary and mobile). Process of continuous sorption and desorption of compounds in contact with the stationary phase is responsible for different migration times and for separation of analytes.

Two dimensional (GC-GC) and two modal (HPLC-GC) chromatography provide even more sofisticated tools for environmental analysis

GC-MS, HPLC-MS and HRMS enable the trace and ultra-trace analysis

Chromatogram
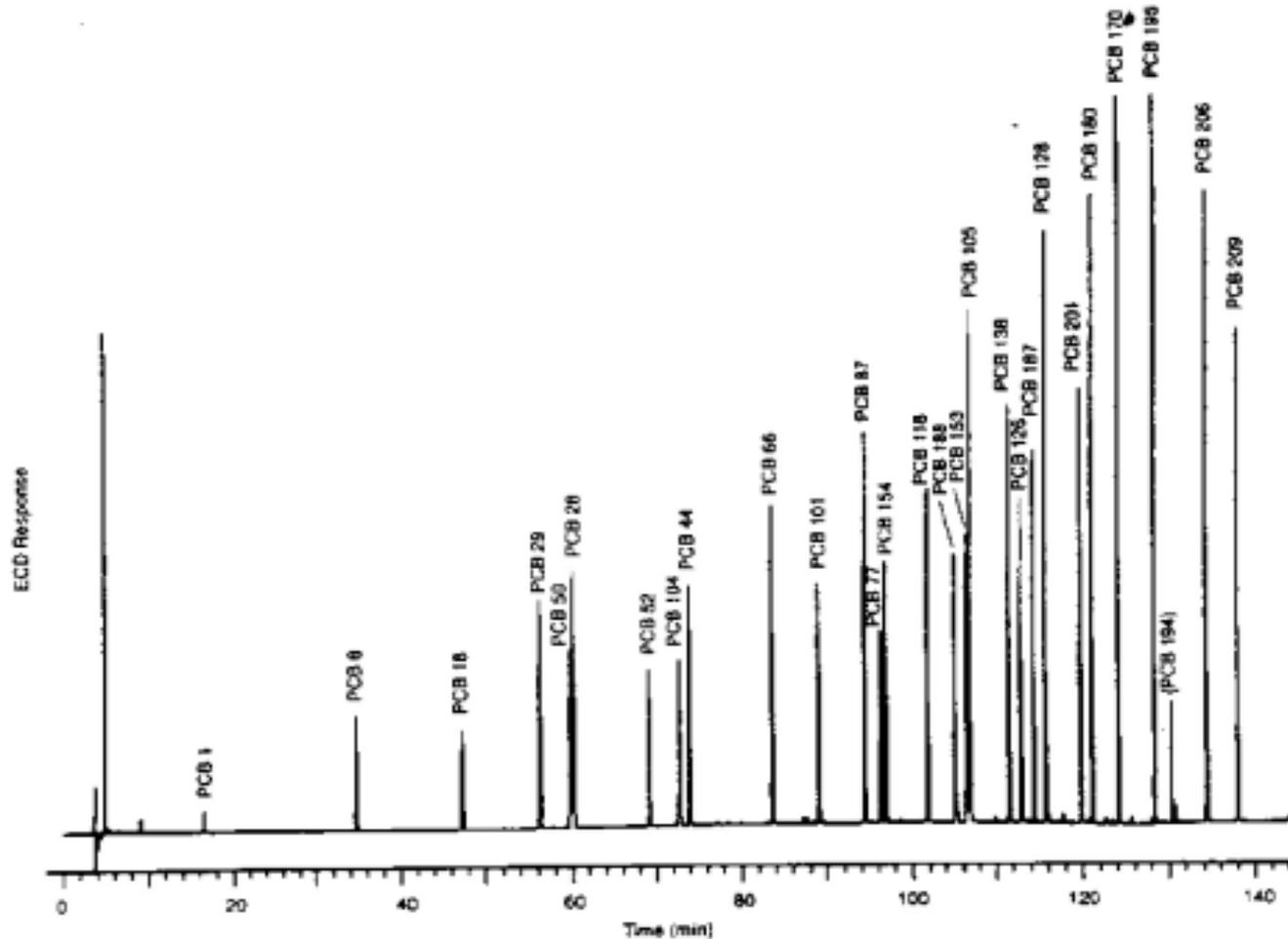
Figure A-1. Chromatogram of NIST SRM 2262 by GC-ECD using a 0.25-mm i.d. x 60-m fused silica capillary column with a 5% phenyl-substituted methylpolysiloxane phase (0.25 μm film thickness) (DB-5, J&W Scientific, Folsom, CA) Temperature Program: 150 °C (40 min) to 220 °C (0 min) at 1 °C/min to 280 °C (25 min) at 3 °C/min.

Mass spectra

Scan 1136 (18.002 min): O2O21.D (-)
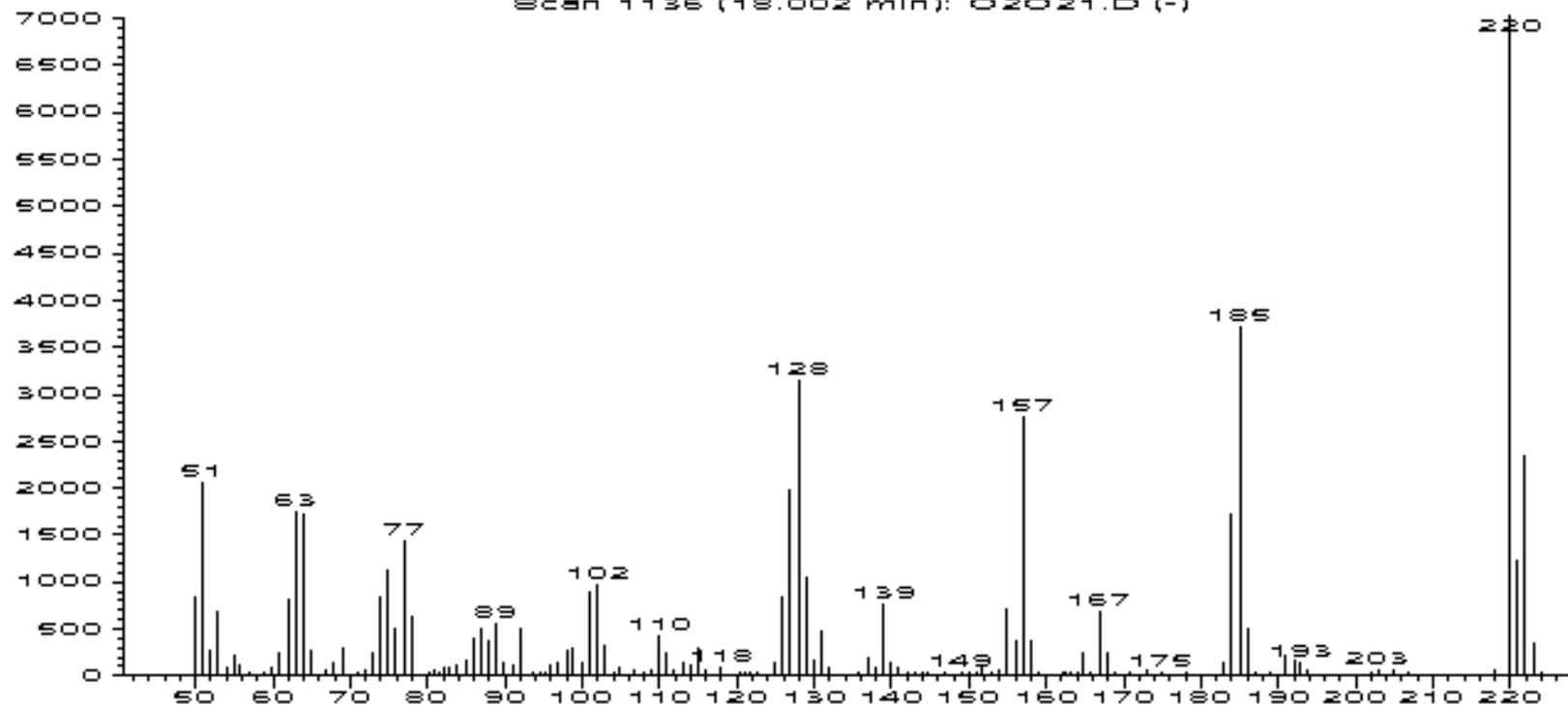
Quality assurance/quality control (QA/QC)

Quality assurance
Preventive measures (quality of facilities, personnel and education, equipment and service, calibration, internal and recovery standards)

Quality control
Control measures (internal – blank and reference material analyses, external – interlaboratory comparison, audit)

Reasons
- repeatibility of measurements
- comparison of results between laboratories
- political and economical importance of results

Terminology

Calibration
Limit of detection and quantification
Sensitivity and specificity
Accuracy, trueness, precision
Method validation and verification
Internal standards
Recovery and surrogate recovery standards
Certified reference materials
interlaboratory calibration tests,
GLP

Standard operational procedure

- General information (terminology, principles, range of use, limitations,
   safety
   procedures, toxicology, waste treatment)
- Directives
- Consumables and chemicals (glass, standards, solvents, reference materials)
- Equipment (sampling and analytical equipment, service)
- Calibration (standards, procedures)
- Analytical scheme (method validation and verification)
- Quality control (internal - blank, reference material, external –
   intercalibration)
- Data interpretation
- Annexes

Mokrá - půdy 2002 - 4

vyhodnoceno: 25.4.2003

Koncentrace ng/g

Číslo vzorku	toluen	02-753	02-752	02-740	02-741	02-742	02-743	02-744	02-745	02-746	02-747	02-748	02-749	02-750	02-751	
Lokalita	GC blank	Lab. blank	RM	454 Hosten	Čihálky	332 Vodojem	Velká Bata1	Velká Bata2	Prostřed kopec	420Vel Bata	Chlumek 1	Chlumek 2	Horák mysl.	Nové pole	jižní CVM	LOQ
Číslo zadava				303S	304S	305S	306S	307S	308S	309S	310S	311S	312S	313S	314S	
Datum odbě				14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	14.11.02	KALIB30
Naváž ka (g)	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0
Ředění	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Naftalen	0,10	1,86	26,74	12,5	6,6	7,5	5,2	5,5	11,8	13,5	7,1	6,6	8,6	5,9	8,5	0,10
Acenaftyle	-	0,02	0,58	0,8	0,3	0,7	0,4	0,5	2,2	1,8	0,6	0,5	1,2	2,4	0,8	0,10
Acenaften	-	0,04	1,22	1,4	0,3	1,4	1,6	0,6	5,3	3,4	2,5	0,8	2,0	5,4	1,2	0,10
Fluoren	-	0,04	2,26	1,7	0,6	1,4	1,3	0,7	4,9	3,8	2,0	1,0	2,2	4,7	1,5	0,10
Fenantren	-	0,12	23,96	24,9	6,4	20,5	18,8	8,4	69,1	59,4	14,2	13,6	29,5	109,3	16,8	0,10
Antracen	-	-	1,12	2,0	0,4	1,9	3,4	1,1	6,1	5,2	2,1	1,4	2,9	16,9	1,8	0,10
Fluoranten	-	-	27,78	68,2	13,7	58,0	42,0	24,2	213,0	162,5	40,7	37,6	82,5	450,2	42,9	0,10
Pyren	-	-	19,38	50,5	9,7	45,6	35,4	20,2	159,3	123,6	32,0	28,6	63,8	377,2	33,0	0,10
Benz(a)ant	-	-	4,60	17,9	2,9	14,4	14,7	9,1	61,5	49,3	18,3	13,1	26,3	206,3	13,6	0,10
Chrysen	-	-	11,50	32,4	7,3	25,6	18,4	12,2	102,6	75,9	22,3	16,8	41,2	204,2	20,0	0,10
Benzo(b)flu	-	-	18,30	61,0	11,7	32,2	23,6	20,4	169,5	128,2	28,0	29,4	67,7	261,1	31,2	0,10
Benzo(k)flu	-	-	6,04	18,1	3,8	14,4	11,0	7,9	56,4	41,9	13,0	11,2	22,4	134,8	11,6	0,10
Benzo(a)py	-	-	8,34	27,6	3,5	23,6	20,3	13,3	92,8	71,6	24,2	18,4	38,4	285,9	21,3	0,10
Indeno(123	-	-	8,22	33,1	6,4	21,4	14,8	11,1	98,7	72,0	22,6	19,6	41,0	216,1	20,7	0,10
Dibenz(ah)	-	-	0,82	2,7	0,6	2,4	1,6	0,9	7,1	8,3	1,8	2,3	4,1	25,8	1,8	0,10
Benzo(ghi)	-	-	11,26	29,7	5,3	20,6	14,8	11,4	83,9	61,4	19,4	16,3	36,0	181,8	18,5	0,10
Suma PA	**0,10**	**2,08**	**172,12**	**384,5**	**79,5**	**291,6**	**227,3**	**147,5**	**1144,2**	**881,8**	**250,8**	**217,2**	**469,8**	**2488,0**	**245,2**	**1,60**
100% D-PA	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	2 000	
ředění	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
D8-naftalen	0%	0%	88%	72%	79%	66%	65%	80%	62%	66%	21%	61%	75%	81%	81%	
D10-fenant	0%	0%	90%	77%	91%	68%	72%	86%	77%	79%	88%	79%	85%	94%	92%	
D12-peryle	0%	0%	86%	74%	34%	67%	73%	86%	83%	83%	89%	82%	93%	101%	96%	

GC blank ..... slepý vzorek přístroje GC-MS - nástřik čistého rozpouštědla do plynového chromatografu

Lab. blank ..... laboratorní slepý vzorek - analyzovaný celým analytickým postupem s čistými rozpouštědly a všemi použitými materiály

GPC blank ..... slepý vzorek GPC chromatografu

blank, GF blank ..... terénní slepé vzorky - pasivní odběr na polyuretanovou pěnu a skleněné vlákno

CRM ..... analýza certifikovaného referenčního materiálu

RM ..... analýza laboratorního referenčního materiálu

NQ ..... nekvantifikováno - analyt byl překryt interferentem

LOQ ..... meze stanovitelnosti

PAHs in Ambient Air - Košetice 1996-2004
Weekly Sampling - Gas Phase

Legend:
- Benzo[g,h,i]perylene
- Dibenzo[a,h]anthracene
- Indeno[1,2,3-cd]pyrene
- Benzo[a]pyrene
- Benzo[k]fluoranthene
- Benzo[b]fluoranthene
- Chrysene
- Benzo[a]anthracene
- Pyrene
- Fluoranthene
- Anthracene
- Phenanthrene
- Fluorene
- Acenaphthene
- Acenaphthylene
- Naphthalene

PAHs [ng/m3]

Sampling Date
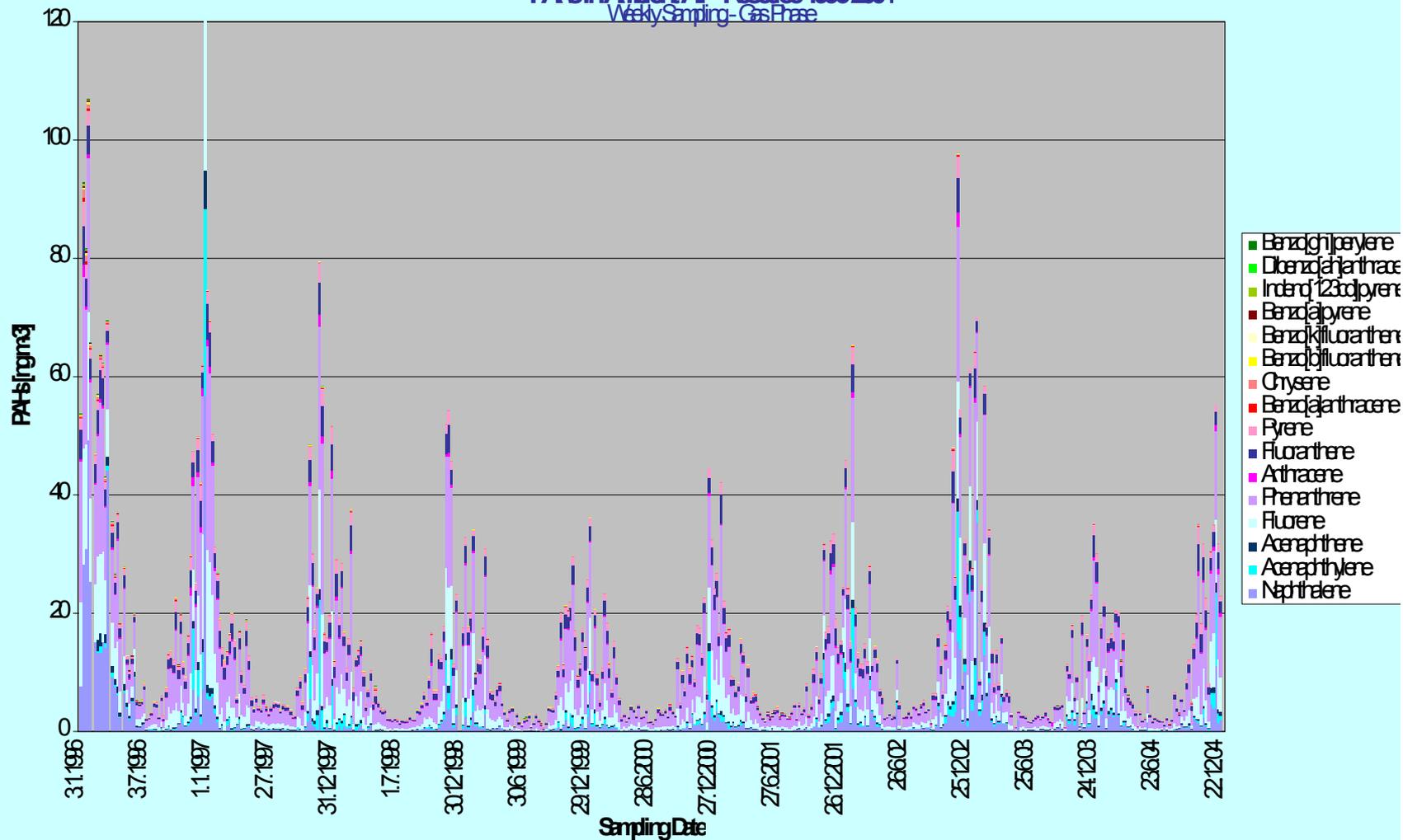
What does it mean "advances" in analytical chemistry?

- **New types of pollutants (not necessary persistent)**

- **New types of combined techniques for instrumental analysis**

- **Analysis of bioavailable ot toxic fractions rather than total analysis (selective sampling or extraction techniques)**

- **Interdisciplinary relations, application of methods from other fields of science (biochemistry, molecular biology, photochemistry, geology, mineralogy, geochemistry)**

- **Summer school 2005**

- **Environmental analytical chemistry**

- **Determination of persistent organic pollutants**

- **GC-MS analysis of polycyclic aromatic hydrocarbons in the air samples**

- 1. High volume air sampling using quartz and polyurethane foam filter
- 2. Automatic extraction of the exposed filters on a Soxtec extractor
- 3. Raw extract clean up and fractionation by a column chromatography
- 4. GC-MS determination of PAHs
- 5. Quantification of GC-MS chromatogram and evaluation of results

1. High volume air sampling using quartz and polyurethane foam filter

Material and chemicals:

- · High volume sampler PS-1
- · Whatman quartz filter (QF) and polyurethane foam filter (PUF), wrapped in two layers of aluminum foil
- · Laboratory gloves
- · Aluminum foil, zip-lock bag
- · Sampling protocol

Operational procedure:

- · Transport the sampler to the sampling site and plug in (220v)
- · Mark the filter cleaning date, beginning of sampling, and the status of the sampler timer to the sampling protocol
- · Insert the filters to the sampler head
- · Switch on the sampler and write down to the protocol the value of the sampler inlet suction pressure
- · After the sampling period, mark the sampler inlet suction pressure value to the protocol again, and turn off the sampler
- · Complete the sampling protocol with the last value of the sampler timer and the time when the sampling was terminated
- · Take the exposed filters out from the sampler head and wrap them into two layers of aluminum foil and a zip-lock plastic bag
- · Transport the filters to the lab in cooler and store them in a freezer (-18 C)
- · Calculate the volume of the air taken by the sampler from a calibration curve, the average inlet pressure value, and the sampling period, mark all the data to the sampling protocol
- · Transport the high volume sampler to the storage place

2. Automatic extraction of the exposed filters on a Soxtec extractor

Material and chemicals:

-
- · Buchi automatic extractor, extraction cartridge
- · Vials (22 ml)
- · Automatic pipette
- · Pasteur pipette
- · Dichloromethane
- · Surrogate recovery standards (D-PAHs, 4 µg/ml)

Operational procedure:

- · Take the quartz filter and polyurethane foam filter out of the foil and spike them with 50 µl of surrogate recovery standard solution
- · Insert each filter separately to the extraction cartridge
- · Fill the bottom vessel of an extractor with dichloromethane (120 ml), add the boiling stones
- · Insert the cartridge with the air sample into the automatic extractor
- · Open the cooling water tap, start an extraction with the program no. 1 (hot extraction - 40 minutes, rinsing - 20 minutes)
- · Take a cartridge with the filter out of an extractor after the program was completed, and concentrate the extract to the final volume of 5 ml using the program no 2.
- · Transfer the extract into the 22 ml-vial using a Pasteur pipette, rinse an extraction vessel twice with a small amount of dichloromethane, and add it to the sample
- · Concentrate an extract further under gentle stream of nitrogen to the final volume of 1 ml
- · Close the vial and store it at a fridge for the further analysis

3. Raw extract clean-up and fractionation by a column chromatography

Material and chemicals:

- · Glass column
- · Vial (22 ml)
- · Mini-vial for GC analysis (2 ml)
- · Calibrated cylinder (25 ml)
- · Pasteur pipette
- · Automatic pipette
- · Cotton (pre-cleaned in dichloromethane)
- · Activated silica gel (pre-cleaned in dichloromethane, activated for12 hours at 150 ℃)
- · Non-activated silica gel (pre-cleaned in dichloromethane)
- · Dichloromethane, hexane
- · Internal standard (terphenyl, 4 µg/ml)

Operational procedure:

- · Add non-activated silica gel into the extract to get the loose consistency of the sample
- · Prepare the glass column:
- o Put a cotton ball to the bottom of a column
- o Add 5 g of an activated silica gel, let it settle by tapping a column with a stick
- o Deposit a sample on the top of silica gel in a column, tap a column again
- · Perform a selective elution: hexane (10 ml) for alkane elution, dichloromethane (20 ml) for PAHs
- · Concentrate the PAHs containing fraction under the gentle stream of nitrogen to the final volume of 1 ml
- · Transfer the sample to the autosampler mini- vial (2 ml)
- · Add an internal standard (terphenyl, 50 µl)
- · Close the vial and store in a fridge until the final analysis on GC-MS

- **4. GC-MS determination of PAHs**

- **Material and chemicals:**

- Gas chromatograph HP 6890 with the mass selective detector, cyclohexane

- **Operational procedure:**

- · Position the vial with the sample to the autosampler of a gas chromatograph
- · Fill the vials on the turret with cyclohexane (a solvent for a syringe needle rinsing)
- · Check on the status of the machine, column, inlet system
- · Open the Chemstation software - Instrument, load DPH-L-AS method
- · Get yourself familiar with the method (inlet, oven, column, modes, SIM)
- · Fill the sequence information
- · Run the analysis

- **5. Quantification of GC-MS chromatogram and evaluation of results**

-

- <u>**Material and chemicals:**</u>

-

- PC with HP Chemstation software

-

- <u>**Operational procedure:**</u>

-

- · Open the Chemstation software – Data analysis

- · Learn about the software, evaluation of chromatogram, mass spectra, spectral library

- · Load the file with proper data, load DPH 53 method

- · Get yourself familiar with the method: calibration table, curves, internal and recovery standards, retention times, target ions and qualifiers, quantification

- · Quantify the results of the air samples

- · Print the chromatogram and a custom report for your record

-