Chapter 3

CRITERIA FOR CHEMICAL METHODS DEVELOPED AND INVESTIGATED IN THIS STUDY

ANALYTICAL DESIGN FEATURES

There is a constant demand for screening methods capable to analyse at lower levels, with shorter turnaround times and lower analysis cost. Methods should be reliable enough to characterise the type and source of contamination, as this forms the basis of sound decisions and action required to protect public health and to improve the quality of the environment. High quality environmental measurements are required for a number of purposes, such as:

- Compliance with legislation
- Characterisation of hazardous waste sites
- Monitoring of the effectiveness of measures taken to reduce contamination
- Monitoring of site remediation
- Decisions and actions regarding waste disposal
- Studies related to the degradation of PAHs in the environment

The need for analytical methods that can provide expedited characterisation of hazardous waste sites is critical. Site remediation is often delayed during the site characterisation step because of slow turnaround times of sample analyses. In addition, once a sample is removed from the location, its chemical integrity is always a concern. Methods that minimise sample handling and transport are needed to improve data quality. The analytical methodology developed in this study is designed to fulfil these needs.

To achieve the objectives for a high quality chemical analysis that is essential for a successful fingerprinting strategy, four important features must be taken into account:

1. Selection of the specific constituent target analytes
2. Selection of analytical methods and performance characteristics
3. Data use, interpretation and assessments
4. Quality assurance and quality control

The close relationship between sampling, analytical determinations, data evaluation and interpretation and environmental management is shown is **Figure 3.1**.

Selection of specific constituent target analytes

This study is focused on coal tar polluted water and soil samples, which contains a heterogeneous mixture of PAHs, alkyl-PAHs and heterocyclic compounds. The analytes targeted for this study are those listed in **Table 2.1.** In the case of the alkyl-PAHs a C_1-PAH indicates a single methyl group attached to the specific PAH, a C_2-PAH the sum of all dimethyl or ethyl isomers and a C_3-PAH the sum of all trimethyl, methylethyl and propyl isomers.

Selection of analytical methods and performance objectives

Contaminated coal tar samples can also contain refined petroleum products (diesels, mineral oils, fuel oils, and lubricating oils). These compounds are co-extracted with the PAHs because they are non-polar and can cause matrix interference.

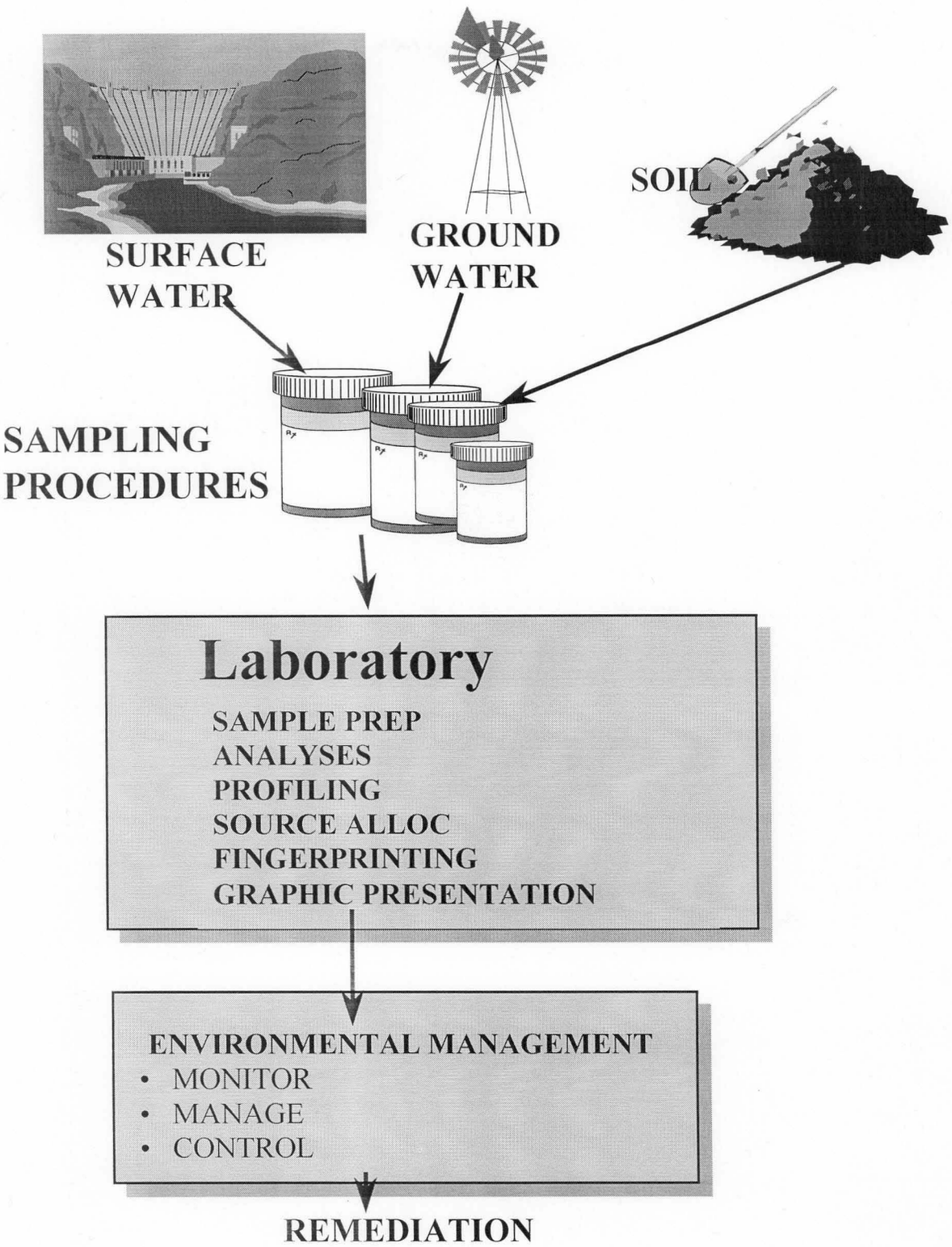

Figure 3.1: Relationship between sampling, the laboratory and Environmental Management

Because the range of matrices in environmental samples is very large it is difficult to develop a "tailor made" strategy for each case. A basic requirement from a quality point of view is that the overall trueness and precision must be adequate for the objective of the measurement. The selection of the analytical method then also depends on the regulatory requirements and other data interpretation objectives. The existing USEPA method were used as a guideline for this study, but considering the inadequacies that were discussed in **Chapter 2**, attention was given to specific modifications and refinements for methods to be more suitable for the characterisation of coal tar pollution. New methods were developed using SPME-GC/MS, mainly because of the advantages associated with this technique, namely simplicity, efficiency, selectivity and sensitivity. Modified and newly developed methods were validated to ensure that it is fit for the intended purpose and a range of performance characteristics was investigated for this purpose. Traceability to a recognised reference (pure substance or certified reference material) was demonstrated where possible.

Criteria for Data Interpretation and Assessment Use

Chemical Characterisation

As shown earlier, the detection of C_1-, C_2- and C_3-isomers in an alkyl homologous series plays an important role in the development of source or weathering ratios. Another assessment use of isomer data is to compare the chemical composition and concentration of spilled, unweathered coal tar with the composition and concentrations of the residual coal tar in the environment. Analytical data obtained from these comparisons allow the analyst to trace the fate, weathering, and environmental partitioning of different fractions of the tar and predict the potential long-term impact of the spilled tar. Reliable determination of trace level contamination is required for these purposes. The objective was, therefore, to achieve a high degree of specificity to distinguish the isomers in an alkyl homologue from other interfering analytes or other compounds, with the required sensitivity.

Hazard Identification

One of the objectives for environmental measurements is to check compliance with legislation. The standard used for hazard characterisation this study is based on the National Primary Drinking Water Regulations and Health Advisories of the USEPA[23] and the Agency for Toxic Substances and Disease Registry (ATSDR)[24]. Health risk based guideline concentrations of PAHs in water can be calculated on an age-weighted exposure distribution. For a lifetime cancer risk of one in a million, the risk-based concentration for benzo[a]pyrene is 0.01 ng/cm^3. For non-carcinogens the guideline concentrations are normally higher, based on exposure to a reference dose (RfD). For naphthalene, for example, the guideline value is 1500 ng/cm^3. Limits for the PAHs that are listed in these regulations are summarised in **Table 3.1.** The table shows data that are available for some parent PAHs, and except for 2-methyl naphthalene, no data are available for alkyl-PAHs. The data was used as a guideline in the development of analytical methodology, where the objective was to achieve a quantification limit of individual PAHs that is lower than the guideline levels specified in the USEPA regulations. For the purpose of a health risk assessment and chemical fingerprinting a high degree of sensitivity and low limits of detection are necessary. Due to the toxicity of, for example, the PAH reference compound benzo[a]pyrene, a detectable concentration of at least 0.01 ng/cm^3 in groundwater samples is required[18]. The methods were therefore developed to meet these sensitivity requirements.

Table 3.1: Drinking Water Regulations and Health Advisories of the USEPA and ATSDR

CARCINOGENS	NONCARCINOGENS	1999 Rank as a ATSDR Priority Hazardous Substance	Cancer Group [a]	Drinking Water Standard MCL ($\mu g/cm^3$) [b]	HEALTH ADVISORIES FOR DRINKING WATER (70 kg Adult)		
					ATSDR MRLs mg/kg/day [c]	USEPA RfD mg/kg/day [d]	USEPA guideline concentration for a 10^{-6} Cancer Risk (mg/cm^3) [e]
	Anthracene	-	D	-	10	0.3	11000
	Acenaphthene	159	D	-	0.6	0.06	2200
	Fluorene	275	D	-	0.4	0.04	1500
	Fluoranthene	101	D	-	0.4	-	1500
	1-methyl naphthalene	-	-	-	0.07	-	-
	2-methyl naphthalene	-	-	-		-	1500
	Naphthalene	75	D	-	0.02	0.02	1500
	Phenanthrene	216	D	-	-	-	1500
Chrysene		115	B2	-	-	-	2.09
Benzo[g,h,I]perylene		-	-	-	-	-	0.42
Pyrene		253	D	-	-	0.03	0.11
Benz[a]anthracene		35	B2	-	-	-	0.092
Benzo[b]fluoranthene		10	B2	-	-	-	0.092
Indeno[1,2,3-cd]pyrene		185	B	-	-	-	0.092
Dibenz[a,h]anthracene		17	B2	-	-	-	0.0092
Benzo[a]pyrene		8	B2	0.0002	-	-	0.0092

Notes:

[a] - Weight of evidence = EPA class designating overall evidence that a substance causes cancer in humans
A = Known human carcinogen
B1 = Probable human carcinogen, limited human data
B2 = Probable human carcinogen, inadequate or no human data
C = Possible human carcinogen
D = Not classifiable as human carcinogen
E = No evidence of carcinogenicity for humans

[b] – Maximum contaminant level (MCL) - The maximum permissible level of a contaminant in water which is delivered to any user of a public water system. MCLs are enforceable standards.

[c] – Minimal Risk Level (MRLs) for Hazardous Substances. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure.

[d] – Reference Dose (RfD). An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime.

[e] – Guideline 10^{-6} Cancer Risk. Health risk based guideline for drinking water corresponding to an estimated lifetime cancer risk of 1 in 1,000,000.

[f] – Lifetime Consumption. The concentration of a chemical in drinking water that is not expected to cause any adverse noncarcinogenic effects for a lifetime exposure.

Quality Assurance (QA) and Quality Control (QC) Objectives

The awareness of QA for environmental measurements has increased considerably during the past few years. This is mainly due to the fact that inaccurate environmental analyses can lead to severe economic and social implications, such as undetected hazards or identification of unreal hazards. The analytical methods developed in this study were required to have certain performance characteristics and to conform to the following Data Quality Objectives (DQO), as summarised in **Table 3.2**.

Table 3.2: Data-quality objectives for groundwater and soil studies

PARAMETER	EXPLANATON OF OBJECTIVES	Data Quality Objectives (DQO) required for this study	Comparative Standard Method DQO's (USEPA, 1986, SW 846 Method 8270)
Accuracy or trueness of the measurements	The main objective was to establish the true concentration of contaminants at low levels and in complex matrixes. CRMs were used to determine the recoveries of PAHs in soil extracts. Spiking techniques were used to determine recoveries for water samples, in which case CRMs are unavailable.	80 - 120% recovery for individual parent PAHs	18 – 137% recovery for p-Terphenyl-d_{14}
Precision	To obtain agreement of measurements under specific conditions, using the same instrument, same analyst and analysing samples in batches.	< 15% RSD	N/A
Quantification limit (QL) for individual PAHs	A Quantification limit of ten times lower than the MCL specified by the USEPA was required.	30 pg/cm^3 (water) 3 µg/kg (soil)	-- 660 µg/kg
Method detection limit (MDL)	Detection limits were specified for each individual PAH (see Table 1.1)	10 pg/cm^3 (water) 1 µg/kg (soil)	 66 µg/kg
Procedural blank	Procedural blanks were used to check the background levels. To ensure reliable results, procedural and field blanks were limited to a maximum concentration of ten times the method detection limit.	10 x MDL	MDL
Duplicate precision	Field replicates were used to control the representativeness, with the maximum relative percent of duplicate values within ± 30%.	< 35 % RSD	N/A
Calibration: %RSD of the RFs	In the case of groundwater analysis the instrument calibrated was optimised in the lower concentration range due to the poor solubility of four- and five-ring PAHs. Relative response factors for individual PAHs are required to have a maximum RSD of 30% over the linear range of the calibration.	< 30 % RSD	N/A
Selectivity	The objective was to distinguish the target analytes from matrix compounds that may have concentrations of up to orders of magnitude higher than the target analytes.	No peak overlap from co-eluting compounds giving mass fragments at the selected mass	N/A
Specificity	A differentiation among various isomers is desired.	Baseline separation	N/A

Quality control procedures

The reliability of the GC/MS method was improved by employing the following quality control procedures before every batch of samples.

GC/MS performance validation

The mass spectrometer was tuned regularly for maximum sensitivity and resolution using a standard tuning procedure, which can be summarised by the adjustment of the following parameters:

Resolution: The optimum resolution was adjusted on the 130 and 131 peaks of the calibration gas mixture of the Saturn 2000 ion trap, by adjusting the axial modulation amplitude.

Sensitivity: The optimum sensitivity was adjusted by setting the RF modulation response to 763 (highest) and 361 lowest, and the filament emission current to 15 µA. The multiplication voltage was tuned automatically by the instrument software to obtain a voltage that is high enough to produce 10^5 electrons from one ion.

Mass calibration: A mass calibration was performed weekly or when the operator manually changes the:

- ionisation time. The ionisation time is normaly computed and set automatically via the automatic gain control (AGC).
- Axial modulation voltage. The A/M voltage must be adjusted to the proper value before a mass calibration. If the voltage is too low, high molecular weight ions will not be observed. If the voltage is too high, the peak width for low molecular weight ions will be broadened and mass misassignments may occur.
- ion trap temperature.

Trap function calibration: This calibration was performed weekly.

SIS calibration: The single ion storage (SIS) amplitude was checked on a regular basis to ensure optimum performance in the SIS mode. SIS eliminates unwanted ions from the trap. Trapped ions exhibit a characteristic frequency of oscillation. This frequency depends on the mass of the ion and the amplitude of the fundamental storage rf field.

After completion of the tuning procedure, the performance verification was then performed using a 40 µg/cm^3 PAH standard. The chromatogram was checked for correct retention times, resolution, peak areas and the mass spectrum was checked for signal to noise ratios and peak intensities.

Instrument calibration and verification procedure

The typical calibration standards for syringe injections were 20, 40, 60, 80 and 160 µg/cm^3 PAHs in water, with 20 µg/cm^3 deuterated PAH internal standards at each calibration level. Calibration standards were run with 2µl injections of each standard containing parent PAH analytes and internal standards. The Calibration data were checked for linearity and relative standard deviation (RSD) and corrections made. The maximum allowable RSD was 30% for the response factors (RF) over the linear range for all target compounds with a minimum RF of 0.05 (response relative to internal standard). The typical calibration standards for SPME analyses were 2, 4, 6 and 8 ng/ml PAHs in water, with 8 µg/ml deuterated PAH internal standards at each calibration level. Calibration standards were run using a sample size of 1.2 cm^3 containing parent PAH analytes and internal standards. The Calibration data were checked for linearity and RSD and corrections made. The maximum allowable RSD was 30% for the response factors over the linear range for all target compounds with a minimum RF of 0.05. A verification standard was run in between samples to check the calibration before and after a

maximum of ten samples using a mid-range standard. Results were checked to be within 30% of the expected values. The software automatically performs the concentration calculations of the verification standard. The instrument was re-calibrated where necessary. Checking the internal standard peak areas for each analysis to be within + 75 and – 75% of those in the daily calibration check, and within a given retention time window of 20 seconds, checked the instrument stability.

Control samples - spiked water sample

A 1000 cm^3 of pure water was spiked with 5 µl of the 2000 $\mu g/cm^3$ PAH standard to obtain a concentration of 10 $\mu g/cm^3$. About 10 cm^3 of methanol was added to keep the PAHs in solution. The control sample was analysed using the standard procedure after every calibration and after every ten samples.

Quantitative analysis using GC/MS

Gas chromatographic separation was carried out using a DB-5 non-polar stationary phase. The mass spectrometer was operated in the full scan mode where all masses between 45 and 450 are acquired or in the SIS mode where only the specified analyte masses are acquired. Quantification of the 16 EPA priority PAHs were performed using:

- the quantification ions specified in **Table 5.1, Chapter 5.**
- response factors (RFs),
- peak areas
- calibrating on the deuterated internal quantification standards
- a linear curve fit, forced through the origin.

The deviation and calibration range tolerance was set at 30%. For the identification of the target analytes, the following criteria were used:

- Retention time window = (±) 0.200 minutes.
- Mass spectrum match threshold = 700
- Minimum peak area = 1000
- Report threshold = 0.10 ng/cm^3
- Signal to noise ratio of the selected ion current = > 3:1
- Maximum uncertainty of the ratio between the molecular ion and qualifier ions = 20%.

Presently alkyl PAH standards for each alkyl group of interest are not commercially available. RFs are, therefore, specified for each degree of alkylation by assignment of the RF of the next closest alkyl homologue group. They are then quantified by grouping the peak areas of individual isomer of each level of alkylation and using the specified RF.

ANALYTICAL CONDITIONS

The following analytical GC/MS conditions were used throughout this study, unless otherwise indicated:

Module: Saturn 2000.40 Mass Spectrometer

Saturn GC/MS Workstation Version 5.2.1
Module Software Version: FF0D
Module Option Keys: EI SIS MS/MS
Setpoints
Trap Temperature: 150 degrees C
Manifold Temperature: 35 degrees C
Transfer Line Temperature: 300 degrees C
Axial Modulation Voltage: 2.8 volts
Air/Water Check
Mass 28 Peak Width: 0.8 m/z
Mass 19 to Mass 18 Ratio: 14.3%
Total Ion Count: 3158 counts
Integrator Zero Set
DAC Setpoint: 100 DACs
Average Counts: 0.5 counts
Electron Multiplier Set
10^5 Gain Value: 2050 volts
Final Gain Value: 2050 volts
RF Full Scale Adjust
DAC Setpoint: 132 DACs
Calibration Ion Used: 614 m/z
Mass Calibration
Method: FC-43

Ion Mass	Apex	Ion Intensity
28	175.8	137
69	433.1	1510
131	822.2	560
264	1658.5	378
414	2601.2	108
464	2916.2	42
502	3155.8	118
614	3865.5	21

Average Calibration Slope: 6.263 DAC/m/z
Standard Deviation: 0.037

Trap Function Calibration
Mass 69 Frequency: 258.900 kHz
Mass 131 Frequency: 257.400 kHz

SIS Calibration
Amplitude Adjust Factor: 60%

Calibration Gas Adjust
Ionization Time: 550 uSeconds
Total Ion Count: 3726 counts

RF Tuning Adjust
Highest Count: 739 counts
Average Count: 351 counts

Segment Number 1:
Description: FIL/MUL DELAY
Emission Current: 0 microamps

Segment Number 2:
Emission Current: 15 microamps
Mass Defect: 0 mmu/100u
Count Threshold: 2 counts
Multiplier Offset: 0 volts
Scan Time: 0.770 seconds
Segment Start Time: 3.00 minutes
Segment End Time: 42.00 minutes
Segment Low Mass: 45 m/z
Segment High Mass: 450 m/z
Ionization Mode: EI AGC
Ion Preparation Technique: NONE

EI-Auto Mode:
Maximum Ionization Time: 25000 µs

Mass Range	Ion. Storage Level	Ion. Time
Scan Segment 1:		
10 to 99	44.0 m/z	100%
Scan Segment 2:		
100 to 199	44.0 m/z	140%
Scan Segment 3:		
200 to 399	44.0 m/z	120%
Scan Segment 4:		
400 to 650	44.0 m/z	35%

Target TIC: 20000 counts
Prescan Ionization Time: 100 µs
Background Mass: 43 m/z
RF Dump Value: 650.0 m/z

Module: 3800 Gas Chromatograph

Front Injector Type 1079

Temp (C)	Rate (C/min)	Hold (min)	Total (min)
300	0	42.00	42.00

Time (min)	Split State	Split Ratio
Initial	On	25

"Advanced Flow Control" for 1 cm^3/min constant flow with pressure pulse injection

Pressure (psi)	Rate (psi/min)	Hold (min)	Total (min)
15.0	0.00	0.10	0.10
7.8	20.00	0.01	0.47
11.8	0.40	0.00	10.47
15.8	0.29	0.00	24.26
19.2	0.34	0.00	34.26
22.6	1.13	4.00	41.27

Column Oven
Stabilization Time: 0.10 min

Temp (C)	Rate (C/min)	Hold (min)	Total (min)
60	0.0	0.01	0.01
130	7.0	0.00	10.01
200	5.0	0.00	24.01
260	6.0	0.00	34.01
320	20	4.80	41.81