Framework Foundation and Guidance For Tier 2 Site-specific Development of Soil Contact Standards for PHC-contaminated Sites: Literature Review

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EXCUTIVE SUMMARY

In June 2001, the Canadian Council of Ministers of the Environment (CCME) released the new Canada-wide Tier 1 Standards for petroleum hydrocarbons (PHCs) in soils. These standards serve as soil quality criteria to assist with the management of site soils contaminated with petroleum hydrocarbons. The ultimate goal of these standards is the protection of human health and the environment. The assessment and management of petroleum hydrocarbons in soils in Canada are the result of the implementation of a three-tiered framework. The objective of this document is to provide a framework for site-specific development of Tier 2 soil contact remedial standards for PHC-contaminated sites, in the event that Tier 1 screening levels are exceeded.

This project was divided into two phases. Phase 1, of which the results are discussed here, comprises a comprehensive review of the existing literature regarding: the fate of PHCs in soil, including specific characteristics that influence bioavailability; the sequestration of different PHC fractions in soil; the tools used to measure bioavailability of PHCs in soil; and, the relationship between acute and chronic estimations of toxicity for soil invertebrates exposed to PHC fractions of crude oil. Phase 2 comprises the development of guidance for a Tier 2 ecotoxicity assessment of site soils and includes guidance for the collection of soil samples for toxicity assessments; a toxicity assessment framework for contaminated site soils; a recommended test battery (test species and methods); procedures to deal with potential PHC mixtures in soil; and, because of the paucity of data, it does not include a discussion of the use of crop yields as an assessment endpoint for development of Tier 2 soil standards. The guidance for Phase 2 is presented in a companion report titled, "Framework and Guidance for Tier 2 Site-specific Development of Soil Contact Standards for PHC-contaminated Sites: Ecotoxicity Assessment". Therefore, the deliverables for this study are two reports that will serve as a framework for the development of a cost-effective approach to a Tier 2 assessment of site soils contaminated with petroleum hydrocarbons.

Derivation of the ecological benchmarks for the soil contact exposure pathway in the development of the Canada-wide (CW) Standards for Petroleum Hydrocarbons in Soil (PHC CWS) was based primarily on data from controlled laboratory ecotoxicity tests with fresh product (e.g., crude oil and three fractions of crude oil). As with other benchmark values, the derivation process for the CW Tier 1 soil contact standards for the petroleum hydrocarbons in soil assumes 100% bioavailability of the product as determined by the total soil concentration of each petroleum hydrocarbon (PHC) fraction measured in soil, using standardized procedures involving solvent extraction techniques. These benchmarks are useful as a "worse-case" scenario and, if these values are met, the risk associated with the contamination at the site is considered acceptable (i.e., minimal). However, the toxicity of fresh product to terrestrial organisms is a function of exposure concentration, exposure duration, and the bioavilability of the PHC constituents comprising the contamination. These benchmarks might overestimate the risk associated with the PHCs in soil at historically contaminated sites simply because the PHCs present in soils at these sites might not be bioavailable. Because the bioavailability of various PHCs can be affected by both "weathering" and "aging" processes, as well as the physico-chemical properties of soil, bioavailability is a key consideration in the context of the standards applicable at higher tiers of the assessment framework.

Scope of the Report

The literature on the bioavailability of petroleum hydrocarbons in soil is discussed in terms of the fate processes and mechanisms that influence the changes in constituent composition and bioavailability of PHCs in soil. The soil and site characteristics that affect or have the potential to influence PHC bioavailability also are discussed and, where possible, evidence for these effects presented. The methods to measure bioavailability have been summarized and different passive sampling devices compared. A section of the report contains a discussion of the practicality of applying acute to chronic ratios of toxicity

to contaminated lands and the final section discusses sampling, collection, handling, and storage of soil samples destined for toxicity assessment.

Fate of Petroleum Hydrocarbons in Soil and Bioavailability

Petroleum hydrocarbons are, for the most part, complex petroleum-based chemical mixtures. Two major changes occur over time after a PHC product has been introduced to soil: 1) a change in the relative constituent composition of the contaminant mixture (weathering); and, 2) a change in the bioavailability of the individual constituents (aging). The fate and behaviour of PHCs in soil depends on the proportional representation of the individual constituents and their physical and chemical properties, as well as, the biotic and abiotic degradation processes that occur within the contaminated soil.

Bioavailability of the individual constituents over time is governed primarily by sorption and/or sequestration into remote, inaccessible (for organism uptake or degradation) regions of soil particles or organic matter. Although both processes involve sorption mechanisms (e.g., adsorption/absorption and desorption), the kinetics of sequestration desorption are very slow; desorption can be so slow that it is considered to be virtually non-existent. These processes occur simultaneously and ultimately govern the availability of contaminants to ecological receptors.

Sorption mechanisms are affected by both the organic or inorganic mineral particles present in the soil. The porosity of inorganic matter plays a significant role in the bioavailability of contaminant molecules and their movement within the soil. Contaminants can become sorbed to internal surfaces of soil particles and organic matter, thus decreasing bioavailability, and there are processes that result in the desorption of contaminants from these surfaces, whereby they become bioavailable again. Contaminant molecules can travel to more remote sites within the inorganic matter and soil particles via diffusion through nanopores (partitioning) that ultimately reduces their bioavailabilty. Moreover, an increase in organic matter content in soil can result in hydrophobic surfaces within these pore spaces that can increase their sorption and retention properties.

Organic matter and soils are heterogeneous. In addition to partitioning theory, sorption to these substrates can be explained by mechanistic models that involve site-specific phenomena. One mechanism is the dual-mode sorption model, whereby contaminant molecules can be distributed to various voids or 'holes' in the organic matter and become entrapped in these sites via adsorption-like interactions. Desorption of entrapped molecules from these holes is very slow thus, although the contaminants are present in soil, they are not bioavailable. A second mechanism is the distributed reactivity model, which suggests there is a physico-chemical change in the organic matter that does not allow desorption of the entrapped molecule. This complex model addresses both the labile and nonlabile compartments; however, most studies indicate something more than a hole-filling mechanism must be occurring. Researchers suggest the sorption of nonpolar contaminants onto high surface area carbonaceous materials to account for fate phenomena, while others suggest sorption of polar molecules to localized sorption sites. Suffice it to say that the mechanisms/models to explain the phenomena of sequestration are not well understood; however, they are discussed in greater detail in Subsection 2.3 of the report.

The interaction of PHCs with soils can result in the formation of non-extractable residues, whereby PHCs covalently bind with organic matter. This process is mediated by microbial activity. These residues are usually considered to be unavailable; however, there is some evidence to suggest that non-extractable residues can eventually be mobilized and become available biologically or chemically.

Many factors can contribute to site-specific changes in PHC bioavailability in soils. Some factors include: soil texture, soil structure, the presence of iron oxides, nature of organic matter, pH, temperature, moisture regime, presence of co-contaminants or non-aqueous phase lipids, salinity, cation exchange capacity, and contaminant properties. Each factor is discussed as it relates to PHC bioavailability. The research, completed to date, suggests that no one factor dominates and multiple factors play significant

roles in site-specific bioavailability with respect to PHCs. Factors such as environmental conditions or weathering, soil characteristics, and route(s) of exposure can ultimately influence and change availability of contaminants via intermediate interaction processes. In short, one must consider multiple factors, some increasingly complex, when determining overall bioavailability of petroleum hydrocarbons.

Chemical and Biological Measures of Bioavailability

Most methods for determining bioavailability of soil contaminants rely on chemical extraction of the readily desorbed fraction of the contaminant(s) from soil. Bioavailability is both species and soil dependent and, as such, no one chemical extraction method will be predictive for all scenarios. The most commonly used methods to measure PHCs in soil involve extraction procedures with solvents, such as acetone and hexane solutions, to obtain the highest percentage recovery. These methods of analyses use exhaustive extraction procedures designed to measure the PHCs that are present in soil and they do not focus on those contaminants that are bioavailable only. There are several non-exhaustive methods developed and tested with petroleum hydrocarbons; however, studies demonstrating a relationship between biological effects (e.g., toxicity) and concentrations determined using non-exhaustive extraction methods are lacking.

Solid phase extraction is one of only two chemical methods that have been extensively validated with historically contaminated (aged) samples of soil. During solid phase extraction (SPE), organic contaminants are extracted from soil using chemical concentration gradient desorption to the aqueous phase followed by sorbtion to hydrophobic polymers that are added to the aqueous soil slurry. However, there are limitations to SPE techniques that can result in either the over-estimation or under-estimation of the amount of PHCs that are bioavailable to organisms. The other validated method, not based on extraction, but involves oxidation by persulfate of bioavailable contaminants, presumes only those PHCs in soil that are bioavailable can be oxidized. This method has only been tested using polycyclic aromatic hydrocarbons, and not with other petroleum products.

Other non-exhaustive extraction methods include the use of *n*-butanol, ethanol:water, methanol:water, and 0.5M NaOH solvent extraction, cyclodextrin macrocyclic compounds, Tenax® beads, super-critical fluid, and subcritical water. Although these methods appear promising, further research is needed to validate and standardize these techniques in terms of their application to soils contaminated with petroleum hydrocarbons.

Biological measures of bioavailability involve using organisms directly for uptake and tissue residue studies, and indirectly through toxicity to terrestrial organisms or changes in microbial activity. Basically, any validated biological test method can be used, but relevance and common sense should be considered. Test organisms include natural assemblages of microbial organisms, soil invertebrates, plants, and even mammals. Bioavailability can vary between organisms and among species. Likewise, routes of exposure and uptake need to be accounted for. Thus, a test battery, using organisms from various ecological niches is recommended. Also, tests must be designed to incorporate possible indirect effects, in order not to over-estimate bioavailability.

The present literature review of current field investigations focused on those evaluating mechanisms of sorption and sequestration, factors affecting bioavailability and methods for estimating bioavailability. Typically, changes in bioavailability are inferred from estimates of soil toxicity at a particular point in time and are rarely measured directly. Several studies have found a decrease, over time, in the bioavailability of different carbon-range fractions of PHCs in soils. Comparisons between field-aged PHC soils and fresh product in laboratory tests showed generally less toxicity to organisms in the field investigations. This suggests that aged PHCs are likely less bioavailable than PHCs as fresh product.

Determining the levels of the biologically available fraction of organic compounds is crucial to the complete assessment of the risks associated with contaminated soils. Passive sampling devices (PSDs) have been recently used to accomplish this. However, limited work has been done to relate the bioavailable fractions that are concentrated within the PSDs to levels associated with adverse biological responses in terrestrial organisms. The efficacies of PSDs, specifically solid phase microextraction (SPME) and semipermeable membrane devices (SPMDs) were compared with respect to their ability to determine the bioavailability of organic compounds in soils, and to correlate the measured levels to those that cause adverse biological effects in earthworms as derived from biological assays. Although there has been substantial research devoted to these methodologies, the results remain inconclusive. Difficulties inherent in the calibration of the systems make the generation of meaningful results challenging.

Passive sampling devices (PSDs) provide an adaptable, cost-effective, portable, and user-friendly method to assess bioavailability of organic contaminants in soils. PSDs are specific to a particular class of compounds (e.g., non-polar chemicals) and, if used properly, they can be used as an initial screening tool. There are basically two passive sampling devices that have been used to assess contaminated soils. The solid phase micro-extraction (SPME) device utilizes a reusable fused-silica fiber that is coated with a non-polar organic phase with a high selective affinity towards organic compounds and as such, they extract and concentrate the analytes of semi-volatile and volatile organic compounds in aqueous and atmospheric samples in one simple step. This method allows for quantification of headspace versus matrix analytes limited only by mass transfer of the analyte within the matrix. The device can increase in applicability by changing the coating specific to contaminants of interest and variations to sampling technique will improve sensitivity. Semipermeable membrane devices (SPMDs) were developed to concentrate several hydrophobic organic compounds from soil, air, or water to a lipid-filled thin-walled, low-density polyethylene (LDPE) dialysis tube similar to the partitioning of organic molecules into endogeneous lipids. Sampling with SPMDs can allow for equilibration within the media (up to several weeks) resulting in time-integrated accumulation. Calibration of the methods, in consideration of the soil properties (e.g., moisture content, organic matter content, porosity), as well as the properties of the chemical(s) or contaminant(s) is time consuming but essential for the generation of meaningful results. Both PSDs utilize either gas chromatography (GC), GC-MS, or HPLC to quantify analytes. Both SMPDs and SPMEs are limited in that they only measure the parent molecule of a contaminant.

Initial field and laboratory studies using both SPMEs and SPMDs to estimate the bioavailability of organic compounds in soil have varying results. SPMEs seem more adequate at detecting and discriminating between lethal and non-lethal concentrations in soil than SPMDs; however, additional research is warranted. Future applicability of SPME devices would require evaluations of efficacy under various soil moisture conditions and soil types.

As mentioned earlier, toxicity is often used a surrogate measure for bioavailability of organic contaminants in soil. However, toxicity is a complex and dynamic process resulting from the interaction of the exposure concentration, the exposure duration, and the bioavailability of the contaminant(s) in soil. Many indirect factors (physical, chemical, and biological) influence the toxicity of contaminants in soil; therefore, these factors should be considered in any risk assessment. Earthworms are a particularly sensitive group of soil organisms because of the nature of their interactions with soil; dermal exposure and ingestion of soil make them very susceptible to the bioconcentration of soil contaminants. Therefore, they are good candidate species for assessing the bioavailability of contaminants, especially metals, in soil. Tissue residues of metals are readily quantifiable. The problem with using tissue residues in earthworms as a surrogate measure for bioavailability of PHCs in soil is that tissue PHC residues are very difficult to distinguish analytically from natural hydrocarbons.

For aquatic organisms, the most widely used model to predict bioavailability and body burdens of contaminants in organisms is the equilibrium partitioning theory (EPT), which assumes uptake occurs via

passive diffusion. This assumption limits the application of EPT to terrestrial organism because other routes of uptake figure prominently. The application of EPT models to earthworms can underestimate actual accumulation levels for highly hydrophobic molecules that are ingested by earthworms. Moreover, some earthworms selectively feed on organic matter or are terrigenous (dirt eaters) and passive diffusion is unlikely to be the major route of entry.

Biological assays are most effective when used as an adjunct to chemical analyses. They reflect environmentally relevant levels of the contaminants in soil (i.e., the bioavailable fractions). The evaluation of contaminated soils typically incorporates a battery of terrestrial organisms, multiple endpoints, and a range of sensitivities. The derived effect-levels when related to the chemical measures of concentrations in soil are reflective of the "total" concentration of the contaminants in soil to which an organisms is exposed, and thus do not directly relate to the fraction of the contaminant that is bioavailable. In contrast, critical body residues and toxicity allow a more accurate estimate of the actual dose associated with a toxic response. Therefore, there is a need for the development of an appropriate tool that has the ability to measure the bioavailable fractions within the soil associated with an observed biological effect.

Acute to Chronic Ratios of PHC Toxicity

Risk assessments and decisions regarding the remediation and management of contaminated sites can be based, in part, on the information derived from toxicity tests. Toxicity tests can be either acute (short term) or chronic (long term). Acute tests can be either instantaneous or over a period of minutes to days, whereas chronic tests can entail exposure durations that are at least a tenth of the life span of a species. Long-term bioassay methods focus on sublethal endpoints (e.g., growth and reproduction) and are the most relevant from an ecological (assessment) perspective. In addition, chronic test methods with soils are considered to be more sensitive in assessing the toxicity of oil contamination than are acute tests. However, most ecological risk assessments are based on acute toxicity data because of the high cost and long duration of chronic tests. It would be advantageous to devise an approach whereby the results of acute toxicity tests could be used to “predict” the results of chronic toxicity tests. One such approach that has been used in the derivation of water quality criteria is the use of the acute-to-chronic ratio approach.

In its simplest form an acute-to-chronic ratio (ACR) is an extrapolation that allows the chronic toxicity of a compound or mixture of compounds to be derived from the results of acute toxicity tests. Such extrapolations should only be made for the same types of tests conducted under the same conditions. The theory behind the ACR concept is that for similar classes of chemicals and similar taxa, acute to chronic ratios established for one species or chemical can be used to estimate the chronic toxicity of the chemical to another species.

The most commonly used approach is the comparison of biological effect concentrations from acute and chronic tests. Several calculations are used to determine the acute to chronic ratio and most require sufficient data for proper prediction. To date, no studies have been conducted to which ACRs had been applied to model petroleum hydrocarbon fractions. Furthermore, few data exist for crude oil toxicity values. Therefore, the level of uncertainty for this application is very high.

Different approaches have been investigated for using ACR in aquatic systems, yet few of these approaches have been applied to terrestrial systems with success because the assumptions to the models do not apply to soil systems. Soils systems are more complex than aquatic systems. Therefore, numerous factors must be incorporated into the models for terrestrial systems when attempting to model chronic toxicity from acute toxicity data. The complexity of the system and the greater number of factors that must be considered has contributed to the failure of developing a suitable ACR approach to deriving soil-quality criteria or benchmark values for terrestrial systems. A fixed ACR approach does not address

these other factors and inevitably increases the uncertainty associated with such an approach. Only the methods utilizing direct biological effect ratios (e.g., LC50/EC50, MATC/LC50, and 1/AF) provide encouraging starting points from which to develop further predictive models in petroleum hydrocarbon contaminated soils. The other approaches that might be applicable are discussed in the report and the reasons for their rejection elucidated.

This document includes a section on guidance specific to collection, transportation, handling, and storage of soil samples from both contaminated (including volatile organic chemicals) and reference (i.e. control) sites that are destined for toxicity assessment. Also, there is framework for developing a sampling plan that includes QA/QC methods.

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APPENDICES

Appendix a

Approaches to acute to chronic ratios

1 INTRODUCTION

1.1 Background

In June 2001, the Canadian Council of Ministers of the Environment (CCME) released the new Canada-wide Tier 1 Standards for petroleum hydrocarbons (PHCs) in soils. These standards serve as soil quality criteria to assist with the management of site soils contaminated with petroleum hydrocarbons. The Tier 1 standards were derived for different hydrocarbon fractions (e.g., four), different exposure pathways, different classes of land use, different types of soil (surface and sub-surface, coarse and fine-grained), and in consideration of the proximity to surface or ground waters. Ultimately, the goal of these standards is the protection of human health and the environment.

One of the exposure pathways is the eco-soil contact pathway. This is intended to protect key ecological receptors that are indicative of soil quality. The Tier 1 levels for this exposure pathway focus on the effects of PHCs by direct contact with the biotic component of a terrestrial ecosystem.

The assessment and management of petroleum hydrocarbons in soils in Canada is the result of the implementation of a three-tiered framework. Tier 1 involves the application of the new Tier 1 national standards that are considered to be protective of human health and the environment at virtually all sites. Tier 2 involves the application of site-specific data or information to adjust, within limits, the Tier 1 standards to calculate Tier 2 levels that accommodate unique site characteristics. Tier 3 levels are generally derived from site-specific ecological or human-health risk assessments, when the assumptions inherent in the Tier 1 values are not appropriate for a site. The level of protection afforded, and the associated underlying guiding principles are preserved throughout this tiered process. However, it is recognized that the successive tiers represent increasing levels of precision, complexity, and cost.

At a site where there is PHC contamination in soil, the general practice is to collect soil samples and have them subjected to chemical analyses to determine the concentrations of the four hydrocarbon fractions using methods stipulated by the CCME. If the analytical results indicate that the PHC levels in soil exceed any one of the Tier 1 standards for that particular soil type and land use, then remedial action is required or, alternatively, a Tier 2 or Tier 3 assessment might be undertaken. For the eco-soil contact pathway, there is currently no mechanism or process to go from Tier 1 to Tier 2, and thus a “full blown” Tier 3 risk assessment must be conducted. The purpose of this project is to provide the foundation for a framework for the site-specific development of Tier 2 soil contact remedial standards for PHC-contaminated sites.

The project was divided into two phases. Phase 1 comprises a comprehensive review of the existing literature pertinent to: 1) site conditions and factors that influence bioavailability of PHCs in soil; 2) the potential role of the phenomena of sequestration in the relative bioavailability of the different PHC fractions in soil; 3) the tools used to measure bioavailability of PHCs in soil, including use of solid-phase micro-extraction (SPME) methods and semi-permeable membrane devices (SPMDs); and, 4) the relationship between acute and chronic estimates of toxicity for soil invertebrates exposed to PHC fractions of crude oil. The relevant literature is summarized herein, along with the implications for a Tier 2 assessment.

Phase 2 of the study involves the development of guidance for: 1) the collection of soil samples at a contaminated site for biological and/or chemical assessments of soil; 2) a recommended approach to toxicity assessment of contaminated soils for Tier 2 soil standard development; 3) a recommended test battery (test species and methods) for Tier 2 soil standard development; and, 4) procedures to deal with PHC mixtures in soil when the constituent distribution indicates that only one or two of the four

hydrocarbon fractions are in exceedence of Tier 1 soil standards. The use of crop yields as an assessment endpoint for Tier 2 soil standard development was not included because of a paucity of reliable data.

The deliverables for this study are two reports that will serve as a foundation for the development of a cost effective mechanism to go to Tier 2 from Tier 1, in the event that Tier 1 screening levels for PHCs are exceeded for the soil contact exposure pathway. It should not be necessary to go to a Tier 3 ecological risk assessment for the development of site-specific remedial standards simply because the ecological soil contact soil objective is exceeded, when there are site characteristics that ameliorate the bioavailability of PHCs, hence toxicity to ecological receptors.

1.2 Scope of Report

The results of Phase 1 of this study, comprising the literature review, are summarized and discussed in this report. The results of Phase 2, comprising the guidance and proposed toxicity assessment framework are presented in a companion document (Stanec Consulting Ltd. 2003).

This report has been divided into several sections. Section 1 contains a summary of the rationale and background for the study and a description of the scope of work. The site conditions and factors (e.g., the phenomena of sequestration) that influence bioavailability of PHCs in soil are discussed in Section 2 in view of the current literature. Section 3 contains a discussion on the chemical and biological measures of bioavailability. An assessment of the potential tools used to measure bioavailability of PHCs in soil, including use of solid-phase micro-extraction (SPME) methods and semi-permeable membrane devices (SPMDs) is presented in Section 4; and, the relationship between acute and chronic estimates of toxicity for soil invertebrates exposed to PHC fractions of crude oil is discussed in Section 5. Section 6 summarizes the protocols and guidance documents available for collection, handling and storage of soils as reference material only, with a focus on the methods and procedures applicable to soils destined for toxicity assessment.

2 BIOAVAILABILITY OF PETROLEUM HYDROCARBONS IN SOIL

2.1 Introduction

Like most regulatory benchmark values, the development of the ecological benchmarks for soil contact in the Canada-wide Standards for Petroleum Hydrocarbons in Soil (PHC CWS) were mainly based on laboratory tests with fresh product (CCME 2000b, ESG International 2003). As with other benchmark values, the use of the Tier 1 values for the PHC CWS assumes 100% bioavailability of the product as determined by the total soil concentration of each petroleum hydrocarbon (PHC) fraction via exhaustive solvent extraction techniques (Alexander 1997, Alexander 2000, CCME 2001). However, there is concern that the use of these regulatory benchmarks and the manner in which the concentrations of petroleum hydrocarbons are routinely measured in soil might overestimate the risk at historically contaminated sites. The use of vigorous extraction techniques with "aggressive" solvents will result in the determination of PHCs that are not readily available to biological receptors especially at historically contaminated sites where there is a decrease in the bioavailability of organic contaminants in many soils (Alexander 1995, Alexander 2000, Reid *et al*. 2000a).

Reviews of a number of studies have shown an apparent decrease in the bioavailability of a variety of organic contaminants, including PHCs, with an increase in the contact time of the contaminant with soil (Alexander 1995, Alexander 1997, Alexander 2000, Reid *et al*. 2000a). This decrease in chemical bioavailability with soil contact time has been termed "aging" (Alexander 2000).

There are several lines of evidence that support the hypothesis of a decline in PHC bioavailability (Alexander 1997). Field and laboratory studies on the disappearance or desorption of organic compounds, including petroleum hydrocarbons, from soil demonstrate that a plot of the dissipation of the compound from soil with time generally has a "hockey stick" appearance. In other words, there is an initial rapid disappearance of the compound with time, followed by a phase of very little or apparently no loss of the compound with increasing time (Alexander 1997, Huesemann 1997, White *et al*. 1999, Cuypers *et al*. 2001). Other studies have demonstrated a corresponding decrease in the microbial mineralization (Alexander 1997, Kelsey and Alexander 1997, White *et al*. 1997 and 1999), earthworm uptake (Kelsey and Alexander 1997, Kelsey *et al*. 1997, White *et al*. 1997 and 1999), and the amount available for extraction using mild solvent extraction techniques (Kelsey *et al*. 1997, White *et al*. 1997) of organic compounds with aging.

Considering that the availability of a compound is intricately linked to the ultimate internal dose, and thus exposure of an organism, it is not surprising that a decrease in uptake or toxicity to invertebrates (Kelsey and Alexander 1997, White *et al*. 1997, Robertson and Alexander 1998, Tang *et al*. 1998), and plants (Scribner *et al*. 1992, Tang *et al*. 1998) has been observed as chemicals age in soils. This phenomenon also has been reflected by decreases in genotoxicity with aging of PAHs in soil (Alexander and Alexander 2000). However, decreases in bioavailability have been found to be, at least sometimes, species-specific (Kelsey *et al*. 1997, Tang *et al*. 1998, Alexander 2000).

Given the role of aging and the observed decrease in bioavailability, the use of regulatory benchmarks that have been developed with data derived from experiments with freshly added (unaged) material (assuming 100% bioavailability), and total contaminant concentration (not the bioavailable portion), appears limited. These benchmarks are useful as a first tier in site assessment as a "worse-case" scenario. Should the site meet those benchmark values, the risk associated with the contamination at the site can be considered acceptable. However, strict use of these benchmarks could grossly overestimate the risk associated with a contaminated site should the bioavailability of the contaminant be less than 100%. Therefore, it is imperative to consider contaminant bioavailability at the site at higher tiers of the assessment framework. Considering that the bioavailability of various PHCs can be affected by aging

(Stroo *et al*. 2000), bioavailability must be considered in the context of the PHC CWS and risk assessment.

2.2 Terminology

There does not seem to be a consensus on the terminology used when discussing the fate of petroleum hydrocarbons, the processes involved, and their bioavailability. Several terms have been used, frequently interchangeably, despite differences in the processes that they describe. For example, the process of sorption and/or sequestration of a chemical, whereby its bioavailability is reduced, has been specifically described as sequestration (Nam and Alexander 1998, Kottler *et al*. 2001), and generally as aging (Nam and Alexander 1998, Alexander 2000, Reid *et al*. 2000a) and/or weathering (Alexander 2000). However, weathering has also been used to describe the change in the relative composition of the contamination (Saeed *et al*. 1998, Neff *et al*. 2000), a process that is quite different from sequestration. Even the term "bioavailability" has different meanings within the literature and among different researchers (Rand *et al.* 1995, Alexander 2000).

For the purpose of this review, the following terms will be used as defined in this section. Be aware that some of these definitions might not apply to other literature, and that it is critical to have a clear understanding of which processes a term is describing when consulting the literature.

Bioavailability: the amount of chemical in soil accessible for uptake and assimilation by organisms (Alexander and Alexander 2000)

Weathering: the relative change in the composition of contamination due to the preferential loss of constituents with time. Note that weathering has been used in various contexts within the literature.

Aging: the time-dependent change in bioavailability of a compound(s) in soil. In this definition, aging includes all sorption and sequestration processes, including the formation of non-extractable residues.

Sequestration: the time-dependent movement of contaminant molecules into remote, inaccessible areas of soil particles and/or organic matter. Sequestration does not involve the formation of covalent bonds.

Non-extractable residues: the time-dependent formation of residues that cannot be solvent extracted from soils, and which can only be removed upon hydrolysis with a strong alkali or acid (Alexander 1999). These residues might involve the formation of covalent bonds between the parent compound, or a metabolite, with the organic matter.

Residual fraction: the contaminant remaining in the soil following weathering and aging. This fraction includes those compounds that are resistant to degradation and other loss mechanisms, as well as, those compounds that are unavailable to organisms for degradation. Therefore, it is the fraction that will remain in the soil with relatively little change in both composition and concentration over an indefinite period of time. This residual fraction is often described as being recalcitrant.

Absorption: the retention of a compound *within* the mass of a solid material (Alexander 1999)

Adsorption: the retention of a compound *on* the surface of a solid material (Alexander 1999)

Desorption: the movement or migration of adsorped entities (e.g., compound) off of the adsorption sites.

Sorption: the combined processes of adsorption and absorption.

K_d: soil-water partition coefficient

K_d = concentration sorbed to soil/concentration in water

A greater K_d signifies greater sorption to soil.

K_{oc}: soil-water partition coefficient standardized to soil organic carbon content;

$K_{oc} = K_d/f_{oc}$ where f_{oc} is the fraction of organic carbon in the soil

K_{om}: soil-water partition coefficient standardized to soil organic matter content;

$K_{om} = K_d/f_{om}$ where f_{om} is the fraction of organic matter in the soil

K_{ow}: octanol-water partition coefficient is the ratio of the chemical concentration in octanol and in water after equilibration of the two phases;

PAHs: polycyclic aromatic hydrocarbons

PCBs: polychlorinated biphenyls

2.3 Fate of Petroleum Hydrocarbons in Soil: Changes in Composition and Bioavailability

"Petroleum hydrocarbons" is a term that describes a wide variety of chemical mixtures and individual chemicals, with the common denominator being that the compounds or mixtures are petroleum derived. Therefore, the fate and behaviour of different PHC mixtures will vary greatly depending on the constituents present in the mixtures and their proportional representation and physical and chemical properties, as well as on the properties of the soil in which they are found.

Following the introduction of a petroleum hydrocarbon mixture to soil, two major changes generally are observed to occur with time: 1) a change in the relative constituent composition of the contaminant mixture; and 2) a change in the bioavailability of the individual constituents. If only a single chemical is involved, then only a change in bioavailability might be observed. Changes in bioavailability are not observed with every soil.

A change in the relative composition of the contaminant mixture (de Jonge *et al*. 1997, Saeed *et al*. 1998, Neff *et al*. 2000) occurs due to the physical and chemical properties of the individual constituents of the mixture and biotic (e.g., biodegradation) and abiotic (e.g., volatilization, chemical and photochemical degradation, leaching, etc.) processes that preferentially remove certain constituents from the soil (Trapp *et al*. 2001). This results in an apparent relative enrichment of remaining constituents in the soil. For example, when crude oil is spilled on soil, there will be a preferential loss of the more volatile, water-soluble, and easily degraded components of the contamination (generally the lower molecular weight compounds) over those constituents that are less volatile, non-soluble in water or non-degradable (generally the higher molecular weight compounds). This results in a relative depletion of the contamination of lower molecular weight compounds, and a relative enrichment in higher molecular weight compounds, though the total concentration of all compounds would decrease.

A change in the bioavailability of the individual constituents over time might also occur. As will be discussed in greater detail in Subsection 2.3, evidence suggests that over time the constituents of the contamination become bound or sorbed and/or sequestered into remote, inaccessible regions of soil

particles or organic matter, such that they become inaccessible to organisms. Thus, the individual chemicals are still within the soil, and can be extracted by exhaustive or aggressive extraction procedures, but they are not generally bioavailable to organisms for uptake.

These two processes, the preferential loss of constituents and the change in bioavailability, occur simultaneously and therefore the rate and extent of one process will affect the rate and extent of the other. Compounds that are highly volatile, water soluble and/or easily degraded may not stay within the soil long enough to become bound and/or sequestered, and thus a decrease in their bioavailability might not be observed (Nam and Alexander 2001). Compounds that are less volatile, water soluble and/or degradable can be bound or sequestered to different extents, depending on the properties of the individual contaminants and the soil, and the dynamics of the particular system (i.e., temperature, moisture levels, winds, microbial populations, etc.). However, the two processes are completely different and it is important to be able to differentiate between the two.

2.3.1 Mechanisms for Reduced Bioavailability

Sequestration of organic compounds is considered to be a sorption-related diffusion-process (Brusseau *et al*. 1991, Huesemann 1997) in which the compound is effectively removed from the soil solution and sequestered into areas within the soil particles that are inaccessible to organisms for uptake and assimilation (Alexander 1997). This process might include a combination of limitations to diffusion, adsorption, and partitioning (absorption) which together prevent the compound from reaching areas where it is accessible or easily desorbed to the soil solution (Luthy *et al*. 1997). Thus, bioavailability is determined by the rate and extent of sorption into these inaccessible areas, and also by the rate and extent of desorption out of these inaccessible areas and back into the available pool.

Sorption and desorption phenomena have been extensively studied; however, the exact mechanisms involved remain largely unknown. The kinetics for both sorption and desorption consist of at least two phases: a fast phase where sorption and desorption are relatively quick; and a slow phase where sorption and desorption are relatively slow (Pignatello 1989, Weber and Huang 1996, Xing and Pignatello 1996 and 1997, Alexander 1997, Kraaij *et al*. 2001). That portion of compound that resides within the domain of the rapid phase is often called the labile fraction, while that within the domain of the slow phase is known as the nonlabile, resistant or irreversible fraction (Pignatello 1989, Alexander 1997, Kan *et al*. 1998). Nonlabile, resistant and irreversible might have more specific meanings and/or mechanistic definitions within a given study; however, all descriptors effectively describe the slow phase of sorption/desorption. Intuitively, one would expect that the rate of desorption to be equal, but opposite, to the rate of sorption. However, in many cases, desorption appears to be slower than sorption (the affinity of the soil for the contaminant seems to be greater during desorption—higher Kd—than during sorption) (Pignatello 1990a). This phenomenon has been termed hysteresis and results in an apparently irreversible fraction that is only very slowly, or perhaps cannot be, desorbed (Pignatello 1989 and 1990a). The amount of compound that is sorbed into this irreversibly-sorbed compartment of the soil increases with increasing time for sorption (i.e., contact time between the contaminant and soil) (Pignatello 1990a), though there appears to be a maximum capacity (Kan *et al*. 1998).

A result of the fast and slow sorption phases is that partition coefficients (K_d) for a contaminant in soil, which are typically determined after a 24-hour equilibration period, might not be accurate (Alexander 1997). The fast phase of equilibration might last days or weeks, while the slow phase could take months or even years (Alexander 1997). Thus, the K_d would tend to increase with time as more and more contaminant is sorbed over time. Therefore, researchers, at least in the area of soil sorption phenomena, use an "apparent K_d" which describes partitioning at a specified point in time (Alexander 1997). It should be noted that values of K_d used in fate and effects models are often assumed to be at equilibrium even if

true equilibrium has not been reached, despite the fact that kinetic expressions for sorption might be more applicable (Pignatello and Xing 1996).

Rather simplistic hypotheses were given initially to explain the fast and slow phases of sorption/desorption in terms of changes in bioavailability. The existence of both a fast and slow phase of sorption indicated that something other than simple adsorption to the surface of soil particles occurred during the sorption/desorption process (Alexander 1997). It was proposed that the fast phase of sorption was adsorption of contaminants to the surface of soil particles. With time, contaminants could diffuse or sorb into remote sites within the soil particles, and that these sites were physically inaccessible to even the smallest organism. The longer the contact time of the contaminant in the soil, the more contaminant and deeper into the particle the contaminant could diffuse. Thus, molecules sorbed close to the surface of the soil particle could quickly desorb and were more available than those that had sorbed deeper into remote sites within the soil particle (Alexander 1997).

The above model is the basic premise for sequestration. It should be noted that with sorption, there are no changes in the contaminant structure, nor are any covalent bonds formed between the contaminant molecules and soil matter. Therefore, the contaminant molecules can be extracted via organic solvent extraction. The use of different solvents and more vigorous techniques can more effectively extract molecules presumably from more remote sites (Alexander 1997).

The above model can also explain both the initial decrease in bioavailability sometimes observed when an organic compound is added to soil, as well as, the progressive decrease in bioavailability observed with aging and sequestration. Many studies have shown that compounds that are sorbed to soil, yet unaged, are less bioavailable than when administered without soil. This has been observed with chemical recovery tests (Conte *et al*. 2001), mammalian dermal and oral uptake tests (Wester *et al.* 1990, Gough 1991, Roy *et al*. 1992), earthworm uptake assays (van Gestel and Ma 1988) and plant assays (Hulzebos *et al*. 1993). Therefore, it appears that even the initial, rapid sorption might influence the bioavailability of the contaminant as compared to its availability via an aqueous solution. As the contaminant ages within the soil, and molecules diffuse to more remote sites, bioavailability decreases further as the molecules are sequestered into those sites inaccessible to organisms.

Soils consist of both organic and inorganic, mineral particles. Researchers have described sorption and sequestration relative to both the organic and mineral phases, sometimes as separate processes and sometimes as co-processes. Regardless, both processes, and various mechanisms of sorption for each, most likely occur concurrently, though the actual contribution of each mechanism would depend on the physico-chemical properties of the individual soil and contaminants present.

Sorption mechanisms can be described either as a sorption phenomenon with inorganic, mineral matter in soils, or as a phenomenon involving organic matter. Proposed mechanisms for each will be given separately. However, it should be noted that all proposed mechanisms are based on inference from experimental results, and not from microscopic observations of where sorbed contaminants are found. A variety of theories have been put forward to explain the large, and sometimes contradictory, array of sorption and sequestration results. No consensus has been reached on which theory is most probably the dominant mechanism, and in all likelihood, several of the mechanisms work concurrently in a particular soil, with the importance of each mechanism depending on the particular soil (Luthy *et al*. 1997).

2.3.2 Sequestration in Inorganic/Mineral Matter

Inorganic matter consists of a variety of surfaces that can act as adsorption sites to contaminants. They include external surfaces, clay interlayer surfaces, and internal surfaces classified by their diameters,

including macropores, mesopores, and micro- or nanopores. No systematic investigation of the roles of these different surfaces has been conducted (Luthy *et al*. 1997).

Despite this, an adsorption hypothesis based on sorption within the smallest pores has been put forward. The intraparticle diffusion theory details the sequestration of contaminant molecules within nanopores within the inorganic particulate (mineral) matter of soil (Alexander 1997). The inorganic fraction of soil contains pores of varying diameters, with a large proportion of these in the nanometre range (Pignatello 1989). No free-living organisms exist that are less than 100 nm, and most bacteria are in fact greater than 1 μm, therefore any contaminants that are sequestered within these small nanopores will be unavailable to organisms (Alexander 1997). If the contaminant is unavailable for uptake, then it is not bioavailable. If the contaminant is not bioavailable, then it is not toxic.

The presence of pores within the inorganic soil fraction does not appear to be sufficient to explain or account for the observed decrease in bioavailability. A study conducted by Nam and Alexander (1998) using model solids demonstrated the requisite of a hydrophobic surface within the nanopores in order to effectively sequester contaminants. Without this hydrophobic surface, contaminants that had diffused into the nanopores could also easily diffuse out and become available for degradation. It is thought that in soils, organic matter might coat the nanopores, thus providing a hydrophobic surface that will sorb and retain the contaminants within the pores (Alexander 1997).

Contaminant molecules can travel to more remote sites within the inorganic matter via aqueous diffusion through nanopores (Brusseau *et al*. 1991); however, this path is not straight, but instead very tortuous (Alexander 1999). In order to diffuse into and along the nanopores, the contaminant molecule is continuously sorbed and desorbed from the nanopore walls to the soil solution in a process analogous to movement through a chromatographic column (Pignatello and Xing 1996). This, coupled with the tortuous path that greatly increases the path length travelled, greatly slows the diffusion of the molecule into and out of soil matter, and is represented by the slow phase of sorption and desorption observed (Brusseau *et al*. 1991, Huesemann 1997, Alexander 1997 and 1999). While the molecule is sorbed within nanopores that are inaccessible to organisms, it is sequestered and not bioavailable. However, once it has desorbed out of these pores, it can become bioavailable again. If the desorption is very slow, the total bioavailable concentration might remain low with time and below a threshold level of concern.

2.3.3 Sequestration in Organic Matter of Soil

Hypotheses have also been put forward regarding the role of organic matter in the sequestration and reduced bioavailability of hydrophobic organic compounds, such as petroleum hydrocarbons. However, unlike with the soil inorganic matter, consensus on the mechanisms of sorption has not yet been reached and further research is required. Much of the work conducted with organic matter has been in an attempt to understand the role of organic matter in slow sorption and desorption rather than for bioavailability per se (Weber and Huang 1996, Xing and Pignatello 1996 and 1997, Gustafsson *et al*. 1997, Graber and Borisover 1998, Huang and Weber 1998, Kan *et al*. 1998 and 2000, Cornelissen *et al*. 2000, LeBoeuf and Weber 2000, Xing 2001). However, as sequestration and bioavailability are considered sorption phenomena, the models proposed can help in the understanding of mechanisms involved in sequestration.

Originally, sorption by organic matter was considered to be a simple partitioning or dissolution (absorption) process, analogous to chemicals partitioning into an organic solvent (Chiou 1989). Organic matter was considered homogenous, and sorption was thus concentration-independent, noncompetitive, and linear (i.e., sorption isotherms were linear) (Xing and Pignatello 1997, Graber and Borisover 1998, Xing 2001). With partitioning, sorbed molecules will be distributed throughout the bulk of the organic matter (Graber and Borisover 1998).

Several studies, however, demonstrated that in many cases, sorption by organic matter was instead concentration-dependent, competitive and nonlinear (Pignatello and Xing 1996, Weber and Huang 1996, Xing and Pignatello 1997, Xing 2001). These results suggest that a fixed, site specific-type of sorption occurs in organic matter (Xing *et al*. 1994) rather than partitioning. If this is the case, contaminant molecules will only be distributed to areas with the specific sorption sites (Graber and Borisover 1998), which might, or might not, be located homogeneously throughout the organic matter. This differs from partitioning where contaminants would be distributed homogeneously.

Several researchers proposed a dual-mode sorption mechanism to explain the apparent incongruence of the partitioning model (Xing and Pignatello 1997, Weber and Huang 1996, Huang and Weber 1998, LeBoeuf and Weber 2000). In these models, organic matter is heterogeneous and consists of two domains that have similar properties to polymers: an expanded, flexible domain that is analogous to a rubbery polymer in which sorption proceeds via partitioning and a condensed, microcrystalline domain that is analogous to a glassy polymer in which sorption involves both partitioning and a pore or "hole-filling" mechanism (Xing and Pignatello 1997). These domains are not distinctly separate but represent a continuum of these properties within the organic matter.

Thus, within both the expanded and condensed domains, partitioning occurs. This partitioning is relatively quick compared to the hole-filling mechanism and is associated with the fast phase of sorption (Xing and Pignetello 1997, Huang and Weber 1998).

In the condensed phase, however, an additional mechanism of sorption is concurrently functioning, which is a hole-filling, adsorption-like interaction that entraps the molecule within the organic matter. The "holes" or pores in the condensed organic matter are thought to be voids within the matrix that are relatively constant, of a size similar to that of the molecule being sorbed, and limited in number (Xing and Pignetello 1997, Xing 2001). Thus, sorption via this site-specific mechanism gives rise to nonlinear sorption isotherms and competitive sorption (Xing *et al*. 1996, Xing 2001), and, as such, it has been observed that competition is related to the amount of condensed organic matter in soil (Li and Werth 2001). The hole-filling mechanism is considerably slower than partitioning; therefore, the overall sorption to condensed organic matter is likewise slower than sorption to the expanded organic matter (Xing and Pignetello 1997). This is supported by studies on PAH availability, where PAHs were more readily available when sorbed by expanded organic matter than condensed organic matter (Cuypers *et al*. 2000).

Two models have been developed based on this "two-domain" organic matter theory. One is the dual-mode sorption model, which concerns itself with sorption by the organic matter only (Xing and Pignetello 1997), and the other is the distributed reactivity model (Weber and Huang 1996, Huang and Weber 1998, LeBoeuf and Weber 2000), which extends the model to include sorption by both inorganic and organic matter.

Xing and co-workers (Xing and Pignatello 1997, Xing 2001) and Weber and co-workers (Weber and Huang 1996, LeBoeuf and Weber 2000) suggest that it is the entrapment and then subsequent slow release of the molecule that limits desorption. Cornelissen *et al*. (1998b) offer that it is the diffusion from the "hole" to the exterior of the organic matter that determines the desorption rate. It is possible that both contribute to the slow desorption of compounds from organic matter.

Another proposed mechanism suggests that following the sorption of a molecule, there is a physico-chemical change in the organic matter itself which results in the molecule becoming entrapped such that it can no longer be desorbed from the organic matter (Kan *et al*. 1998). Kan *et al*. (1998) proposed this model to explain fast and slow desorption, and the hysteresis that is often observed. In this model, molecules are associated with one of two compartments in the soil organic matter: a labile compartment

in which compounds reversibly desorb; and a nonlabile compartment in which compounds are irreversibly sorbed due to the changes in the organic matter following sorption. Therefore, desorption from the nonlabile compartment is no longer opposite and equal to sorption, and hysteresis is observed. This irreversible compartment was found to have a finite capacity, such that once it was filled, desorption of any molecules in excess was reversible (Kan *et al*. 1998).

It was also shown that the irreversible compartment could be further divided into two phases based on desorption rates: a slow phase with a desorption half-life between 2 and 7 days; and a very slow phase with a half-life ranging from months to years (Kan *et al*. 1998). A slow and very slow phase of desorption was also observed by Cornelissen *et al*. (2000) though this work was not incorporated into any particular model.

Studies by Chen *et al*. (2000) investigating competitive sorption and its effect on desorption from the irreversible compartment provide further evidence that something more than a hole-filling mechanism (as proposed in the dual-mode and the distributed reactivity models above) is occurring. A hole-filling mechanism implies that sorbed molecules can be displaced by a suitable competitor(s), thus increasing desorption. However, this was not the case with sediments in which the labile, easily desorbed fraction was previously removed. The authors concluded that this indicated that the irreversible fraction was not a simple hole-filling domain, and that the results were more consistent with the irreversible fraction model.

Other researchers have suggested the sorption of nonpolar contaminants onto high surface area carbonaceous materials (HSACM) (e.g., soot, charcoal, etc.), which are wide spread in the environment, might explain the observed nonlinear sorption isotherms of contaminants (Gustafsson *et al*. 1997, Chiou and Kile 1998). The affinity of these particles for contaminants is much greater than the affinity of organic matter (Gustafsson *et al*. 1997, Chiou and Kile 1998). It is proposed that sorption to HSACM is a surface adsorption (Chiou and Kile 1998) or a hole-filling (Xia and Ball 1999) phenomenon.

Another, little studied mechanism has been proposed that suggests that the sorption of polar molecules (such as phenol) by organic matter might be explained by the formation of localized sorption sites through the interruption of polar contacts within soil organic matter (Graber and Borisover 1998). Polar molecules are able to disrupt these contacts, however, nonpolar molecules do not.

2.3.4 Formation of Non-extractable Residues

A fraction of the contaminant in soil might become non-bioavailable due to complexation reactions that bind the contaminant (or a metabolite very similar to the parent compound) to soil organic matter such that it is (presumed to be) unavailable to organisms (Alexander 1999). The exact nature of this binding is unknown; however, the defining attribute of compounds in this fraction is that they cannot be extracted by extensive solvent extraction techniques, but only upon hydrolysis with strong alkali or acid (Alexander 1999). Thus, these bound compounds or substances are sometimes termed non-solvent extractable residues (Northcott and Jones 2001a).

It is thought that the contaminants are complexed via the formation of covalent bonds between the contaminant (or metabolite) and organic matter (Alexander 1997). Evidence suggests that this process is mediated by microbial action (Macleod and Semple 2000, Roper and Pfaender 2001). However, studies conducted by Northcott and Jones (2001a) and Macleod and Semple (2000) have also indicated that non-solvent extractable residues can be formed under sterile conditions, with Northcott and Jones (2001a) suggesting that the residues within this fraction were not covalently bound. A cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance spectroscopy (CPMAS ^{13}C NMR) on non-extractable residues of pyrene in sediments also indicated that the non-extractable fraction was not covalently bound, but instead adsorbed or encapsulated in the sediment humin (Guthrie *et al*. 1999).

As in sequestration, the fraction of the compound found in the non-extractable compartment increases with an increase in the contact time of the contaminant with the soil (Macleod and Semple 2000, Northcott and Jones 2001a). These residues are generally considered to be unavailable by regulatory agencies (Alexander 1999), and since they are not extracted with routine solvent extraction methodologies, they are generally ignored. However, there is evidence that non-extractable residues can be mobilized and become available biologically (Gevao *et al*. 2001) or chemically (for extraction) (Amellal *et al*. 2001).

The formation of non-extractable residues has been observed with several petroleum hydrocarbons and/or their metabolites, including phenols, catechols, quinones (Alexander 1999 and references therein), and PAHs (Ressler *et al*. 1999, Macleod and Semple 2000, Northcott and Jones 2001a, Roper and Pfaender 2001). Thus, this might contribute appreciably to a reduction in bioavailability but only if the complexation or binding of the residues cannot be reversed or is sufficiently slow to not be of concern. Northcott and Jones (2000) suggest that natural humic degradation processes, as well as enzymatic reactions (i.e., organism digestive fluids) might enhance the formation of non-extractable residues, or alternatively, promote their release. Further research is required in this area.

2.4 Soil and Site Factors Affecting Bioavailability of Petroleum Hydrocarbons

As can be seen, sorption, sequestration and the formation of non-extractable residues of petroleum hydrocarbons are complex, and not entirely understood, processes that occur in a highly variable and heterogeneous medium, soil. Several site-specific factors, many of which are not well understood, can affect sorption, sequestration and the formation of non-extractable residues, resulting in changes in bioavailability. However, it is difficult to predict how these site-specific factors will affect bioavailability. Very few studies, with the exception of those conducted by Alexander and co-workers (Hartzinger and Alexander 1997, Nam *et al*. 1998, White *et al*. 1998, Chung and Alexander 1999, Kottler *et al*. 2001, Northcott and Jones 2001a and b) specifically studied the effect of various environmental and chemical properties on aging and bioavailability. Most studies were investigations on sorption phenomena, and were not conducted with aged materials or they did not consider bioavailability (Pignatello and Xing 1996, Xing *et al*. 1996, Chen *et al*. 2000, Braida *et al*. 2001, Li and Werth 2001). However, in as much that sequestration is a sorption phenomenon, those factors that affect sorption most likely will affect sequestration and bioavailability to some extent as well.

While time is definitely a factor controlling the extent of sequestration and thus bioavailability, it will not be discussed further in this section. It is well known that an increase in the contact time results in an increase in the amount sequestered (Alexander 1995, Alexander 1997, Kelsey and Alexander 1997, White *et al*. 1997 and 1999, Alexander 2000, Reid *et al*. 2000a) or residing in the non-extractable fraction (Macleod and Semple 2000, Northcott and Jones 2001a). This, in turn, is attributed to a decrease in the bioavailability (Kelsey and Alexander 1997, White *et al*. 1997) and extractability of the compound (Kelsey *et al*. 1997, White *et al*. 1997).

Site-specific factors have been found to affect the sorption, sequestration and/or bioavailability of petroleum hydrocarbons in soils (Table 2.1). Most studies were conducted with nonpolar, hydrophobic compounds, which make up the bulk of petroleum contamination. This section will only consider those site-specific factors that affect the bioavailability of petroleum hydrocarbons; other factors might change the bioavailability of other types of contaminants. As a result, any of the listed factors might prove useful in the derivation of site-specific Tier 2 levels for petroleum hydrocarbons in soils, though some factors will be more strongly correlated to changes in bioavailability than others. This will be discussed at the conclusion of this section.

Table 2.1: Site-Specific Factors That Might Affect the Bioavailability Of Petroleum Hydrocarbons in Soils.

Factor affecting Bioavailability	Effect on Bioavailability	Reference
Soil Texture	Finer particle-size fractions have higher sorption capacity due to higher surface area and/or higher OC content	Carmo *et al.* 2000, Mulder *et al.* 2000, Amellal *et al.* 2001,
	Swelling clays and sorption to internal surfaces: contradictory results	Carmichael and Pfaender 1997
	Clay content might contribute significantly more than OM	Luthy *et al.* 1997 and references therein
	Soil texture plays a major role in PAH availability	Mingelgrin and Gerstl 1983; Amellal *et al.* 2001
Soil Structure/Aggregates	Soil structure, composition and aggregation can affect which sorption process dominates	Luthy *et al.* 1997, Amellal *et al.* 2001
Precipitate formation	Encapsulation of OM/clay with sorbed contaminant within inorganic precipitates might reduce bioavailability	Luthy *et al.* 1997
Presence of iron oxides	Sorption of phenols influenced by presence of iron oxides	Artiola-Fortuny and Fuller 1982
Organic Matter	Source of OM (i.e. algae, lignin, crude oil) might affect sorption kinetics	Luthy *et al.* 1997, Bayard *et al.* 2000
	Diagenesis can change physico-chemical properties of OM which might affect sorption kinetics	Luthy *et al.* 1997, Young and Weber 1995, Johnson *et al.* 1999 and references therein
		Li and Werth 2001 and references,
	Condensed OM has higher aromaticity and atomic C/H values	Perminova *et al.* 2001
	Partitioning coefficients differ for different humic substances	Braida *et al.* 2001
	Variability in sorption might be caused by quality of OM, size of OM particles, or sequestration of OM in inorganic aggregates	Nam *et al.* 1998, Billeret *et al.* 2000,
	Amount of OM important for sorption of PAHs; may have threshold level	Conte *et al.* 2001
	Percent aromaticity and polarity affects sorption	Xing *et al.* 1994, Johnson *et al.* 1999, Nanny and Maza 2001, Perminova *et al.* 2001
	No correlation with OM polarity and aromaticity	Cornelissen *et al.* 2000
	Presence of polysaccharides in OM unlikely to contribute to sequestration	Hartzinger and Alexander 1997
pH	Increased desorption at lower pH for halogenated aliphatic hydrocarbons	Pignatello 1990b
	No effect of pH on desorption from irreversible compartment	Chen *et al.* 2000
	No effect of pH from 5-8; increase sorption only at pH 2	Perminova *et al.* 2001
	Observed both pH dependent and independent sorption of benzene and pH dependent sorption of phenol	Nanny and Maza 2001
	pH dependent conformation changes in humic acids observed	Chien and Bleam 1998
	Protonation of humic material at low pH	Conte and Piccolo 1999
Temperature	OM can display a phase transition temperature for transitions from a condensed state to an expanded state which could affect sorption behaviour	Xing and Pignatello 1997; LeBoeuf and Weber 1997
	Desorption requires energy and thus is temperature dependent	Pignatello and Xing 1996
	Temperature increase to 30°C probably doesn't increase desorption	Cuypers *et al.* 2001

Table 2.1: Site-Specific Factors That Might Affect the Bioavailability Of Petroleum Hydrocarbons in Soils.

Factor affecting Bioavailability	Effect on Bioavailability	Reference
Moisture regime	Moisture regime affects PAH sorption and sequestration	Chiou 1989, Kottler *et al.* 2001, White *et al.* 1997,1998,
	Hydration can affect sorption via competition and/or changes in OM structure	Kottler *et al.* 2001 Graber and Borisover 1998
Presence of co-contaminants	Competition between chemicals has been observed which affects the sorption/desorption of co-solutes	Li and Worth 2001, White *et al.* 1999, Xing 2001
	Soil lipids can compete with PAHs, decreasing sorption	Kohl and Rice 1999
Presence of oil/NAPL	Presence of oil changes partitioning behaviour	Walter *et al.* 2000, Dragun 1998
Salinity	Changes in salinity can affect sorption	Dragun 1998, Perminova *et al.* 2001
Contaminant properties	Sorption often concentration-dependent	Pignatello and Xing 1996, Braida *et al.* 2001, Chung and Alexander 1999
	Molecular descriptors for molecule size, surface area and hydrophobicity related to sorption	Brusseau 1993, Hu *et al.* 1995, Braida *et al.* 2001, Northcott and Jones 2001a, Xia and Ball 1999
	Molecular length, log Kow and molecular size (as molecular connectivity index) not correlated to sequestration with aging	Kottler *et al.* 2001

OC—organic carbon PAH—polycyclic aromatic hydrocarbons
OM—organic matter C/H—carbon:hydrogen ratio
Log Kow—logarithm (base 10) of the octanol-water partition coefficient

2.4.1 Soil Texture and Structure

Soils are generally classified by the distribution or relative amounts of three different particle size classes (sand, silt and clay) in soil. This particle size distribution then defines the texture of the soils. With regard to surface area, the surface area for these three particle size classes increases in the order sand<silt<clay. Texture refers to the amount of the individual particles in the soil; however, these particles can become associated with each other and form aggregates. It is the size and shape of the aggregates that define the structure of a soil (Dragun 1998).

Both the texture and the structure of soils have been found to affect the sorption and/or bioavailability of nonionic organic compounds, such as PHCs. In comparative studies, an increase in sorption (Carmo *et al*. 2000, Amellal *et al*. 2001) and decreases in bioavailability (Billeret *et al*. 2000, Mulder *et al*. 2000), mineralization, and recovery efficiencies (Carmichael and Pfaender 1997) have been observed with the finer soil fractions (i.e., clay, silt) as compared to the coarser fractions for various PAHs. Carmichael and Pfaender (1997) also observed an increase in the fraction of PAHs sequestered in the finer soil fractions following an extraction of the PAHs, while Visser *et al*. (2001) observed higher PHC residuals in a clay soil than sand and loam soils following a laboratory degradation study. These all suggest that sorption to fine soil fractions might decrease the bioavailability of PHCs. However, in unaged soils with phenanthrene, White *et al*. (1997) observed that earthworm uptake was greater in soils with higher clay content, though no correlation for bioavailability or extractability with clay content was discerned following aging of the soil. The reasons for these results are unknown.

It is reasoned that the finer-particle sizes have a higher capacity than coarser particles to sorb hydrophobic compounds due to their higher surface area for sorption (Carmo *et al*. 2000, Mulder *et al*. 2000, Amellal *et al*. 2001). Mingelgrin and Gerstl (1983) suggest that, despite the lower sorption affinity of clays, sorption to clays might contribute more to the sorption of compounds in soil than sorption to organic matter because the fraction of clay in a soil is generally much greater than the fraction of organic matter.

However, it has also been suggested that the finer fractions might have a greater organic carbon content which could contribute to the sorption (Carmo *et al*. 2000). Clays are often associated with organic matter forming organo-clay aggregates (Dragun 1998) and Amellal *et al*. (2001) found that the clay fraction of their soil had an organic carbon content of 27%. This increased surface area with associated organic coating might provide a larger organic surface area for sorption (Carmichael and Pfaender 1997). Alternatively, Amellal *et al*. (2001) suggested that a combination of surface adsorption and partitioning within clay aggregates might explain the high concentration of phenanthrene that they found in the clay fraction. Regardless of which mechanism is dominant, a decrease in the bioavailability, as measured by mineralization of phenanthrene was observed in the four largest aggregate sizes tested (White *et al*. 1997). However, this same study (White *et al*. 1997) was unable to correlate sequestration to the clay content of the soils.

Many clay particles are structured such that they can swell and expose interlayer surfaces as sorption surfaces. Observations seem to be equivocal as to whether sorption of nonpolar organic compounds occurs within these interlayers (Parfitt and Greenland 1970 as cited in Luthy *et al*. 1997, Farrell and Reinhard 1994, Huang *et al*. 1996)

Soil processes that occur over time might also influence bioavailability. Inorganic precipitates might form and encapsulate both organic matter and clay surfaces that have sorbed contaminants. If this encapsulation occurs after the contaminant has been sorbed, the contaminant molecules might become effectively trapped and therefore not bioavailable (Luthy *et al*. 1997).

Sorption of nonpolar organic compounds in organic matter of soils is considered to be the major sorption mechanism for these compounds (Calvet 1989, Alexander 2000). Thus, it is reasonable to believe that bioavailability of nonpolar organic compounds, such as PHCs, would be correlated to the quantities and properties of the organic matter.

2.4.2 Presence of Iron Oxides

One study noted that the sorption of monohydroxybenzene derivatives (i.e., phenols) was primarily influenced by the presence of iron oxides in soil (Artiola-Fortuny and Fuller 1982). This could be due to more sites on iron oxides that are capable of hydrogen bonding with organics and/or to the ability of iron oxides to react with both anions and cations. Phenols are capable of hydrogen bonding, and since they are also weak acids, they can exist as both anions and neutral molecules (Artiola-Fortuny and Fuller 1982). An increase in sorption might result in a decrease in bioavailability, though this requires further study.

2.4.3 Organic Matter

Several studies have shown that sorption and/or bioavailability is affected by the amount of organic matter in soil (Nam *et al*. 1998, Billeret *et al*. 2000, Conte *et al*. 2001). Nam *et al*. (1998), in a study specifically investigating sequestration and the reduction in bioavailability of phenanthrene with aging and organic matter content, deduced that there was a threshold level of organic matter that was required for sequestration (2% organic carbon). Sequestration was not observed in samples with organic carbon (OC) contents below 2%, and while sequestration was evident at organic carbon contents higher than 2%, it did not become more pronounced with increasing OC content in the soil. Interestingly, OC content did

not appear to affect bioavailability (as measured by mineralization) in samples that were not aged. A strong relationship between the soil organic matter content and sequestration and/or bioavailability is far from universal; members from the same research group as Nam and co-workers found little correlation between soil organic matter content and sequestration for a suite of soils (Chung and Alexander 1998). White *et al*. (1997) also found that the amount of organic matter in soils did not correlate with the observed decrease in earthworm uptake and bacterial mineralization of phenanthrene. Other soil properties, or a combination of properties, might be of more importance than organic matter alone.

Sorption to soils has sometimes been related to the organic carbon normalized partition coefficient, Koc. Chiou *et al*. (1998) found that the log Koc for three PAHs remained constant for a suite of soils, suggesting that sorption is related to the amount of organic carbon. Perminova *et al*. (2001) also related sorption to Koc, but found that the partitioning coefficient depended on the type of humic substance tested (i.e., humic acid versus fulvic acid), and that the affinity of both humic and fulvic acid for PAHs depended on their source. Similarly, others have suggested that the source of the organic matter should be taken into consideration (Chin *et al*. 1997, Luthy *et al*. 1997) and that soils with coal tar derived organic matter (Bayard *et al*. 2000) or other carbonaceous organic matter (e.g., charcoal, coal) (Gustafsson *et al*. 1997, Chiou and Kile 1998) might have substantially different sorption affinities.

Other properties of organic matter have also been implicated in sorption and bioavailability. As discussed previously in Subsection 2.3.3, organic matter is thought to consist of both an expanded and a condensed domain that have different sorption mechanisms and affinities. Organic matter diagenesis (the alteration of organic matter with time) is thought to change expanded organic matter into a more condensed form (Young and Weber 1995, Huang and Weber 1998, Johnson *et al*. 1999, LeBoeuf and Weber 2000). Condensed OM has a higher degree of aromaticity and atomic C/H values (Johnson *et al*. 1999, Li and Werth 2001). An increase in the partition coefficients (Kd and Koc) has been noted for condensed organic matter versus expanded (Johnson *et al*. 1999, LeBoeuf and Weber 2000) which was attributed to the greater aromaticity and decreased polarity of this fraction (Johnson *et al*. 1999). The degree of aromaticity (Chin *et al*. 1997, Nanny and Maza 2001, Perminova *et al*. 2001) and polarity (Xing *et al*. 1994) of soil organic matter has been correlated to the sorption of aromatics. However, contrary to these studies, Cornelissen *et al*. (2000) could not find a correlation for desorption of chlorobenzenes or polychlorinated biphenyls with soil or sediment aromaticity or polarity. Chin *et al*. (1997) also found that the correlation with aromaticity depended on the source of the organic matter. As well, Chefetz *et al*. (2000) investigated both the aromaticity and aliphaticity of soils, and found that both moieties contributed to the sorption of PAHs, and therefore the aliphaticity of the organic matter should not be ignored. It should be noted that the aromaticity of humic acids has been found to increase with depth in soils (Chen and Pawluk 1995).

The above suggests that the factors controlling sorption and sequestration by organic matter are not yet well understood and further research is required. Often, studies on sorption and/or sequestration are conducted on whole soils with attempts at determining *a posteriori* correlations with soil properties. Without a method for controlling variation in all factors other than the one of interest (e.g., aromaticity) conflicting results can be expected. However, the conflicting results do indicate that factors other than aromaticity, affect sorption and sequestration behaviour. Factors, such as the amount, type, and physico-chemical properties of the organic matter in the soil can affect the sorption affinities, and potentially the bioavailability of petroleum hydrocarbons in soils.

One constituent of organic matter that was found not to contribute to the sequestration of phenanthrene was the presence of polysaccharides (Hartzinger and Alexander 1997).

2.4.4 Soil pH

The results of studies on the effect of soil pH on sorption of nonpolar organic compounds have been highly variable. Some researchers have observed a decrease in sorption at lower pH values (Pignatello 1990b), others have observed an increase in sorption (Nanny and Maza 2001, Perminova *et al*. 2001). Sorption has been observed to be either pH independent or pH dependent depending on the source of the humic acid tested (Nanny and Maza 2001). These data suggest that the effect of pH on sorption and possibly bioavailability might be soil and compound specific. For some compounds that are weak acids, such as phenol, which has a pKa of 9.8, pH can have a large effect on sorption, though for phenol this would occur only at high pH values (Nanny and Maza 2001). Such values are unlikely to occur in most environments.

Nevertheless, from existing studies and observations, it is reasonable to assume that pH might affect sorption. pH-dependent conformational changes in humic acids have been observed by Chien and Bleam (1998), and the protonation of humic materials is expected at low pH values in soil (Conte and Piccolo 1999). Protonation would cause an increase in the hydrophobicity, thus affecting sorption (Nanny and Maza 2001). While changes in pH have yet to be correlated to changes in the bioavailability of petroleum hydrocarbons, it can be presumed that an observed change in the sorption of PHCs with pH might translate into changes in bioavailability.

2.4.5 Temperature

Since desorption, which is involved in bioavailability, is an energy-requiring process, it has been suggested that temperature might influence this process, with desorption increasing with increasing temperatures (Pignatello and Xing 1996). However, Cuypers *et al*. (2001) did not believe that a temperature increase from room temperature to 30°C would affect desorption of PAHs, citing a study by Bonten *et al*. (1999a and b) and that desorption of PAHs from the rapid phase significantly increases only at temperatures above 65°C. The effects of temperature on desorption of other PHCs in soil, or those that are found within the slowly-desorbing fraction, are unknown.

Temperature might affect sorption by organic matter, depending on the characteristics of the organic matter. As discussed in Subsection 2.3, organic matter consists of expanded and condensed domains that are analogous to rubbery and glassy polymers, respectively. Like these polymers, the organic matter should display a phase transition temperature, the temperature at which the glassy/condensed state becomes rubbery/expanded. If this occurs within the range of environmentally relevant temperatures, then change in temperature might result in a change in the sorption characteristics of the organic matter as it becomes more or less condensed (LeBouef and Weber 1997, Xing and Pignatello 1997). This could be significantly dependent on the source and characteristics of the organic matter at a particular site.

2.4.6 Moisture Regime

It has been observed that the extent of sequestration (White *et al*. 1997, 1998, Kottler *et al*. 2001) and the predominant sorption mechanism (Chiou 1989) are affected by either the moisture level or a moisture regime of wetting- and drying-cycles in soil. Chiou (1989) noted that sorption behaviour was more congruent with an adsorption mechanism when soils were dry, while hydrated soils demonstrated behaviour expected for a partitioning-type of mechanism. To explain this phenomenon, it was hypothesized that the adsorption mechanism occurred when soils were dry, however, when soils were wet, water molecules competed for adsorption sites and thereby suppressed adsorption, in which case partitioning into organic matter becomes predominant. Kottler *et al*. (2001) observed greater sequestration with aging in soils that were dry at the time of contamination with phenanthrene as compared to moist

soils. Similar to Chiou (1989), Kottler *et al*. (2001) proposed that competition between water and phenanthrene molecules might have limited sorption under moist conditions.

Alexander and co-workers (White *et al*. 1997 and 1998, Kottler *et al*. 2001) looked specifically at the effect of wetting and drying cycles, both before and during contaminant addition, as well as, during aging of the contaminated soil, on sequestration and bioavailability of phenanthrene and di(2-ethylhexyl)phthalate (DEHP). Significant effects of wetting and drying on sequestration were observed, though the nature of the effects (i.e., an increase versus decrease in sequestration) depended on the age of the contamination, when the wetting and drying cycles occurred, and on the chemical itself.

White *et al*. (1998) and Kottler *et al*. (2001) observed that phenanthrene in soils, that were subjected to wetting- and drying-cycles during aging, was less bioavailable than phenanthrene in contaminated soils aged at a constant moisture level. This decrease in bioavailability, however, was only observed for aging periods of less than 60 days, and disappeared if the aging period was longer (White *et al*. 1998). White *et al*. (1998) also noted that wetting- and drying-cycles had an opposite effect on the sequestration of DEHP. It was hypothesized that this could be due to differences in the physico-chemical properties of the two chemicals resulting in different mechanisms of sorption for the two compounds. If this is true, similar observations might be made with petroleum hydrocarbons that span a range of physico-chemical properties. Kottler *et al*. (2001) also determined that the time when the wetting- and drying-cycles occurred could affect sequestration. The occurrence of the cycle prior to contamination led to greater availability of subsequently aged phenanthrene. It was thought that changes in aggregation, surface area and/or porosity brought on by the changing moisture levels could be responsible for the effect on sequestration.

White *et al*. (1998) observed that bioavailability of phenanthrene was enhanced if previously contaminated and aged soils were subjected to wetting- and drying-cycles. The same mechanisms that enhanced sequestration for samples undergoing the cycles during aging could also aid in the reverse process for contaminants that are already sequestered.

These results demonstrate how environmental conditions such as moisture levels can affect the sequestration and bioavailability of petroleum hydrocarbons and should be considered during site assessments. Further research is required to discern the mechanisms that lead to changes in sequestration, and how they can be used to predict bioavailability at a contaminated site.

2.4.7 Presence of Co-contaminants

Competition between co-contaminants has been observed in several studies (Xing and Pignatello 1997, White *et al*. 1999, Li and Werth 2001, Xing 2001), which can result in suppression in sorption of at least one of the competing compounds. White *et al*. (1999) investigated the effect of co-contaminants specifically on the bioavailability of sequestered phenanthrene and found that the addition of a competing PAH (pyrene or anthracene) increased the bioavailability of phenanthrene as measured by extraction and microbial degradation. While the mechanisms underlying this observed increase in availability are not understood, it was suggested that it might be caused by a competitive displacement of the sequestered phenanthrene by the competing PAHs.

Not only can anthropogenic molecules compete for sorption sites within soils, but apparently lipids that are naturally found in soil can as well. Kohl and Rice (1999) studied the impact of soil lipids on PAH sorption and found that the amount of PAH sorbed to soil increased when lipids were removed from the soil. Their results suggested that the soil lipids were competing for adsorption sites with PAHs. Soil lipids might be an important factor to consider, as the lipid content of most agricultural soils ranged from 1.2-6.3% of the soil organic matter (Stevenson 1992 in Kohl and Rice 1999).

2.4.8 Presence of Oil or Non-aqueous Phase Liquids

Apart from competition effects, the presence of significant amounts of oil or non-aqueous phase liquids (NAPLs) in soil can affect soil sorption dynamics by introducing an additional phase into the soil compartment into which nonpolar organic compounds can partition (Dragun 1998, Walter *et al*. 2000). This has been demonstrated with PAHs and various oil contaminants, with partitioning behaviour depending on the properties of the oil (Walter *et al*. 2000). Changes in the partitioning behaviour of the petroleum hydrocarbons within the soil will change the sorption of the PHCs by the soil, and thus change the sequestration and bioavailability.

2.4.9 Salinity

Few studies have investigated the effect of salinity on sorption and bioavailability of petroleum hydrocarbons; however, it is known that salinity can affect sorption coefficients according to the Setschenow equation. Nevertheless, this effect is often minor (Dragun 1998). Studies conducted by Perminova and coworkers did observe a reduction in PAH sorption with an increase in ionic strength up to 10-2 M (Yashchenko *et al*. 1999 as cited in Perminova *et al*. 2001). Again, changes in sorption might, but not necessarily, be correlated to changes in bioavailability.

2.4.10 Cation Exchange Capacity

Cation exchange capacity (CEC) generally influences the sorption of cationic molecules. As such, the CEC of soils most likely will not influence the sorption of most petroleum hydrocarbons since the majority of PHCs are nonpolar organics. However, some petroleum constituents might be more polar (i.e., phenols, heterocyclics) and their sorption could be affected by the CEC of the soil. This is an area requiring further study. Artiola-Fortuny and Fuller (1982) did investigate the role of CEC on sorption of phenols, and while it was correlated to the sorption of some phenols, generally it was not the major influencing factor for sorption.

2.4.11 Contaminant Properties

While the usefulness of contaminant properties might be limited for the prediction of petroleum hydrocarbon bioavailability due to the typically large number of constituents comprising these complex products, each with varying physico-chemical properties, they might be useful in certain cases.

Several studies indicate that the affinity a soil has for a nonpolar hydrophobic compound (including PAHs) is greater at lower initial concentrations of the solute than at higher concentrations (Weber and Huang 1996, Xing *et al*. 1996, Chung and Alexander 1999, Braida *et al*. 2001, Xing 2001). This has been demonstrated in both experiments on sorption (Weber and Huang 1996, Xing *et al*. 1996, Braida *et al*. 2001, Xing 2001) and sequestration (Chung and Alexander 1999). It is hypothesized that this is caused by a decrease in the total affinity of the soil as sorption sites become "filled" at higher solute concentrations (Xing *et al*. 1996). These sorption sites would be the voids or "holes" in either inorganic or organic matter that can contribute to nonlinear sorption. The time required to reach equilibrium is also affected by the initial concentration, with the higher solute concentration attaining equilibrium more quickly (Braida *et al*. 2001). Thus, the degree of sequestration and reduction in bioavailability is influenced by the initial concentration of the contaminant(s) in soil.

Various molecular descriptors that describe a variety of contaminant properties have also been tested for correlation with sorption and sequestration. Northcott and Jones (2001a) found that the amount of PAHs forming non-extractable residues increased with the molecular weight and hydrophobicity (K_{ow}) of the PAH. Xia and Ball (1999) found a similar positive correlation with the partitioning coefficient and K_{ow}.

Braida *et al*. (2001) related molecular size and hydrophobicity to the sorption rate, and hypothesized that the sorption of larger and more hydrophobic PAHs is slower due to retarded diffusion via micropores that are similar in size to the sorbing PAH. Brusseau (1993), however, found that only equilibrium sorption constants were correlated to K_{ow} and that, under non-equilibrium conditions, K_{ow} was a poor descriptor for sorption.

Some researchers have looked at specific molecular descriptors. Researchers have investigated the role that molecular size (as measured by the first-order valence molecular connectivity index and the van der Waals volume) and surface area in influencing sorption (Brusseau 1993, Hu *et al*. 1995). All descriptors were correlated to sorption, with the first-order valence molecular connectivity index having the highest correlation (Hu *et al*. 1995). Hu *et al*. (1995) concluded that the rate-limited sorption behaviour might be explained by the size and structure of the molecule being sorbed. However, Kottler *et al.* (2001) more specifically investigated how some of these molecular descriptors (e.g., molecular connectivity index, log Kow, molecular length) affected sequestration of PAHs, and all were poorly correlated with sequestration. This indicates that while sorption is related to sequestration and aging, the effect of factors on sequestration should not be based solely on data derived from sorption investigations.

2.5 Conclusion Regarding Factors Affecting the Bioavailability of Petroleum Hydrocarbons

Several environmental and site-specific factors have been implicated in the sequestration and/or aging, and thus bioavailability of petroleum hydrocarbons in soil. However, several of the observed correlations with site factors have also had contradictory results in other studies, indicating that there might not be one dominant factor that controls bioavailability in soils. As well, many of the above relationships were derived from experiments investigating sorption and not sequestration and/or bioavailability. The value of the relationships based on the effects on sorption need to be assessed. At least one study, which specifically investigated sequestration (Kottler *et al.* 2001), contradicted results obtained in a purely sorption-related study (Hu *et al*. 1995).

The factors showing the most promise for use in site-specific assessments include soil texture and clay content, organic matter content and its properties, and soil structure. These are all properties that are measurable, and which have shown good correlations with either sorption or sequestration. Factors such as pH, temperature and salinity have also been shown to affect sorption and sequestration, but the results have been more ambiguous, especially with respect to petroleum hydrocarbons. Finally, factors such as the presence of oil and/or co-contaminants, contaminant properties, and moisture regimes might affect bioavailability; however, these factors are either difficult to control (i.e., moisture regime), or too complex to model for sites contaminated with such a complex mixture as PHCs (i.e., presence of co-contaminants or contaminant properties). However, most of the above studies were not designed to specifically investigate the effect of site-specific factors on bioavailability and/or sequestration and/or did not investigate changes in bioavailability with aging. Specific studies must be conducted to investigate the relationship between the site-specific factors and changes in bioavailability, both with and without aging, and to determine the underlying mechanisms involved that result in changes in the bioavailability of the petroleum hydrocarbons.

It should be kept in mind that many of the above factors can also affect the abiotic and biotic components of soil systems, which in turn can affect the sequestration process. Environmental conditions such as temperature, moisture levels, nutrient levels, pH, and soil texture affect microbial populations and rates of biodegradation while wind conditions and temperature can affect the volatilization of some contaminants. In other words, these factors can influence the weathering process. Weathering processes, including biodegradation, will change the length of the contact time a contaminant has with the soil, and thus the time available for it to become sequestered and/or bound to the non-extractable fraction. Nam and

Alexander (2001) demonstrated that the amount of a contaminant that becomes sequestered is dependent on the initial rate of biodegradation in soil as this will determine the time that the contaminant is able to interact with the soil. When biodegradation rates are low, significantly more contaminant is sequestered.

Finally, the route of exposure should be considered when determining bioavailability of petroleum hydrocarbons. Many studies utilize bacterial degradation or mild solvent extraction as a method for correlating factors with bioavailability. These factors do not always correspond with the bioavailability of a compound to organisms, such as earthworms (Gevao *et al*. 2001). Microbes are exposed to contaminants in the aqueous phase, whereas organisms such as earthworms will be exposed via both the soil solution and ingestion of soil particles. The process of ingestion and digestion might affect desorption and the release of residues as the soil passes through the gut of an organism (Gevao *et al*. 2001). This is supported by work conducted with marine polychaetes, where it was found that PAHs were solubilized more into the digestive fluid of the polychaete than into seawater, resulting in a greater potential for bioaccumulation (Weston and Mayer 1998). Similar interactions between biota and soil contaminants likely occur with other organisms.

3 MEASURES OF BIOAVAILABILITY

In order to reliably predict risk posed by petroleum hydrocarbons, methods must be available that can determine the bioavailability of, and thus exposure of organisms to, petroleum hydrocarbons in contaminated soil. Several different techniques have been used in the past, which can be divided into two categories: physico-chemical extractions and biological methods.

3.1 Chemical Methods for Determining Bioavailability

3.1.1 Non-exhaustive ("Mild") Solvent Extraction

Regulatory agencies currently base site and remediation acceptability on contaminant concentrations as determined by vigorous and exhaustive extraction (Kelsey and Alexander 1997). This is true for petroleum hydrocarbons in soil as well, where for the Canada-wide Standards for Petroleum Hydrocarbons in Soil, petroleum hydrocarbons are extracted by vigorous means (e.g., acetone:hexane) such as Soxhlet extraction (CCME 2001). The performance of these procedures is based on obtaining the highest percent recovery possible, and the methods do not reflect that only part of the total PHCs measured in soil is bioavailable. As discussed previously, this can lead to an over estimation of risk since the analytical approach only, does not reflect the bioavailability of the contaminant (Kelsey and Alexander 1997, Kelsey *et al*. 1997).

It is hypothesized that PHCs are sorbed to soil along a continuum ranging from a labile fraction (sorbed near surface and subject to rapid desorption) and a nonlabile fraction (sequestered in remote regions of soil particles and/or chemically bonded to organic matter, and therefore subject to slow desorption) (Alexander 1997). It is also known that different organic solvents will have different affinities for contaminants and extract different amounts (Kelsey *et al*. 1997). Therefore, the assumption for non-exhaustive solvent extraction is that the solvent used will only remove the easily desorbed, labile, and bioavailable fraction from soil.

In order for non-exhaustive solvent extraction methods to be predictive of ecological effects, the results of such tests need to be correlated with the results of biological assays. However, bioavailability is both organism and species dependent and, as such, no one chemical extraction method will be predictive for all scenarios (Reid *et al*. 2000a).

Often, only a single solvent (or solvent mixture, e.g., methanol:water) is used (Kelsey and Alexander 1997, Kelsey *et al*. 1997); however, some researchers use a sequential extraction method wherein progressively stronger solvents or solvent combinations are used to extract progressively more of the sorbed chemical (Kelsey and Alexander 1997, Macleod and Semple 2000, Northcott and Jones 2001a). In this way, different chemically determined "bioavailable" fractions are related to the toxic response of organisms in the soil.

Several non-exhaustive methods have been developed and tested with petroleum hydrocarbons as a means of measuring bioavailability, though none have been strictly validated nor standardized. Many have been correlated to biological effects.

The results of these studies indicate the complexity of determining a suitable solvent for estimating bioavailability. The extraction of different PAHs by various solvents has correlated well with the results of biological tests in some studies. Tang and Alexander (1999) tested a number of different solvents individually and found that all extractions were highly correlated ($r^2 > 0.89$) to earthworm and plant uptake. Kelsey and Alexander (1997) found some qualitative correlation between the earthworm uptake

of PAHs and extraction with ethanol/water, though the progressive decrease in uptake by the earthworms was not reflected by the extraction at the longest aging period. Alexander and Alexander (2000) correlated bioavailability, as measured by genotoxicity, to concentrations of PAHs extracted by *n*-butanol. Kelsey *et al*. (1997) also observed a correlation with *n*-butanol and both earthworm and microbial bioavailability of phenanthrene. Several other solvents did not correlate well with either species, or correlated with one species but not the other (Kelsey *et al*. 1997). This underscores the need to validate any solvent extraction method with the species of interest before applying the method.

It should be noted, however, that a strong correlation between an extraction method and a biological response does not necessarily mean that the relationship is directly predictive of bioavailability (Reid *et al*. 2000b). The slope and the intercept of the relationship must also be considered with a 1:1 relationship (intercept of zero and slope of one) being the most desirable (Reid *et al*. 2000b). This is often not the case, as seen in Tang and Alexander (1999), where there is no mention of the type of relationship (i.e., 1:1 or otherwise). The correlation between butanol and a biological response noted by Kelsey *et al*. (1997) was not 1:1.

A correlation between an extraction method and a biological response does not always result in better predictive capabilities. Krauss *et al*. (2000) used two different non-exhaustive extraction techniques, methanol/water and 0.5 M NaOH, as well as an exhaustive extraction technique, and found that the non-exhaustive techniques did not improve the prediction capabilities over the exhaustive extraction for the bioavailability of 20 PAHs to earthworms. As well, it is possible that an extraction technique might correlate better for a certain "aging" period, but not as well for another (Kelsey and Alexander 1997, Reid *et al*. 2000b).

In many cases, butanol is considered to be a mild extractant for use in non-exhaustive extraction, while dichloromethane is considered to be a strong extractant to use for exhaustive extraction (Macleod and Semple 2000, Reid *et al*. 2000a). As such, differences in the amount of hydrocarbon extracted when the two are used, are expected and have been observed in many cases (White *et al*. 1997, Kelsey *et al*. 1997, Nam *et al*. 1998, Tang *et al*. 1998). However, studies by Northcott and Jones (2001a) and Reid *et al*. (2000b) did not find any differences in the extraction capabilities of the two solvents. These have been attributed to several possible reasons, such as differences in the organic carbon content of the soils used, differences in the extraction methodologies, and/or differences in the methods used to spike the soils with the contaminant for testing purposes (Northcott and Jones 2001a).

In summary, the use of non-exhaustive solvent extraction techniques for estimating the bioavailability of organic compounds shows potential; however, much work is needed to validate and standardize this as a technique. Differences in solvents used, experimental and extraction methodologies, soils and chemicals tested, and biological endpoints can, and have, resulted in apparent contradictory observations. Further research should be conducted in this area, including validation of the methods across various soil types and contamination, as well as across species.

3.1.2 Solid Phase Extraction Techniques

Solid phase extraction is one of only two chemical methods that have been extensively validated with historically contaminated (aged) samples. The other validated method is persulfate oxidation (Cuypers *et al*. 2001). During solid phase extraction (SPE), organic contaminants are extracted from soil with the aid of hydrophobic polymers; organics are allowed to desorb from soil to the aqueous phase, then they are sorbed by the polymer(s). Several different polymers have been used, including Tenax® polymeric beads (Pignatello 1990a, Macrae and Hall 1998, White *et al*. 1999, Cuypers *et al*. 2001), XAD polystyrene resin beads (Carroll *et al*. 1994, Northcott and Jones 2001b), octadecyl-modified silica (C18) disks (Sijm *et al*. 2000, Krauss and Wilcke 2001), and polyethylene tube dialysis (PTD) (Macrae and Hall 1998). Semi-

permeable membrane devices (SPMDs) and solid-phase micro-extraction (SPME) are also solid phase extraction techniques that have been used to estimate bioavailability, and they are covered in more detail in Section 4.

Solid phase extraction is based on extraction of the readily desorbed, and presumably the bioavailable, fraction of the contaminant from the soil via initial desolution into the aqueous phase of a soil slurry. The driving force for desorption is the chemical concentration gradient between the soil and water phases. Addition of hydrophobic polymers removes the contaminant from the water phase, thus maintaining the chemical gradient of the contaminant between the soil and water phases and encouraging further desorption of the readily available contaminant (Cuypers *et al*. 2001, Krauss and Wilcke 2001, Northcott and Jones 2001b). The solid-phase extractant can be added in excess to act as an infinite sink for adsorption of released contaminant (Northcott and Jones 2001b), or can be sequentially replaced to maintain the concentration gradient (Pignatello 1990a, Cornelissen *et al*. 1998a, Cuypers *et al*. 2001). In this manner, the total amount of the easily desorbed fraction that might be potentially bioavailable is determined. Alternately, Krauss and Wilcke (2001) used C18 disks as a tool to "mimic" uptake by organisms and thus did not determine the total amount of contaminant that was desorbable, but instead only the amount adsorbed by the disks at equilibrium with a soil slurry. As in the experiments on factors affecting bioavailability, often experiments with solid-phase extraction procedures were conducted to investigate desorption phenomena (e.g, Pignatello 1990a, Cornelissen *et al*. 2000, Kan *et al*. 2000) and not bioavailability *per se*.

The use of solid phase extraction techniques for estimating bioavailability assumes that the contaminant is bioavailable via the aqueous phase/pore water only. If there are other routes of exposure other than solely pore water, the SPE might underestimate bioavailability. Likewise, if uptake by an organism is the endpoint of interest to indicate bioavailability or if the organism is able to biotransform the contaminant, then SPE techniques might overestimate uptake (Sijm *et al*. 2000). Thus, it is important to be aware of the limitations of these methods and to understand the system under study.

Tenax® polymer beads have been used extensively in desorption and bioavailability experiments. This technique is generally easy to work with, relatively inexpensive (Macrae and Hall 1998), and has an affinity for organic contaminants that is similar to that of organic carbon (Cornelissen *et al*. 1997).

Experiments have demonstrated that desorption of petroleum hydrocarbons from contaminated soils, as determined with Tenax® beads, correlates well with microbial bioavailability, and can be used to predict the fraction of PHC that will be degraded during bioremediation (Cornelissen *et al*. 1998a, Macrae and Hall 1998, White *et al*. 1999, Cuypers *et al*. 2001). Tenax® beads are considered a good model for desorption of contaminants during biodegradation as Tenax® acts much like microbes in maintaining the concentration gradient between the soil and the aqueous phase (Cornelissen *et al*. 1998a). While most work has been with microbial bioavailability and biodegradation, White *et al*. (1999) related desorption as measured with Tenax® to the uptake of phenanthrene by earthworms. They observed a decrease in uptake that correlated to a decrease in Tenax® extraction.

Cuypers *et al*. (2001) specifically studied the predictive powers of Tenax® (as well as persulfate oxidation, Subsection 3.1.4) for total petroleum hydrocarbon bioavailability to micro-organisms. It was observed that Tenax® removed slightly less of the low molecular weight hydrocarbons and slightly more of the high molecular weight hydrocarbons than were microbially degraded. This was explained by both differences in the extraction and degradation times, as well as by the recalcitrance (non-degradability) of some higher molecular weight hydrocarbons that could still be extracted though not degraded. It should be noted that several researchers noted a decrease in extraction as measured by Tenax® with increased aging of compounds in soil (White *et al*. 1999, Cuypers *et al*. 2001).

XAD resin is another solid phase extractant that has been used in a similar manner to Tenax® (Carroll *et al*. 1994). Northcott and Jones (2001b) utilized XAD beads to determine desorption kinetics of several PAHs over time (aging). Bioavailability was not determined, but if the extraction by XAD is considered to represent the available fraction, then sequestration was not observed in their study. This would contradict other studies with PAHs (White *et al*. 1999, Cuypers *et al*. 2001). Similarly, Carroll *et al*. (1994) used XAD beads to study the desorption of PCBs from sediments, and while the fast desorbing fraction was considered to be the labile, bioavailable fraction, no tests on bioavailability were conducted. Therefore, further research is needed to determine if extraction with XAD is correlated to bioavailability.

The use of C18 disks has been suggested as another method to estimate the bioavailability of contaminants (Sijm *et al*. 2000), and has been successfully used to estimate biota-soil accumulation factors (BSAFs) for PAHs and PCBs to earthworms even though the data suggest that routes of exposure other than via the soil solution were present (Lake *et al*. 1996, Krauss and Wilcke 2001). Uptake by earthworms of DDT and its metabolites was also correlated with extraction by C18 disks (Tang *et al*. 1999). A comparison of extraction with the C18 disks to that with a mild solvent extraction (methanol-water) demonstrated that the bioavailability of PAHs and PCBs was better predicted by the C18 extraction (Krauss and Wilcke 2001).

Macrae and Hall (1998), in a comparative study on the effectiveness of various solid-phase extraction techniques (SPMDs, Tenax®, and polyethylene tube dialysis) to estimate bioavailability used a method called polyethylene tube dialysis (PTD) that was essentially a SPMD in reverse. With PTD, a slurry of contaminated soil or sediment is placed within a polyethylene dialysis tube, and the contaminant is extracted out of the tube into the hydrophobic solvent pentane. With SPMDs, a polyethylene tube is filled with a hydrophobic compound, triolein, and contaminants are extracted from a water or slurry sample into the tubing. It was found that bioavailability of PAHs appeared to be greater when the PTD method was used compared to SPMDs and Tenax® extraction, and the authors concluded that PTD extraction was a more stringent method that could best be used for risk assessments of contaminated soils and sediments. It was further suggested that the other two methods were more applicable for determining the degree of biodegradation that would be expected at a contaminated site (Macrae and Hall 1998).

A similar method with dialysis tubing was used by Woolgar and Jones (1999) to determine near equilibrium aqueous concentrations of PAHs from a diversity of contaminants (e.g., various non-aqueous phase liquids, sewage sludge, coal tars). Again, the study material, as a water slurry, was placed inside the dialysis tubing, and desorption of the contaminant out of the tubing and into water was measured. Desorption from soils was not measured; however, it might be possible to modify this method for determining desorption and possibly bioavailability of contaminants from **soils. Validation of the method for soils and determining bioavailability would need to be done.**

3.1.3 Cyclodextrin-based Extraction

Cyclodextrins are highly soluble, macrocyclic compounds with a hydrophobic organic interior. Their use was investigated by Reid *et al*. (2000b) as a potential extractant for labile nonpolar organic contaminants for the determination of contaminant bioavailability. A study comparing the extraction capabilities of dichloromethane, butanol and a novel, water-soluble sorbent with a hydrophobic interior, cyclodextrin, was undertaken in order to determine if cyclodextrin could be used to provide a more predictive estimate of the bioavailability of phenanthrene to microorganisms. In this study, all extractants used correlated well to microbial mineralization (and thus bioavailability); however, only the cyclodextrin extractant demonstrated a 1:1 relationship with the concentration of mineralizable phenanthrene, and was therefore directly predictive of bioavailability. Both dichloromethane and butanol overestimated the actual bioavailability. Similar to observations with Tenax® (Cornelissen *et al*. 1998a), Reid *et al*. (2000b)

believe that cyclodextrin extraction mimicked the desorption processes that determine microbial bioavailability of contaminants and thus degradation.

3.1.4 Persulfate Oxidation

Unlike the previous methods, persulfate oxidation is not based on contaminant extraction. Rather, it is presumed that only bioavailable contaminants can be oxidized by persulfate, providing an estimate of the bioavailable fraction (Cuypers *et al*. 2001). This method was specifically investigated for its predictive properties of both field-aged PAHs (Cuypers *et al*. 2000) and total petroleum hydrocarbon (Cuypers *et al*. 2001) bioavailability to micro-organisms. It was found that persulfate oxidation provided a good estimate of PAH bioavailability (Cuypers *et al*. 2000); however, it was unable to predict the bioavailability of total petroleum hydrocarbons due to the inability of persulfate to oxidize hydrocarbons with high ionization potentials, such as aliphatic hydrocarbons (Cuypers *et al*. 2001).

3.1.5 Super-critical Fluid Extraction

Super-critical fluid extraction (SFE) is a technique that uses super-critical fluids as solvents for extraction. The advantage of SFE over other solvent-based extractions is that the solubility of the solute into the solvent can be changed by several orders of magnitude by varying the pressure of the system (Young and Weber 1997). SFE with pure carbon dioxide has been used to predict the bioavailability of aged PAHs in soil from a manufactured gas plant site (Hawthorne and Grabanski 2000). SFE is considered to be a good method for studying both bioavailability and sorption/desorption phenomena as changes in extraction parameters can result in extraction of contaminants from progressively more sequestered and less bioavailable domains of the soil (Björklund *et al*. 1999, Hawthorne and Grabanski 2000). Also, unlike solvent extractions, SFE will not significantly change the organic matrix during the extraction (Hawthorne and Grabanski 2000). Hawthorne and Grabanski (2000) demonstrated that SFE under the mildest extraction conditions studied correlated well with the degree of biodegradation observed after one year of bioremediation of the soil. While these results are promising, further validation of the method with more soils and bioavailability of PAHs to other organisms is required. This research is forthcoming (Hawthorne and Grabanski 2000).

While super-critical fluid extraction appears promising, it is not ideal (Johnson and Weber 2001). For some compounds (i.e., phenanthrene) SFE desorption kinetics and energetics differ from those in aqueous desorption (Johnson and Weber 2001 and references therein).

3.1.6 Subcritical Water Extraction

Johnson and Weber (2001) developed a method using a subcritical water extraction method to estimate the long-term release of contaminants soils and sediments. Subcritical-water extraction is an activation energy based technique that utilized superheated water (subcritical refers to water above 100°C but kept in a liquid state by increasing the pressure). Desorption rate data derived from the subcritical extraction were modeled to estimate activation energies, and these were then used to predict desorption at other temperatures. This method predicted long-term release rates of phenanthrene well.

3.2 Biological Methods for Determining Bioavailability

Biological methods are any tests (bioassays) using organisms themselves to determine or estimate the bioavailability of contaminants in a site soil. Some of these bioassays, such as measures of contaminant uptake by organisms (i.e., tissue concentrations), directly determine contaminant bioavailability to organisms, while others, such as soil toxicity and changes in microbial activity are indirect assays from

which the level of bioavailability cannot be directly measured but instead is inferred (MacMillen *et al*. 2003).

Several different biological test methods have been successfully used to estimate bioavailability of petroleum hydrocarbons in site soils and their associated risk (Table 3.1). This list is not exhaustive, and basically any validated biological test can be used. While the testing of soil extracts with aquatic species has been used in the past, the relevance of the results of such tests for terrestrial species is questionable and therefore these tests are neither recommended (van Gestel *et al*. 2001) nor discussed further in this report. Several biological tests with terrestrial organisms are available, and some are standardized (Løkke and van Gestel 1998, Stephenson *et al*. 2002).

Several of the above studies were laboratory studies conducted specifically to determine the effect of sequestration of PAHs by soil during aging on the bioavailability of the PAHs to soil organisms. Studies by Kelsey and Alexander (1997), Kelsey *et al*. (1997), Nam *et al*. (1998), and White *et al*. (1997, 1998, 1999a) found that the availability of PAHs, as measured by mineralization and uptake by earthworms and plants, decreased with aging of the contaminant in soil. These results were often correlated to a concurrent decrease in contaminant extractability by mild solvent extraction (Kelsy *et al*. 1997, Nam *et al*. 1998, White *et al*. 1997, 1998) or contaminant desorption (White *et al*. 1999) with aging. Sijm *et al*. (2000) also described a method (soil availability ratio—SARA) that utilizes uptake by organisms to determine the bioavailable fraction of contaminants in site soils.

Several of the methods in Table 3.1 have also been used to determine the bioavailability and/or associated risk of petroleum hydrocarbons in site soils (Saterbak *et al*. 1999, Billeret *et al*. 2000, Filimonova and Pokarzhevski 2000, Stroo *et al*. 2000, Charrois *et al*. 2001, van Gestel *et al*. 2001, Visser *et al*. 2001) or in soils following remediation (Marwood *et al*. 1998, Saterbak *et al*. 2000). As noted by Charrois *et al*. (2001), the results of the biological test are often not predicted by the total chemical concentration in the soil.

Table 3.1 Biological Test Methods Used in Determining Bioavailability of Petroleum Hydrocarbons in the Laboratory and Field.

Test Organism	Test Endpoints	Reference
Microbial	Degradation and/or mineralization	Kelsey *et al.* 1997, White *et al.* 1997, 1999a, Nam *et al.* 1998, Macleod and Semple 2000, Reid *et al.* 2000b, Visser *et al.* 2001
	Respiration	Filimonova and Pokarzhevski 2000, Visser *et al.* 2001
	Carbon cycling	Visser *et al.* 2001
	Nitrogen cycling	Visser *et al.* 2001
	Genotoxicity	Alexander and Alexander 2000
	SOS-Chromotest	Marwood *et al.* 1998
	Toxi-Chromotest	Marwood *et al.* 1998
	Microtox or bioluminesence	Harkey and Young 2000, Stroo *et al.* 2000, Visser *et al.* 2001
Soil fauna (microbial and invertebrate)	Litter decomposition, bait lamina test	Van Gestel *et al.* 2001, Visser *et al.* 2001
Macro- and mesofauna	Abundance and diversity	Visser *et al.* 2001
Springtails	Survival, reproduction	Van Gestel *et al.* 2001, Visser *et al.* 2001
Earthworm	Uptake	Ma *et al.* 1995, Kelsey and Alexander 1997, White *et al.* 1997, 1998, 1999, Tang *et al.* 1998, Sijm *et al.* 2000, Stroo *et al.* 2000,
	Survival, reproduction, avoidance, growth	Saterbak *et al.* 1999, 2000, Stroo *et al.* 2000, Charrois *et al.* 2001, Van Gestel *et al.* 2001, Visser *et al.* 2001
Enchytraeids	Survival, reproduction	Filimonova and Pokarzhevski 2000
Plants	Germination, root elongation, growth	Marwood *et al.* 1998, Saterbak *et al.* 1999, 2000, Van Gestel *et al.* 2001, Visser *et al.* 2001
	Uptake	Tang *et al.* 1998
Mammalian	Uptake	Stroo *et al.* 2000
	Enzyme biomarkers (EROD)	Billeret *et al.* 2000
	Dermal uptake (human cadavers)	Stroo *et al.* 2000

There are several advantages to using biological measures over chemical measures of bioavailability. Biological measures:

- integrate effects of soil type and aging on bioavailability (Saterbak *et al*. 1999, Charrois *et al*. 2001);
- integrate interactions occurring among multiple contaminants in the site soil (Charrois *et al*. 2001), such as those typically found with petroleum hydrocarbon contamination (both toxicity interactions and physico-chemical interactions such as competition for sorption sites);
- take into consideration multiple exposure routes other than via soil solution depending on the assay (Sijm *et al*. 2000). As was observed with the solid-phase extractions discussed in Subsection 3.1.2, SPE measures bioavailability as the fraction that can be desorbed to the aqueous phase, only;
- can account for both biotransformation and detoxification reactions within organisms (Sijm *et al*. 2000), as well as the formation of toxic biodegradation products (Charrois *et al*. 2000) that can alter the risk associated with the contamination. This is not a measure of bioavailability *per se*, but can be an important factor in risk assessments; and,
- can provide information on the general biological quality of contaminated soils by integrating effects due to the contaminant and soil properties, including physical changes in the soil properties induced by the contamination; these changes are not necessarily related to bioavailability (Saterbak *et al*. 1999, van Gestel *et al*. 2001).

Bioavailability can be organism- (Kelsey *et al.* 1997, White *et al.* 1997) and species- (Guerin and Boyd 1992) specific. Thus, it is recommended that a test battery of organisms be used to evaluate the bioavailability and risk associated with a contaminated site (van Gestel *et al.* 2001). The use of a test battery can account for organism and species differences in bioavailability, uptake, and routes of exposure (Stroo *et al.* 2000, van Gestel *et al.* 2001). It is recommended that the test battery include species from various ecological niches to increase the relevancy of the assessment of bioavailability and risk at a site and, for even greater relevancy, site-specific species should be included if possible (van Gestel *et al.* 2001). Other considerations when utilizing these tests and interpreting the results include the tolerance of the test species for the site and/or test conditions (van Gestel *et al.* 2001). Erroneous estimations of bioavailability and risk might be made if the test species is intolerant to the test conditions (including site soil properties), regardless of the contaminants present. Similarly, the possibility that a contaminant can be non-bioavailable but still affect soil properties such that the soil is rendered uninhabitable for a species, needs to be considered (van Gestel *et al.* 2001). This includes the development of hydrophobicity in soils with the weathered petroleum hydrocarbons, as observed by Visser *et al.* (2001).

With biological assays, it is important to understand how the test organism interacts with the test system, and what is being measured. As was observed with some of the chemical extraction methods, microbial degradation correlated well with the amount of desorption from soil into the aqueous phase (Cornelissen *et al.* 1998a, Reid *et al*. 2000b, Sijm *et al.* 2000). However, this relationship does not account for other routes of uptake (Lake *et al.* 1996, Krauss and Wilke 2001).

The use of microbial degradation and/or mineralization is an indirect method of estimating bioavailability of petroleum hydrocarbons (Reid *et al.* 2000a) and might underestimate the bioavailability of PHCs to other organisms. Researchers have noted that mineralization of PHCs might underestimate the true bioavailability PHCs in soils (Huesemann 1997). Estimations of bioavailability based on PHC mineralization assumes that all of the PHCs that are not mineralized are sequestered and hence not

bioavailable. However, it has been shown that a fraction of this non-mineralized residual following degradation consists of recalcitrant compounds that are not biodegradable, and which might be bioavailable to other organisms (Huesemann 1997, Cuypers *et al.* 2001). Therefore, assessments that consider only microbial biodegradation as an estimate of bioavailability or risk could seriously underestimate both of these parameters. This underscores the need for understanding the test system and the benefits of using a test battery including different test species and methods.

Likewise, other indirect measures of bioavailability, such as toxicity to a test organism, might over estimate bioavailability. This is true when a factor other than contaminant bioavailability affects the response of the test organism adversely (van Gestel *et al.* 2001). However, for the determination of the overall risk of a site soil to biological entities, biological tests might be more appropriate because they do incorporate factors other than contaminant toxicity into their responses.

3.3 Field Studies of PHCs in Soil

This review has focused on studies with petroleum hydrocarbons that were designed to explain mechanisms of sorption and sequestration, factors affecting bioavailability and methods for estimating bioavailability. Several laboratory studies have demonstrated the potential for PHC sequestration and decreased bioavailability with time. However, a number of field studies also have demonstrated a reduction in the bioavailability of PHCs in soils, especially with regard to aging. Changes in bioavailability are rarely measured directly. More frequently they are inferred from estimates of soil toxicity at a particular moment in time. Inferences on bioavailability were made from a few studies. Stroo *et al*. (2000) specifically studied bioavailability of PAHs in the field.

In Canada, the Canada-wide Standards for Petroleum Hydrocarbons in Soil (CCME 2000a) provide benchmark remedial values based on four carbon-range fractions for petroleum hydrocarbons. Limited ecological testing has been conducted with the specific fractions; however, a field study was conducted with crude oil and to determine degradation and toxicity of the four hydrocarbon fractions under field conditions (Visser *et al*. 2001). While this study did not specifically study bioavailability, the results indicate that a reduction in the bioavailability of the fractions occurred over time. The results of a three-month laboratory biodegradation and toxicity study with crude oil indicated that the PHCs were less available and less toxic in a clay soil when compared to a sandy soil. This could be due to the high surface area of the clay minerals. A reduction in the toxicity of PHC-contaminated soils to plant and invertebrates was observed in field studies compared to laboratory studies with fresh product for fraction 3, and in one instance, fractions 2 and 4 (Visser *et al*. 2003). These results indicate that similar concentrations of aged PHCs were not as toxic as freshly added PHCs, which suggests that the aged PHCs were likely less bioavailable. The significance of these results is that the Canada-Wide Standards developed with fresh product will be protective.

Stroo *et al*. (2000) investigated the bioavailability of contaminants in soils from a former manufactured gas plant (MGP) with a test battery. They demonstrated a wide range of bioavailabilities of PAHs depending on the soil. The aged soil and lampblack soil showed the lowest bioavailabilities of all soils tested and this was consistant for all bioassays. The PAHs in lampblack soil were unavailable. It is interesting to note that lampblack soil has a sooty texture and appearance. High surface area carbonaceous materials such as soot have been implicated in increased sorption (Gustafsson *et al*. 1997, Chiou and Kile 1998).

Charrois *et al*. (2001) studied the bioavailability of creosote constituents to earthworms using weathered soils and biotreated soils. From their study it was deduced that the bioavailability of the creosote PAHs were reduced when a residual nonaqueous-phase liquid was present. No determinations on the effect of sequestration and aging were made in this study.

Aged soils from a manufactured gas plant site were studied by Harkey and Young (2000) and subjected to both exhaustive and mild extraction techniques and biological testing (Microtox®). There was little correlation between the toxicity observed with the soils and the PAH concentrations that were determined by the exhaustive extraction. This suggests that differences in the bioavailability of the contaminants in the soil controlled the toxicity of the soil.

Salanitro *et al*. (1997) investigated crude oil contamination and the effects of bioremediation on reducing toxicity; they did not directly measure changes in bioavailability. A residual fraction of petroleum hydrocarbons remained in the soil following bioremediation. The authors suggested the residual PHC fraction was sequestered and unavailable. They considered these residues to be solely recalcitrant, nonbiodegradable compounds because they were neither toxic to soil organisms (plants and earthworms) nor leachable.

3.4 Summary

It is clear that sorption of petroleum hydrocarbons to soils, and subsequent sequestration and formation of non-extractable residues, can lead to changes in the bioavailability of these compounds and thus affect the risk associated with these compounds. Unfortunately, much of the research has not specifically investigated the effect of sorption and aging on the bioavailability of compounds to soil organisms. The exact mechanisms involved in the sorption and sequestration process are still under study; however, several models have been proposed that describe these processes. Further research is required to more precisely determine the mechanisms of sorption and sequestration. It is highly likely that more than one mechanism occurs concurrently and, once the mechanisms have been determined, research should be conducted to determine the contribution of each mechanism to the total sorption and sequestration by the soil. Further research also is required to elucidate which site-specific, environmental factors control these processes, especially how they relate to changes in bioavailability initially and over time, in order to accurately and reliably develop models that can be used to predict bioavailability in the field.

Several chemical and biological methods have been developed to estimate the bioavailability of contaminants in soils. Most of the chemical methods require further validation and standardization before they can be used in a regulatory context. As well, both chemical and biological methods have limitations. Chemical methods might underestimate bioavailability to organisms via routes of exposure other than the aqueous phase. Some biological methods might underestimate bioavailability of recalcitrant, nondegradable compounds (e.g., mineralization tests), or overestimate bioavailability, if factors other than the contaminant bioavailability influence the biological response (i.e., soil properties).

The interaction between the concurrent processes of weathering and aging should be investigated further. At sites such as those with complex PHC mixtures, the degree and rate of weathering might significantly affect the degree of sequestration. As well, the distinction between bioavailable compounds and those that are recalcitrant and nondegradable is important. Recalcitrant compounds are not necessarily unavailable compounds. A compound can be recalcitrant to microbial degradation, yet remain available to other organisms for uptake. However, some recalcitrant compounds might also become sequestered and/or bound to soil over time, resulting in the formation of non-bioavailable, recalcitrant residues.

The routes of exposure of the organism to the contaminant must also be considered when predicting bioavailability or interpreting bioavailability data. Differences in the availability of a contaminant to different organisms have been observed, and could be related to differences in the routes of exposure. Much of the work on the bioavailability of PHCs has been conducted with mico-organisms, and the data might not be directly applicable to higher organisms with more complex exposure pathways.

Changes in the bioavailability of PHCs due to aging and site-specific factors should be considered during higher tiers of risk assessments (i.e., Tiers 2 and 3). Failure to do so could result in the use of overly conservative remediation values, and an increase in the cost of remediation and/or amount of soil lost. Soil is a valuable resource that should be conserved. Destruction of soil fertility or structure occurs when destructive remediation technologies are applied needlessly to soils that are actually non-toxic. Stroo *et al*. (2000) estimated a 2.5 to 4 million dollar reduction in cleanup costs at a former gas manufacturing site, if the risk assessment and remediation decisions were based on the results of bioavailability studies rather than benchmark levels. Field studies that specifically investigate changes in PHC bioavailability rather than solely bioremediation should be conducted to provide field-relevant data on aging and the bioavailability of PHCs in soils. At this time, effects on bioavailability in the field generally must be inferred from studies that did not specifically investigate this phenomenon.

4 ESTIMATING TOXICITY AND BIOAVAILABILITY OF ORGANIC COMPOUNDS IN SOIL

4.1 Introduction

Organic contaminants (e.g., polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorines) are ubiquitous in soil environments. They have the potential to exert lethal and sub-lethal effects on ecological receptors. Earthworms are a particularly sensitive group of soil organisms because of the nature of their interactions with soil. Because they often ingest soil and are in constant contact with the soil, they are susceptible to the bioconcentration of soil contaminants. They also serve as a food source for higher trophic levels (e.g., worm-eating birds) and contribute to the potential for the biomagnification of these compounds. Therefore, determining the levels of the biologically available fraction of organic compounds is crucial to the complete assessment of the risks associated with contaminated soils.

Traditional soil assessments incorporate the use of vigorous chemical extraction to measure total concentrations of toxic chemicals in soils, and do not include an assessment of the bioavailability of the contaminant in soil (Kelsey *et al*. 1997, White *et al*. 1997, Bierkens *et al*. 1998). Advances in the risk assessment of soil have led to the use of biological toxicity tests. These tests are suitable for the direct measurement of environmentally relevant toxic concentrations in soil. However, the use of organisms is time consuming, expensive, and usually requires several concentrations and many replications to determine a reliable exposure concentration-response relationship. As a result, passive sampling devices (PSDs) have been suggested and recently used as an alternative to assess the bioavailability of organic compounds in soil (Wells and Lanno 2001, Lanno 2002). PSDs are able to concentrate the bioavailable fraction of several organic contaminants in aquatic and atmospheric samples, and soil suspensions (Arthur and Pawliszyn 1990, Petty *et al*. 1993, Strandberg *et al*. 1997, Eriksson *et al*. 1998, Rantalainen *et al*. 1998, Rantalainen *et al*. 2000). They are designed to mimic biological systems by providing a medium into which selective hydrophobic contaminants can sequester (Parkerton *et al*. 2000, Sijm *et al*. 2000, Wells and Lanno 2001), much like the partitioning of hydrophobic chemicals across the lipid membranes of terrestrial organisms. However, only a few researchers (Wells and Lanno 2001, Lanno 2002) have attempted to relate the bioavailable fractions that are concentrated within the PSDs to levels associated with adverse biological responses in terrestrial organisms.

The purpose of this section of the report was to investigate and compare the efficacy of PSDs, specifically solid phase microextraction (SPME) and semipermeable membrane devices (SPMDs) to: 1) determine the bioavailability of organic compounds in soils; and, 2) correlate the measured levels to those that cause adverse biological effects in earthworms as derived from biological assays. Included in the following subsections are discussions on the use of PSDs (SPMEs and SPMDs) to measure the bioavailable fractions in soil and the relationship between the results of their application and soil toxicity to earthworms. The section concludes with an overview of soil assessment with PSDs and their future implications for assessing the risk associated with contaminated soils.

4.2 Bioavailability

The toxicity of an organic contaminant in soil is a function of the exposure concentration, the exposure duration, and the bioavailability of that contaminant in soil. The bioavailability of a compound in soil is that portion of the compound that is freely available for uptake into an organism through various routes of exposure (e.g., dermal absorption). Bioavailability can be measured by responses in an organism (e.g., mortality, metabolism, reproduction, etc.) and is specific to the contaminant, the exposure matrix (e.g., aqueous or non-aqueous media), the route of exposure, and the target organism likely to be affected

(Kelsey *et al*. 1997, Wells and Lanno 2001). As discussed earlier, bioavailability is a complex process involving various relationships between the environmental concentrations present within the soil, and the portion available for uptake by organisms (Sijm *et al*. 2000).

As discussed earlier, several physical, chemical, and biological factors influence the availability of an organic compound to organisms in soil. Therefore, these influential factors should be considered when assessing the bioavailability and the associated toxicity of a contaminant to terrestrial organisms in soil.

Organic compounds may undergo several fates upon introduction into soil environments such as volatilization, binding to soil particles, leaching to groundwater, biodegradation or transformation by microbial organisms, or transfer to macroinvertebrates (e.g., earthworms) via dermal sorption or ingestion. The exact fate of organic contaminants is a function of the chemical itself, the properties of the surrounding soil and the soil organisms.

The hydrophobicity of a chemical plays a significant role in the fate of that chemical. Highly hydrophobic compounds with low water solubility are more likely to associate with soil particles rather than with pore water (Belfroid *et al*. 1996). Cerniglia (1992) found that highly hydrophobic compounds were associated with increased persistence in soil. Increased persistence of hydrophobic contaminants is likely a result of repulsion from the pore water to adsorption to the surface of soil particles. Further soil-contaminant interactions are a result of diffusion and/or weak dipolar forces (Pignatello and Xing 1996) on or within soil micropores. Sorption and desorption processes eventually lead to the partitioning or movement of the hydrophobic chemicals from accessible to less-accessible sites within the organic matter and soil micropores (intra-particle diffusion) resulting in decreased bioavailability of the chemical (Linz and Nakles 1997, Robertson and Alexander 1998, Reid *et al*. 2000a). The initial rates of sorption and desorption occur rapidly, but slow down due to diffusion limitations such as steric hinderance within the soil organic matter and micropores (Pignatello and Xing 1996, Hatzinger and Alexander 1997). As a result, aged hydrophobic organic contaminants (i.e., increased residence time of the contaminants in soil) will have a greater unavailability due to slow desorption rates and movement through micropores, and hence inaccessability to microbial organisms or macro-invertebrates. The sequestration of selected polycyclic aromatic hydrocarbons (PAHs), phenanthrene and naphthalene, over time in soil resulted in a diminution of the quantity of these chemicals available to earthworms (Kelsey and Alexander 1997). In addition, vigorous extraction of the two chemicals did not correlate, but over-estimated the fraction of chemical bioavailable to earthworms (Kelsey and Alexander 1997). Additional studies demonstrated that phenanthrene, anthracene, flouranthene and pyrene were less available for uptake by earthworms and microbes with increasing residence time of the PAHs in soil (Tang *et al*. 1998). The accumulation of chlorobenzene in aged contaminated field soils was considerably less than in freshly contaminated soil, indicating less bioavailability in historically field-contaminated soils (Belfroid *et al*. 1995). White *et al*. (1997) and Kelsey *et al*. (1997) also observed that bioavailability of phenanthrene, chrysene, and atrazine declined with increasing residence time in the soil, but that bioavailability was also markedly species- and organism-dependent.

Soil particle size also influences the bioavailability of organic compounds in soil. Smaller particles contain higher concentrations of contaminants due to a greater surface area to mass ratio (Belfroid *et al*. 1996). A review by Belfroid *et al*. (1996) suggests however, that sorption over different particle size classes is partially controlled by the organic matter (or carbon) content of the soil. Selective feeding behavior may also influence the relative bioavailability and uptake of a contaminant. Earthworms will typically feed upon organic matter and prefer smaller particles, thus increasing the uptake of the contaminant in the organism relative to the bulk concentration in the soil (Belfroid *et al*. 1996). A study by Ma *et al*. (1995) demonstrated that food deprived earthworms consumed greater amounts of contaminated soil increasing the bioaccumulation of PAHs; however, when uncontaminated food was added, bioaccumulation decreased by two- to five-fold. The avoidance behavior of earthworms may also

influence the bioavailability and uptake of a contaminant in soil. In addition, the burrowing activity of earthworms through contaminated soil may increase exposure, availability and uptake of the contaminants to the earthworms (Belfroid *et al*. 1996).

The concentration of the contaminant in the soil is also influential in assessing bioavailability; increasing concentrations of pentachlorophenol, phenanthrene, and pyrene have been associated with increased sorption to soil, less desorption to the pore water, and decreased bioavailability unless ingested by earthworms (Divincenzo and Sparks 1997, Chung and Alexander 1999). Similar effects were observed for other organic compounds such as toluene, naphthalene, 1,2,-dichlorobenzene, and DDT (Kan *et al*. 1998).

4.3 Kenetics of Bioavailability of Organic Contaminants in Soil

The previous sections described several physical, chemical, and biological (e.g., earthworm behavior) factors that affect the bioavailability of an organic compound in soil. However, to understand the bioaccumulation of these compounds by an earthworm, consideration must be given to the kinetic processes that allow the compound to partition into the organism. The main routes of exposure of earthworms to hydrophobic organic contaminants in soil may occur via dermal absorption (e.g., through the skin from pore water), or through the direct ingestion and passage of the contaminated soil or organic material through the gut.

The equilibrium partitioning theory (EPT) is the model most widely used to predict the bioavailability and body burden of contaminants in aquatic and terrestrial organisms (Belfroid *et al*. 1994). The EPT was originally proposed for estimating bioaccumulation in aquatic environments; however, a review by Belfroid *et al*. (1996) provides support for the applicability of the EPT to terrestrial environments (e.g., soil). The theory is based on the assumption that organic contaminants sorbed to soil particles are in equilibrium with the pore (i.e., interstitial) water, the same aqueous phase to which terrestrial organisms are exposed (Sijm *et al*. 2000). Equilibrium between the aqueous (i.e., interstitial water) and the non-aqueous phases (i.e., soil) is described by a sorption coefficient (K_p):

$$K_p = C_p / C_w$$

where C_p = concentration of contaminant sorbed to soil

C_w = concentration of contaminant in interstitial water

K_p is synonymous with K_d which was mentioned earlier. The concentration of the contaminant in the organism is a function of the concentration of the contaminant in the interstitial water, and the lipid content of the organism. The EPT involves the combination of two distinct partitioning processes; (1) partitioning of the compound to pore water; and (2) partitioning of the compound from the pore water to the lipid membranes of an organism (Belfroid *et al*. 1996) (Figure 4.1). Therefore, using the sorption coefficient to determine the relative concentration in the interstitial water, the concentration of the contaminant in the lipid can be predicted (Belfroid *et al*. 1996). The relationship between the concentration in the interstitial water and the organism lipid is referred to as the bioconcentration factor (BCF). As sorption is largely based on the log of the octanol-water partitioning coefficient (K_{ow}) of the contaminant, the BCF of the contaminant is also based on the log K_{ow}.

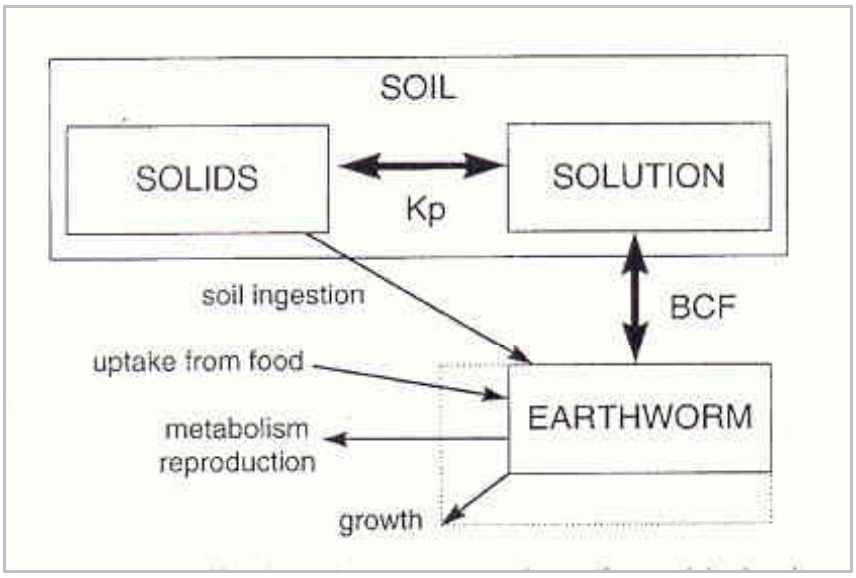

Figure 4.1. Processes that affect the concentration of organic contaminants in earthworms. Thick arrows represent the equilibrium partitioning theory (where Kp = sorption coefficient and BCF = bioconcentration factor), and thin arrows represent factors that influence the validity of the theory (Jager 1998).

The EPT model assumes that the uptake of organic contaminants occurs via passive diffusion of the dissolved contaminants present in the interstitial water across the lipid membranes of the organisms (Belfroid *et al*. 1996, Ma *et al*. 1998). Studies demonstrated that the bioaccumulation of hydrophobic contaminants (e.g., PAHs) was similar for earthworms exposed to contaminated soil relative to exposure in contaminated water only (Belfroid *et al*. 1996, Ma *et al*. 1998, Jager *et al*. 2000). This suggests that uptake via the interstitial water is the most predominant route of uptake, as described by the EPT. However, in the case of high molecular weight PAHs, BCFs deviate from that predicted by the EPT (Ma *et al*. 1998). Belfroid *et al*. (1995) observed that uptake from soil ingestion was significant for highly hydrophobic chemicals (log K_{ow} > 5), leading to an underestimation of the actual accumulation levels based on the levels predicted by the EPT (Belfroid *et al*. 1995). The EPT fails to consider additional routes of uptake, other than that from pore water, and as such, an additional relationship between the soil and the organism, described as the biota-to-soil-accumulation factor (BSAF), should be considered. When soil ingestion by earthworms was examined it was concluded that although soil may be the source of contamination, the pore water might remain a relevant exposure route (Belfroid *et al*., 1995). Pore water ingested with the soil may enhance desorption of the compound from the ingested soil particles resulting in the absorption of the freely dissolved compounds across the gastro-intestinal tract. Modified versions of the EPT have been developed (Belfroid *et al*. 1995, Belfroid *et al*. 1996) that incorporate additional routes of uptake.

Additional deviations in BCFs also arise from the selective feeding behavior of the earthworms, biotransformation within the earthworm, different K_p values from aging or sequestration, the presence of other contaminants (e.g., oil), and the type of organic matter (Belfroid *et al*. 1996; Jager 1998). Consequently, the relationship between the concentration of a contaminant in pore water and soil will determine the bioavailability and the resulting concentrations in the organism (Sijm *et al*. 2000). Assessing the bioavailability of organic compounds in soil is imperative to the risk assessment and prioritization of remediation for contaminated soils (Lanno 2002).

4.4 Methods to Study Bioavailability

As mentioned earlier, terrestrial risk assessments have relied on the use of vigorous extraction and chemical characterization, and do not typically include an assessment of the bioavailability of the contaminant in soil (Kelsey *et al*. 1997, Bierkens *et al*. 1998). As a result, soil extraction techniques have focused on "total" concentrations, which may overestimate exposure and therefore, the risk (or toxicity) of organic compounds in soils (White *et al*. 1997, Kelsey *et al*. 1998). As several factors influence the bioavailability (Subsection 2.4), and consequently the uptake of a contaminant in soil, chemical analysis of "total" concentration alone is not a reliable means of assessing toxicity. Attempts have been made, with limited success, to use mild solvent extractions to measure the bioavailable concentrations of organic compounds in soil (Kelsey *et al*. 1997, White *et al*. 1997, Tang and Alexander 1999). In most cases, mild extraction demonstrated decreased bioavailability with increasing residence time; concurrent extraction with vigorous chemicals demonstrated minimal diminished "total" concentrations in the aged soils (Kelsey and Alexander 1997, White *et al*. 1997, Chung and Alexander 1999). In one particular study, sequestration of phenanthrene and pyrene increased with time, but the percentage sequestered decreased with increasing PAH concentration. Therefore, the concentration of the chemical can affect sequestration via the saturation of sorption sites within soil (Chung and Alexander 1999). In all cases, mild extraction over-estimated the accumulation of organic contaminants in earthworms and the mineralization of these compounds by bacteria (Kelsey and Alexander, 1997; White *et al*., 1997; Chung and Alexander, 1999). In addition, different solvents were required for different contaminants and test species, potentially increasing the cost and time associated with such tests, decreasing their capacity for use in environmental risk assessments.

Biological assays are most effective when used as an adjunct to chemical analyses. They are suitable for the direct measurement of environmentally relevant toxicity of the contaminants in soil (Keddy *et al*. 1995, Bierkens *et al*. 1998). Bioassays involve exposure of a subject (e.g., organism) to a stimulant or an absorbed dose, to elicit an effect measurable by a change in a biological characteristic or state (e.g., mortality) (Lanno and McCarty 1997). The evaluation of contaminated soils typically incorporates a battery of terrestrial organisms, multiple endpoints, and a range of sensitivities (Keddy *et al*. 1995, Beirkens *et al*. 1998). The battery of terrestrial test organisms typically ranges from ecologically and economically relevant plant species, to various species of soil invertebrates (e.g., earthworms, springtails). In general, test organisms are exposed to a series of exposure concentrations resulting from the direct amendment of soil with a liquid or solid contaminant, from the dilution of field-contaminated soil with a negative control soil, or from soils collected along a gradient of contamination. The effect levels are calculated using appropriate statistical procedures based on the initial exposure concentrations derived from chemical analyses. As a result, the derived effect levels are reflective of the "total" concentration of the organic contaminants in soil, and thus do not directly relate to the fraction of the contaminant that is bioavailable. The "total" concentration of the contaminant in soil is correlated with an observed biological effect in the organism. In contrast, critical body residues allow a more accurate estimate of the actual dose associated with a toxic response, accounting for the contaminant's bioavailability in the soil as well as various uptake routes (McCarty and Mackay 1993, Lanno and McCarty 1997). Critical body residues normalize toxicity values, for a common toxicant, among species as the concentration in the organism is related to a common toxicity endpoint and mechanism of action (Lanno and McCarty 1997). In this case, the "total" concentration in the organism is associated with an observed biological effect. The ability to correlate the accumulated dose (i.e., the critical body residue) with the "total" contaminant concentration in soil may give an indication as to the fraction of the compound in soil that is bioavailable to a particular organism. Therefore, there is a need for the development of an appropriate tool that has the ability to measure the bioavailable fractions within the soil associated with an observed biological effect in terrestrial organisms. To date, potential methods include the use of passive sampling devices (PSDs) that are based upon the partitioning mechanisms involved with the accumulation of organic compounds within organisms.

4.4.1 Passive Sampling Devices

Passive sampling devices (PSDs) provide an alternative means to assess the bioavailability of an organic contaminant within soil. The devices are cost-effective, portable, easy to deploy, applicable to a wide variety of soils, and are compatible with various analytical instruments (Lanno 2002). They are meant to mimic biological systems by providing a medium into which selective contaminants can partition from the pore water or air (Parkerton *et al*. 2000, Sijm *et al*. 2000, Wells and Lanno 2001). The passive sampling devices are specific to a particular class of compounds (e.g., non-polar chemicals) but are not specific to the compounds in that particular class (Wells and Lanno, 2001). The passive sampling devices have applicability as an initial screening tool in the risk assessments of soils, provided the chemical measures can be correlated with adverse biological effects in soil-dwelling organisms.

4.4.2 Solid Phase Micro-extraction

Solid phase micro-extraction (SPME) is a rapid, reusable, and solventless extraction method used to detect and quantify semi-volatile and volatile organic compounds in aqueous and atmospheric samples (Arthur and Pawliszyn 1990, Eriksson *et al*. 1998). The SPME device uses a fused-silica fiber that is coated with a non-polar organic phase (e.g., polydimethyl siloxane) inside a syringe (Figure 4.2). The fiber has a high selective affinity towards organic compounds and as such, extracts and concentrates the analytes of interest in one simple step (Zhang and Pawliszyn 1993). Extraction of the analytes occurs via diffusion and absorption by immersing the fiber into a water sample or exposing the fiber to the headspace of a soil sample (Arthur and Pawliszyn 1990, Eriksson *et al*. 1998, Parkerton *et al*. 2000). Within a few minutes the sampling is completed and the fiber is injected into a gas chromatograph (GC), gas chromatograph-mass spectrometer (GC-MS), or a high performance liquid chromatograph (HPLC) whereby the analytes are thermally desorbed and quantitated (Arthur and Pawliszyn 1990, Parkerton *et al*. 2000).

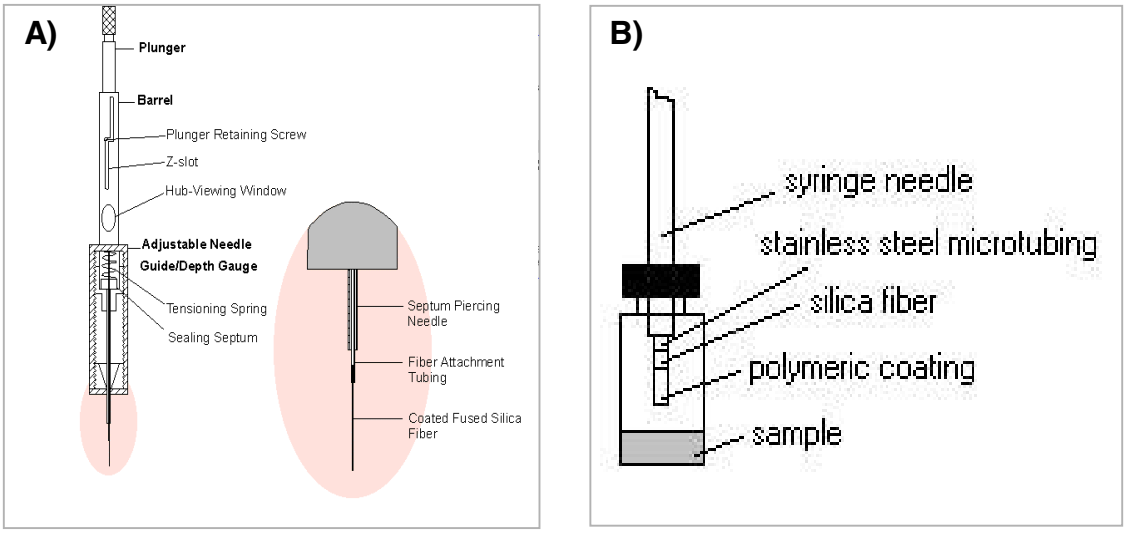

Figure 4.2. A) A solid phase microextraction (SPME) device and B) one where the coated fused silica fiber is immersed into the aqueous sample or exposed to the headspace of an aqueous or solid (e.g., soil) sample for extraction of volatile and semi-volatile organic compounds (adapted from Pawliszyn, 2001).

Headspace SPME analyses functions on the basis of the organic compounds partitioning between (1) the matrix and headspace, and (2) between the headspace and the fiber coating (Zhang and Pawliszyn 1993). Consequently, the efficiency of the SPME may be limited by the rate of mass transfer of the analyte within the matrix, and the release of the analyte from the matrix to the headspace (Zhang and Pawliszyn 1993). In aqueous phases, the direct immersion of the fiber and absorption of the analytes depends of the distribution coefficient of the organic compound between the stationary phase (i.e., the fiber) and the aqueous phase (e.g., water) (Arthur and Pawliszyn 1990). Increased applicability of SPME to a variety of volatile and semi-volatile organic compounds can be achieved with the use of different coatings specific to the selective absorption of the organic compounds of interest (Arthur and Pawliszyn 1993, Wells and Lanno 2001). Variations to the sampling technique can improve the sensitivity of SPMEs. For example, continuous mixing of the samples, sampling at elevated temperatures (e.g., 40°C), and adding modifiers to facilitate the release of the analytes into the headspace improve sensitivity of the method (Zhang and Pawliszyn 1993). In contrast, James and Stack (1996) found that an ambient temperature of 22°C was optimal for the headspace sampling of aromatic and chlorinated hydrocarbons; absorption to the SPME fiber decreased with increasing temperature. Sensitivity can also be improved by reasonably minimizing the headspace volume above the soil layer while maximizing the soil sample volume (James and Stack, 1996). The addition or presence of water in the soil matrix (e.g., soil slurry) may enhance the release of volatile analytes into the headspace for absorption to the fiber (James and Stack 1996).

Several studies have used SPMEs to measure organic contaminants (e.g., petroleum hydrocarbons, organochlorines) via absorption from soil suspensions or soil headspace analyses (Zhang and Pawliszyn 1993, James and Stack 1996, Martos *et al*. 1997, Eriksson *et al*. 1998, Prosen and Zupančič-Kralj 1998). Analyses of diesel-contaminated soil and water demonstrated that SPMEs were suitable to monitor the biodegradation of and changes in the concentration of organic volatiles and semi-volatiles over time (Eriksson *et al*. 1998). Longer straight chain hydrocarbons (e.g., >C14) concentrated to greater amounts in the gas phase of water samples relative to soil samples, mainly due to low volatility and greater adsorption to soil particles. As a result, SPME fibres must be carefully selected to maximize extraction in the media of interest; in this case soil samples required longer sampling times and a thinner fibre thickness relative to that used for the water samples (Eriksson *et al*. 1998). Headspace analyses using SPMEs have also been successful in concentrating benzene, toluene, ethylbenzene, and xylenes (collectively referred to as BTEX compounds) and PAHs (e.g., naphthalene, acenapthalene, phenanthrene, chrysene, perylene) in sand and sewage sludge (Zhang and Pawliszyn 1993). SPMEs have also been successfully used for the rapid quantification of residual volatiles (e.g., aromatic hydrocarbons and chlorinated hydrocarbons) in landfill areas (James and Stack 1996). The scientific literature demonstrates the applicability of these devices as a screening tool for the rapid identification and semi-quantification of a wide range of volatiles (e.g., hydrocarbons). However, the applicability of these modified techniques in assessing the actual bioavailability of these compounds in soil has yet to be addressed.

4.4.3 Semipermeable Membrane Devices

Semipermeable membrane devices (SPMDs) were developed by scientists at the US Geological Survey's Columbia Environmental Research Center for the passive sampling of bioavailable (i.e., dissolved and vapour phase) non-polar contaminants (Petty *et al*. 2000). SPMDs are able to concentrate several hydrophobic organic compounds including PAHs, polychlorinated biphenyls (PCBs), and organochlorine pesticides (Petty *et al*. 1993). Huckins *et al*. (1990) provide an exceptional review of the original conception and use of these bioconcentration devices, in addition to proposing modifications that are currently used today, to more realistically mimic the partitioning of organic molecules into the lipids of organisms. The SPMDs consist of layflat, thin-walled, low-density polyethylene (LDPE) dialysis tubing filled with a known amount of neutral lipid (Petty *et al*. 2000, Wells and Lanno 2001). Triolein is generally used as the model for neutral or storage lipids in organisms, but other lipids (e.g., lecithin) may

be used as well (Huckins *et al*. 1990). The LDPE membrane of the SPMDs contains transient pores with a diameter of up to 10 Å, meaning that dissolved organic chemicals with a molecular mass less than about 600 can permeate and partition into the lipid of the SPMD (Strandberg *et al*. 1997, Petty *et al*. 2000). The capacity of SPMDs to concentrate hydrophobic contaminants is related to the compound's K_{ow}; therefore, the greater a compound's K_{ow}, the greater the capacity of the SPMD for that chemical (Petty *et al*. 2000). The ability of SPMDs to sample within or above soil samples also depends upon the soil's properties (e.g., moisture content, organic matter content, porosity) in addition to the physical properties of the chemical contaminant (e.g., water solubility, vapour pressure) (Strandberg *et al*. 1997).

To estimate the concentration of freely available organic compounds, the SPMDs are deployed and allowed to equilibrate within the media (e.g., soil) for several weeks, resulting in time-integrated accumulation and values (Strandberg *et al*. 1997, Wells and Lanno 2001). With respect to soil environments, SPMDs are generally buried within the soil (5 - 15 cm below the soil surface) without disturbance until required for analysis (Zabik *et al*. 1992, Strandberg *et al*. 1997, Wells and Lanno 2001). Processing of the SPMDs consists of (1) removing debris from the membrane; (2) organic solvent dialysis of the lipid and membrane (usually in hexane); (3) size-exclusion chromatography; (4) chemical class fractionation (for the analysis of several contaminants, e.g., PAHs, PCBs, or organochlorines); and (5) detection and quantification of contaminants via chromatography (e.g., GC, GC-MS, HPLC) (Figure 4.3) (Petty *et al*. 2000). Johnson *et al*. (2000) provide a complete guide and protocol for the preparation, use, deployment, processing, and analysis of SMPDs in water, sediment, soil, and air.

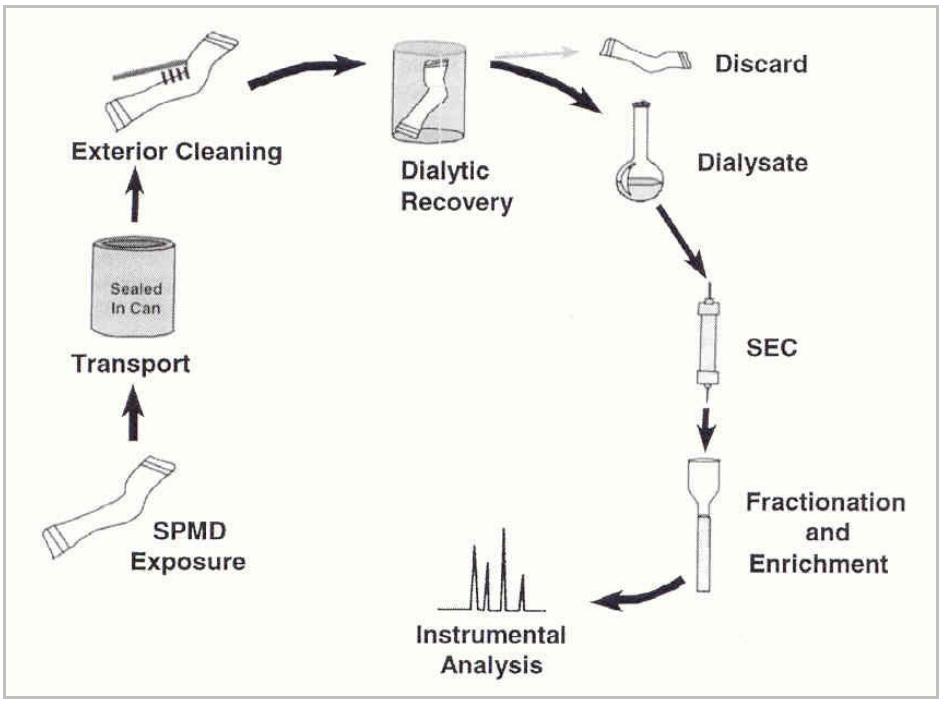

Figure 4.3. The key aspects of SPMD sample processing and analysis (Petty *et al*. 2000). (SEC refers to size-exclusion chromatography)

SPMDs are applicable to a variety of environmental systems including air, water, sediment, and soil (Petty *et al*. 1993, Rantalainen *et al*. 2000). SPMDs have been used to sample for pesticides in the air phase of soil using different types of sorbents (e.g., 2,2,4-trimethylpentane, C18 bonded-phase silica sorbent, or XAD-4 resin) with varying success (Zabik *et al*. 1992). The use of C18 bonded-phase silica sorbent and XAD-4 resin were effective in accumulating pesticides from soil; 2,2,4-trimeethylpentane was most effective but inadequate in sampling as approximately 40 to 80% of the solvent in the SPMD

was lost over time (Zabik *et al*. 1992). The SPMDs were able to accumulate various pesticides within soil and differentiate between low and highly contaminated areas within field trials at a waste site (Zabik *et al*. 1992). However, in each case, the amount of pesticides accumulated in the passive sampling devices were variable and less than those amounts extracted from soils using traditional extraction methods. As a result, the authors concluded that the SPMDs were useful for providing qualitative data for site characterizations. The SPMDs were able to discriminate between areas of low and high contamination but the authors neglected to consider the risk associated with the contaminated soils and whether the accumulated pesticides were the bioavailable portions of the contaminants in soil. Johnson *et al*. (1995) used SPMDs filled with C18 to characterize the distribution of soil contaminants (PCBs) applicable in the field for contaminated site assessments. The SPMDs sampled the PCBs in the vapour phase and accumulation increased with soil contaminant concentration and decreased soil moisture content (Johnson *et al*. 1995). The calibration of the passive sampling devices in the laboratory was inadequate in predicting soil concentrations in the field. As a result, field-based calibration (e.g., collecting concurrent soil samples of low, medium, and high concentration from SPMD deployment locations) is required to accurately characterize soil concentrations (Johnson *et al*. 1995). Rantalainen *et al*. (1998) used SPMDs containing triolein as a screening tool for organic contamination (chlorohydrocarbons) in soils to identify point sources of contamination among contaminated lakeshore soil. Strandberg *et al*. (1997) used SPMDs containing triolein to sample bioavailable organochlorine (PCB congeners) contaminants in, and evaporating from composts. The authors however, failed to differentiate between the accumulated and 'bioavailable' levels, unless it is assumed that the accumulated portion is bioavailable; biological studies to confirm uptake or accumulation levels in terrestrial organisms relative to the SPMDs were not referred to. Nonetheless, the SPMDs were able to accumulate contaminants from both the air and liquid phase in the compost in addition to semiquantitatively characterizing the congeners.

Several of the described studies have implemented SPMDs as screening tools in the rapid assessment and characterization of contaminants in soils. However, these studies neglect to address whether the measured concentrations in the SPMDs correlate with actual bioavailability of these substances to terrestrial organisms. To date, Wells and Lanno (2001) and Lanno (2002) provide the first published studies that attempt to correlate environmentally available fractions to adverse biological responses in earthworms in soil.

4.5 Passive Sampling Devices as Estimators of Bioavailability and Predictors of the Toxicity

4.5.1 Solid Phase Microextraction

Limited references with respect to the use of SPMEs with correlation to biological responses were found. A study by Lanno (2002) employed the use of earthworm lethality tests, SPMDs, and SPMEs to examine the bioavailable fraction of petroleum hydrocarbons (PHCs) in recently oil-contaminated field soil. However, the analyses of the PHC-contaminated soil by SPMEs have not been completed to date, and therefore the results are not yet published (Lanno 2002).

An additional study by Wells and Lanno (2001) attempted to correlate the amount of phenanthrene extracted by SPMEs to critical body burdens in earthworms as measured by lethality in soils of varying organic matter content. SPMEs were deployed in soil suspensions containing various concentrations of phenanthrene and earthworm lethality tests were conducted concurrently (Wells and Lanno 2001). The SPMEs extracted less phenanthrene from soils containing a greater amount of organic matter (Wells and Lanno 2001). This may provide a method to assess varying bioavailability in soils, provided that measured concentrations can be correlated with biological responses via some sort of calibration curve. In addition to discriminating between soil types of varying organic matter content, the authors

demonstrated that the SPMEs could discriminate between phenanthrene levels over a range of concentrations. Critical body phenanthrene residues paralleled phenanthrene accumulation by SPMEs with greater accumulation in soil containing less organic matter. SPMEs were suitable for the detection and discrimination between lethal and non-lethal phenanthrene concentrations in soil (Wells and Lanno 2001).

4.5.2 Semipermeable Membrane Devices

Lanno (2002) attempted to use SPMDs to estimate the bioavailability of petroleum hydrocarbons (PHCs) in a recently oil-contaminated soil relative to earthworm lethality studies. SPMDs were initially deployed for 14 days during a preliminary study to examine the efficacy of using this technique in field soil. The SPMDs were placed at a depth of 15 cm below the soil surface at various locations ranging from non-contaminated reference sites to the contaminated site at which a pipeline broke. The results from these preliminary studies indicated that the SPMDs accumulated detectable amounts of PAHs, mainly phenanthrene and benzo[a]pyrene. As a result, additional SPMDs (22 in total) were deployed for the duration of the actual study. In contrast to the preliminary studies, the dialysates from these SPMDs indicated no detectable PAHs via analysis by HPLC or GC-MS. Discrepancies arose in the moisture content of the soils as a result of sampling times; the preliminary studies were conducted under periods of heavy precipitation whereas the subsequent study was conducted under dry conditions. As a result, the ability of SPMDs to accumulate dissolved contaminants reliably over time may be limited by soil conditions (e.g., wet versus dry soil). The experimental results derived from the SPMDs were variable and as a result, no comparisons or inferences could be made with respect to earthworm toxicity.

The previously mentioned study by Wells and Lanno (2001) also attempted to correlate the amount of phenanthrene extracted by SPMDs to critical body burdens in earthworms as measured by lethality in soils of varying organic matter content. In general, the SPMDs accumulated phenanthrene from all of the spiked soils, but could not discriminate between lethal and non-lethal exposure levels relative to the experimental controls. Nonetheless, the SPMDs were able to detect differences in the phenanthrene content among soil types of varying organic matter. SPMD accumulation of phenanthrene was consistent in linearly accumulating the substance within the first 16 days of the test, but variable thereafter. Earthworm mortality occurred within the first eight days of exposure, corresponding to the linear uptake phase exhibited by the SPMDs. However, the SPMDs continued to accumulate the phenanthrene from the soil thereafter, confounding the ability to correlate the results to earthworm lethality. As a result, SPMDs had limited applicability to earthworm biological responses.

4.5.3 Efficacy of Passive Sampling Devices in Estimating Bioavailability and Predicting the Toxicity of Organic Compounds in Soil

It appears that SPMEs are slightly more effective than SPMDs in estimating the bioavailability of selected organic pollutants in soils. In addition, based on the limited research to date, SPMEs offer a more suitable approach to relating the bioavailable fraction of the organic contaminants to biological responses (i.e., mortality) in earthworms. The use of SPMEs and SPMDs to estimate bioavailability of organic compounds is limited to a few studies and, as such, additional scientific studies are warranted. However, before the efficacy of these techniques can be improved, certain aspects of the technology must be improved.

Several types of SPME fibers and phases are available and this increases the applicability of the approach (Wells and Lanno 2001). Although inexpensive and reusable, limitations for field-use include the fragility of the SPME fiber, and susceptibility to damage, vandalism, or theft (Wells and Lanno 2001). Future applicability of SPME devices to the field must include an assessment of efficacy under various soil moisture conditions in addition to the variety of soil types that naturally exist. SPMEs must be used

with caution when extrapolating to test organisms (e.g., earthworms) as they only detect the parent compounds and cannot account for biotransformation products (Wells and Lanno 2001). Nevertheless, SPME devices seem to be a promising screening tool for the initial assessment of contaminated soils and, upon further investigation, the technology might become suitable for estimating ecological risk.

SPMDs on the other hand, were quite variable in their ability to estimate the bioavailability of PHCs and PAHs (e.g., phenanthrene) in soil. SPMDs are adequate for concentrating organic chemicals from soils (Zabik *et al*. 1992, Strandberg *et al*. 1997, Rantaleinen *et al*. 1998), but the SPMD concentrations cannot be related to biological concentrations that are toxic to the organism. SPMDs are relatively sensitive to cross-contamination and must be handled, stored, and deployed carefully. Additional care must be taken to minimize analytical interferences as well (Petty *et al*. 1993). Similar to SPMEs, SPMDs are limited in that they only measure the parent compound of a contaminant; they do not measure biotransformation products. The proper use of SPMDs entails an equilibration period of several weeks and is advantageous in that it provides a time integration of chemical accumulation.

4.6 Future Directions

Passive sampling devices offer a new and innovative alternative to the conventional chemical and earthworm assays in assessing the ecological risk associated with contaminated soils. Several of the studies investigated demonstrated that SPMDs and SPMEs were useful in accumulating a wide variety of organic chemicals in both the field and laboratory. However, further research is required to assess their potential to predict the toxicity of such contaminated soils to biological organisms (e.g., earthworms). As a potential screening tool in soil risk assessment, they can be used to guide the extent to which additional toxicity testing (e.g., bioassays) or remediation are required. Consequently, SPMDs and SPMEs may help to focus the efforts for the development of realistic cleanup guidelines for regulatory purposes (Lanno 2002). SPME- and SPMD-based measures, if calibrated to biological responses, also have the long-term benefit of reducing the reliance on live test organisms and the cost of estimating bioavailability via organism-based screening tests (Lanno 2002).

In conclusion, SPMEs and SPMDs are rapid, inexpensive tools used to derive a quantitative measure of the contaminants present within soil. With additional research, these devices have the potential for correlation to biological responses and, as such, may prove to be an adequate tool to assess bioavailability, and hence toxicity of organic chemicals to ecological receptors.

5 ACUTE TO CHRONIC RATIOS OF PHC TOXICITY

5.1 Introduction and Objectives

Traditional approaches to the quantification of petroleum hydrocarbon (PHC) fractions in soil have involved the use of chemical analytical techniques. However, chemical analysis on its own is insufficient to provide insight into the potential ecological risk of contaminated soils since it does not allow for an integration of the combined effects of the mixture of all chemicals present at a polluted site (van Gestel *et al*. 2001). An assessment of soil quality after an oil spill and/or remediation effort might be obtained by evaluating the toxicity of the affected soil organisms (Dorn *et al*. 1998). The prediction of the response of a complex system to a specific toxicant inevitably involves some uncertainty. Assessment decisions regarding the remediation and management of contaminated sites can be based on the information derived from toxicity tests. Toxicity tests can be either acute or chronic, depending on the duration of the test. The duration of acute tests are relatively short; and can be either instantaneous or conducted over a period of minutes to days. Conversely, the durations of chronic tests are relatively long; they should entail exposure durations that are at least a tenth of the life span of a species.

Understanding and evaluating chronic toxicity of chemicals is essential to our ability to arrive at sound decisions about the state of the environment (Sun *et al*. 1995). Long-term bioassay methods focus on sublethal endpoints (e.g., growth and reproduction) and are the most relevant tests from an ecological perspective (van Gestel *et al*. 2001). In addition, chronic bioassays or test methods with soils are considered to be more sensitive in assessing the toxicity of oil contamination than are acute tests (van Gestel *et al*. 2001). However, due to the high cost and long duration of chronic tests, resources and time constraints often ensure they are not routinely undertaken. As a result ecological risk assessments are most often based on toxicity data obtained from acute standardized laboratory tests (van Gestel *et al*. 2001, Roex *et al*. 2000). It would, therefore, be advantageous to devise an approach whereby the results of acute toxicity tests could be used to "predict" the results of chronic toxicity tests using statistical techniques (Sun *et al*. 1995). We reviewed the existing approaches regarding the use of acute to chronic ratios in aquatic and terrestrial systems to determine which of these methods is most applicable to soils contaminated with petroleum hydrocarbons.

5.2 Acute to Chronic Ratios

In its simplest form an acute-to-chronic ratio (ACR) is an extrapolation factor that allows the chronic toxicity of a compound or mixture of compounds to be derived from the results of acute toxicity tests.

The theory behind the ACR concept is that for similar classes of chemicals and similar taxa, acute to chronic ratios established for one species and chemical can be used to estimate the chronic toxicity of the chemical to another species (U.S. EPA 1991). Such extrapolations should only be made for the same types of tests conducted under the same conditions. An ACR is simply an extrapolation factor to derive environmental quality criteria to assure the safety of populations in the field and, consequently, of the ecosystem itself. When applying these extrapolation factors several approaches can be used (Roex *et al*. 2000).

A commonly used determinant of acute toxicity is the median lethal concentration (LC50). An LC50 is the concentration of a substance that is estimated to be lethal to 50% of the test organisms. Kenaga (1982) reported that the LC50 is not useful for predictions of chronic toxicity (U.S. EPA 1989). The uncertainty in predicting chronic toxicity from acute toxicity data for a given species has been observed to be less than the uncertainty in predicting acute toxicity between species (U.S. EPA 1989). In its most basic form, an ACR could be considered the ratio of the LC50 of an acute test and the EC50 of a chronic

test, where the EC50 is the median effective concentration or the concentration of a substance that is estimated to cause some defined effect to 50% of the test organisms after prolonged exposure.

One widely used approach for summarizing chronic toxicity experiments is the use of maximum acceptable toxicant concentration (MATC) limits. The MATC is a hypothetical value whose upper and lower limits are represented by the lowest-observed-effect concentration (LOEC) and the highest no-observed-effect concentration (NOEC) in a test, respectively (Sun *et al*. 1995). The MATC is the geometric mean of the LOEC and the NOEC. The difference between acute and chronic tests is determined by the lifespan of the test organism (van Gestel *et al*. 2001), but in some cases in the literature this appeared arbitrary. For instance, van Gestel *et al*. (2001) applied an arbitrary value of ≥ 5 days as the cut off exposure duration for acute tests. Van Gestel *et al*. used 14-day earthworm toxicity tests in conjunction with assays using extracts taken from oil-contaminated soil. The application of this acute cut off point meant that all tests performed with soil elutriates were classified as acute and all tests using whole soils were classified as chronic. Due to the differences in these approaches, it was not surprising that the authors found a marked difference between the performance of the chronic and acute assays.

The vast majority of information dealing with ACRs describes approaches that have been applied in aquatic systems. A review of the literature revealed a few studies in which ACRs for terrestrial systems were used to model a variety of heavy metal compounds; however, no studies were found in which ACRs had been applied to model petroleum hydrocarbon fractions. Few data exist on the toxicity of crude oil in soils (Saterbak *et al*. 1999). This means that the level of uncertainty is currently quite high and comparisons between acute and chronic data are limited and the data preclude extrapolation of values to other species and conditions. The application of ACR method will become more relevant as additional studies are conducted to increase the existing data and reduce the uncertainty. Most information available currently has been obtained from toxicity tests with either contaminated-site soils or soils from remediated sites, using non-standardized test methods and conditions (van Gestel *et al*. 2001, Saterbak *et al*. 1999, Wong *et al*. 1999). Consequently, most available toxicity data concerning oil pollution in terrestrial systems is restricted to acute toxicity tests on a limited number of organisms such as earthworms and micro-organisms and a very limited amount deals with sublethal effects (van Gestel *et al*. 2001).

5.3 Calculations of Acute to Chronic Ratios

In aquatic systems, several initiatives have been taken to model ACRs over the past thirty years. The simplest approach is to find a ratio between acute and chronic data by applying the following equation;

$$ACR = LC50/EC50$$

Mount and Stephan (1967) produced another early approach to quantifying ACR, in which they introduced a procedure of estimating chronic toxicity by using an application factor (AF).

$$AF = MATC/LC50$$

The AF is the ratio of the maximum acceptable toxicant concentration (MATC) to acute median concentration (LC_{50}) (Sun *et al*. 1995). Kenaga (1982) presented real data examples for predicting chronic toxicity from acute test results based on the analysis of acute-to-chronic ratios (ACR). The ACR derived in Kenaga's study is the inverse of the AF.

$$ACR = 1/AF$$

Both of these approaches require sufficient data from both acute and chronic toxicity tests as a basis for their predictions (Sun *et al*. 1995). The use of fixed AFs implies that the relationship between acute and chronic toxicity is considered to be independent of the test species and the test compound. The ratio of acute and chronic effect levels for different species is assumed to be the same, regardless of differences in life histories. In addition, every compound is supposed to have the same acute-to-chronic ratio, regardless which life history attribute is affected by chronic exposure. These assumptions may result in under or over estimation of the toxicity in the field when using the fixed extrapolation factors (Roex *et al*. 2000).

5.4 Modeling Approaches Using ACR in Aquatic Systems

An extrapolation factor is usually used to derive environmental quality criteria to assure the safety of populations in the field and, consequently, of the ecosystem itself. Below this safe level, the toxicant should have no adverse effects on the ecosystem (Roex *et al*. 2000). Kenaga (1982) found that using this approach, toxicity data did not necessarily correlate with a large ACR.

Roex *et al*. (2000) hypothesized that a fixed relationship exists between the acute and chronic effect concentrations within a class of compounds because of a similar mode of action. The study examined organic toxicants and metals grouped according to their mode of action. The LC50 was used for the acute value (24 or 48 hours for invertebrate) and the chronic endpoint was the LOEC(r) defined as the lowest-observed-effect concentration at which the intrinsic rate of population increase (r) is affected. Linear regression by a least-square approximation was used to establish a relationship between the LOEC(r) and the LC50. In the data set used in this study, acute toxicity tests provided a good estimate of the chronic toxicity endpoint LOEL(r). The average ACR calculated in this study (6.03 ± 3.97) was in good agreement with the extrapolation factors that are used in risk assessment (10) between LC50 and the no-observed-effect concentrations (NOEC).

Another approach developed for use in aquatic vertebrates is accelerated life testing. Sun *et al*. (1995) employs this theory to model aquatic toxicity data using a software program, which can predict chronic lethality using acute toxicity test results. This approach uses a number of statistical tests such as regression, least squares estimates, and asymptotic normality to create a user friendly computerized program. When a database of various chemicals and fish species was analyzed and the calculated values of prediction were compared to the MATC obtained from actual chronic toxicity experiments, the technique provided relatively accurate predictions. This model is particularly useful in predicting chronic no-effect concentrations for survival of fish species that are difficult to culture under chronic testing conditions (Sun *et al*. 1995). One of the limitations of this approach is that only lethality is examined and not other important chronic endpoints such as reproduction. This model is also based on a number of assumptions including: 1) that the mode of action of the chemical is simple; 2) that the biological mechanisms for lethality are the same at high and low doses; and, 3) that the model fits the data well.

Lee *et al*. (1995) developed a statistical method for predicting chronic lethality from acute toxicity tests using multiple regression techniques. Two basic models were formulated to represent the response surfaces of probit (percent) mortality as a function of toxicant concentration and acute exposure time. The data requirements for these models are similar to those for any acute estimation study. This model requires that data quality be sufficient to permit estimates with small standard errors, so a minimum of two conditions is recommended. First, at least five concentrations or doses resulting in mortalities >10% and <90% over a fixed exposure time are desirable. Second, several observation times should be represented, and a minimum of four is proposed. The range in times of exposure should be adequate to permit estimation of the trend in lethality within a concentration. The authors suggest that this approach is applicable to a wide variety of chemical classes and chronic test types and, that when acute data quality allow, it should be chosen to predict chronic NOEC for lethality. This model can be applied to the results

of the acute invertebrate terrestrial tests only if the number of observations that are recorded are increased.

Brix *et al*. (2001) used ACRs to determine species sensitivities distributions (SSD) for different aquatic taxonomic groups exposed to copper. Because there are so few chronic toxicity data for most stressors, the chronic SSD is often estimated from the acute distribution using a chemical-specific ACR. The ACRs in this study used the acute LC_{50} divided by the MATC. This value was then used to determine the SSD. The authors conclude that their approach provides an improvement in the methodolgy for assessing risks of copper in the aquatic environment.

Heger *et al*. (1995) used fish and daphnid $LC(EC)_{50}$ and NOEC data from tests with prolonged exposures to calculate the acute-prolonged ratio (APR). Generally for chemicals and pesticides, they recommended that an APR ratio of 100 be used. The authurs concluded that, in addition to the substance itself, toxic effects also depend on the test species and the chosen endpoint. Länge *et al*. (1998) cast some doubt on the methods used in the Heger study. They felt that the APR of 100 is too high and concluded that an ACR of 73 would safely predict the NOEC for 90% of the substances tested.

5.5 Approaches in Terrestrial Systems

The rate and extent that a chemical is released from the soil into the vapour and or aqueous phases may change over time (Saterbak *et al.* 1999). By definition, reduced availability of a chemical within a soil correlates with a lower dose and/or reduced exposure to the chemical of the ecological receptor. Decreased availability of a chemical might also alter its mobility and transport in the environment. If soil ecotoxicity tests are to gain acceptance for routine use in site risk assessment and help to define clean up standards, additional work is needed to verify that tests based on responses of invertebrates provide reliable information about the biological quality of the soil.

Soils are a more complex system than water. There are a number of factors that should be integrated into any chronic models derived from acute data. Some of these factors are: 1) the soil type; 2) the length of time that the contaminant has been in the soil (e.g., soil contact time); and, 3) the interspecies differences in sensitivities. These factors contribute to the difficulties in developing soil-quality criteria or benchmark values that are not overly conservative (Saterbak *et al.* 1999).

Saterbak *et al.* (1999) developed and evaluated acute and chronic assays with earthworms using field soils contaminated with a variety of petroleum products, from different regions of the United States. The sensitivities of the various assays used were compared and soil ecotoxicity assays and testing strategies in the context of risk-based framework recommended. The data support the hypothesis that different taxa respond differently to hydrocarbons and that a universal hydrocarbon parameter that can be used to predict toxic effects on soil communities has yet to be identified.

Fuchsman *et al.* (1999) developed a probabilistic model to predict effects threshold concentrations for chlorinated benzenes in sediment using acute to chronic ratios. In this study the distribution of ACR values was developed using literature derived data for which the LC_{50} of an acute test (96 or 48 h) could be paired with a measure of test organism response after chronic exposure (at least 6 days). Acute and chronic values were paired if they were derived within the same study (implying comparable test conditions) for a single chemical and test species. The acute value was divided by the chronic value to yield an ACR for each pair of tests. In this study the ACR values were normally distributed after log transformation, and the minimum and maximum values were specified as the log of 1.0 and the mean plus two standard deviations, respectively.

5.6 Examinations of the Relationships Between Acute and Chronic Estimates of Toxicity for Invertebrates Exposed to Oil Contaminated Soils

Van Gestel *et al.* (2001) compared the effectiveness of acute and chronic bioassays for the ecological assessment of soils historically contaminated with mineral oil. The division between acute and chronic exposure in this study was arbitrarily set as <5 days which meant that all tests performed on elutriates were classified as acute and all on solid soil substrates as chronic. Using this approach all of the tests using soil invertebrates are chronic. This study confirmed that the chronic tests were more sensitive than the acute tests in determining the potential toxicity of oil-polluted soils, but no attempts were made to model the relationship or extrapolate the reason for these differences.

Dorn *et al.* (1998) used acute tests to assess toxicity of soils spiked with various crude oil fractions. Only one of these tests used a terrestrial invertebrate species, the 14–day lethality test using *E. fetida.* The results of the laboratory spiking experiments showed large differences in the toxicity of the various oil fractions. The heavy crude, consisting primarily of high molecular weight hydrocarbons and essentially devoid of volatile components, was less toxic to the species tested than the lighter crude oils. The lighter oils were more toxic and this is thought to be associated with the ease of uptake and increased bioavailability of the lower molecular weight compounds. The most encouraging factor was that the test variability obtained from this study was within the range observed for aquatic toxicity tests. Considering the heterogeneous nature of soils, the observed precision presents encouraging opportunities for the future use of these bioassays (Dorn *et al.* 1998). In addition the development of models comparable to those outlined in aquatic systems might well be feasible.

Wong *et al.* (1999) used the physicochemical properties of eight hydrocarbon-contaminated soils to predict toxicity to earthworms (*Eisenia fetida*). The toxicity of the soils was assessed using the chronic endpoints of avoidance, survival, and reproduction. No acute tests observing lethality were conducted. Physical properties were measured and hydrocarbon contamination was characterized by total petroleum hydrocarbons, oil and grease, and GC boiling point distribution. A variety of statistical approaches such as univariate and multivariate statistical methods were applied to the data along with multivariate one-component partial least squares models. The application of these models was found to be predictive 42 and 29%, of the time, respectively. This was the first attempt at using quantitative models to relate soil chemical parameters and ecoxtoxicolgy. The models were developed with a limited data set (seven to eight soils) and are valid only for the range of chemical concentrations used in this study. This study provides an encouraging starting point from which to develop further predictive models which relate acute toxicity tests of invertebrates in petroleum hydrocarbon contaminated soil to chronic values by partially utilizing ACRs.

5.7 Application of the Acute to Chronic Ratio Approach Using Estimates of Toxicity for Soil Invertebrates Exposed to Oil Contaminated Soils (PTAC Phase 1 Toxicity Data)

The data available for petroleum hydrocarbons in soil (i.e., those provided by the Petroleum Technology Alliance Canada – PTAC) were used to evaluate the various approaches to ACRs. Table 5.1 contains the summary data for invertebrates and plants (root and shoot length). Values were listed according to the types of test, species, and soils with which the experiments were undertaken. Table 5.2 shows a summary of the ACRs that were achieved. At this time there are numerous gaps in the PTAC data set so values within groups were not available for extrapolation. Table A.1 (Appendix A) summarizes each of the approaches to ACRs outlined in this subsection. Each approach was examined to determine whether it would be applicable to the existing PTAC database. For the invertebrate data, it was possible to determine ACRs for some of the data using the following methods: 1) dividing the LC_{50} by the LD_{50}; 2) determining the Application Factor (AF) which is the ratio of the MATC to LC_{50}; and 3) determining the

inverse of the AF and by applying the risk assessment extrapolation factor of ten times the LC_{50}. For the plant data ACRs were determined only by dividing the LC_{50} by the LD_{50} and by applying the extrapolation factor. Each of these approaches are known to be highly variable and often inaccurate. The extrapolation factor yields a value that grossly overestimates the real chronic data that has been obtained.

All other methods discussed such as the linear regression approach (Roex *et al.* 2000), the accelerated life testing (Sun *et al.* 1995), the probit analysis (Lee *et al.* 1995), the acute-prolonged ratio method (Heger *et al.* 1995), the probabilistic model (Fuchsman *et al.* 1998) and the partial least means square combined with univariate and multivariate statistical approaches (Wong *et al.* 1999) are not at this time possible. These approaches were rejected for one of the following reasons: 1) they require a software programme to run the models that were specifically designed by the authors of the studies; 2) they require a larger data set than is presently available; 3) they require more frequent observations; or, 4) they require additional values which have not presently been calculated or cannot be calculated from the available data.

5.8 Conclusions and Recommendations

In contrast to aquatic systems, terrestrial systems are more complex and therefore a greater number of parameters must be considered. The major statistical problems encountered in predicting chronic lethality from acute toxicity data included the choice of model, a method for efficient estimation of its parameters (Sun *et al*. 1995) and the paucity of data. There are a variety of factors that should be accounted for when extrapolating to chronic toxicity from acute data; a fixed ACR approach does not address these other factors. Fixed extrapolation factors, such as the risk assessment recommended value of 10 times the LC_{50} or the even higher 100 times the LC_{50} put forward by Heger *et al*. to deal with pesticides (Heger *et al*. 1995), are vastly over conservative.

The prediction of the response of a test species to a complex contaminated soil system will inevitably involve some degree of uncertainty. Many studies recommend that to obtain useful information on potential ecological risks of polluted or remediated soils the use of a battery of tests is preferable (van Gestel *et al*. 2001). This would include a number of different test species representative of the ecosystem to be protected. As more tests are conducted under standardized conditions and the database of available data increases, then the uncertainty will decrease. However, for ACRs to be effective, the test conditions must remain relative so that comparisons can be made.

The limitations to applying the ACR approach to test data for PHCs in soil are summarized as follows:

1) Currently, the use of ACRs are not good predictors of experimental chronic toxicity data and the probability of grossly over estimating or under estimating the long-term effects of PHC fractions in soil is high;

2) The ACRs are highly variable, even within a data set in which the experimental conditions were matched and well known to the authors. For instance the application of the univariate, multivariate and partial least mean squares approach used in Wong *et al.* (1999) only achieved a 42% and 29% predictive power;

3) Relatively subtle differences in methods of analysis can lead to quite different results;

4) Using taxa interchangeably in tests will increase variability;

5) ACRs do not account for the effects of incremental doses;

6) ACRs do not compensate for differences between responses observed in organisms in laboratory tests and those observed in field populations;

7) Ecosystem and indirect toxic effects such as synergistic contaminant effects and food-chain interactions are not accounted for; and,

8) ACRs do not quantify uncertainties.

We conclude that ACRs on their own are not a useful predictive tool for determining chronic toxicity of PHC fractions. It is possible that as the PTAC data set increases and the uncertainty surrounding the interactions of PHC fractions in various soil types decreases, there might well be an application for some form of ACR modeling. Direct testing of remediated soils, although less instantaneous is likely the most accurate and appropriate approach to determining the toxicity of a given site. The use of ACRs in conjunction with direct testing could, in the future, yield a more accurate approach to PHC soil testing which encompasses the various confounding factors.

Table 5.1 Acute to chronic ratios for Invertebrate species (data courtesy of PTAC; ESG International Inc. 2003)

Test Method	Species	Parameter	[1] Soil	[2] Mogas	[3] F2	[4] F3	[5] F4	[6] Crude
Acute LC50 Earthworm and Springtail	*Eisenia andrei*	7-d mortality	Clay loam	1.07	1.03	NV	Not toxic	3.98
	Eisenia andrei	14-d mortality	Clay loam	1.07	0.53	22.36	Not toxic	3.98
	Eisenia andrei	7-d mortality	Sandy loam	0.63	NA	NA	NA	NA
	Eisenia andrei	14-d mortality	Sand loam	0.40	NA	NA	NA	NA
	Eisenia andrei	7-d mortality	Artificial soil	1.23	1.19	Not toxic	NA	5.73
	Eisenia andrei	14-d mortality	Artificial soil	1.15	1.15	NV	NA	5.25
	O. folsomi	mortality	Clay loam	NA	2.92	5.97	Not toxic	4.86
	O. folsomi	mortality	Sandy loam	4.19	NA	NA	NA	NA
	O. folsomi	mortality	Artificial soil	5.96	3.23	6.67	Not toxic	7.59
Chronic IC50 Earthworm and Springtail	*Eisenia andrei*	No. juveniles	Clay-loam	NA	0.49	0.78	4.4	1.63
	Eisenia andrei	No. juveniles	Clay-loam	NA	0.49	0.78	4.4	1.63
	Eisenia andrei	No. juveniles	Sandy loam	NV	NA	NA	NA	NA
	Eisenia andrei	No. juveniles	Sandy loam	NV	NA	NA	NA	NA
	Eisenia andrei	No. juveniles	Artificial soil	NV	NA	NA	NA	NA
	Eisenia andrei	No. juveniles	Artificial soil	NV	NA	NA	NA	NA
	O. folsomi	No. juveniles	Clay loam	NA	1.47	1.49	NA	NV
	O. folsomi	No. juveniles	Sandy loam	2.04	NA	NA	NA	NA
	O. folsomi	No. juveniles	Artificial soil	2.89	NA	NA	NA	NA
Acute/Chronic Ratios (ACR) LC50/IC50	*Eisenia andrei*	7-d mortality	Clay loam	NV	2.10	NV	NV	2.44
	Eisenia andrei	14-d mortality	Clay loam	NV	1.08	28.82	NV	2.44
	Eisenia andrei	7-d mortality	Sandy loam	NV	NV	NV	NV	NV
	Eisenia andrei	14-d mortality	Sand loam	NV	NV	NV	NV	NV

Table 5.1 Acute to chronic ratios for Invertebrate species (data courtesy of PTAC; ESG International Inc. 2003)								
Test Method	**Species**	**Parameter**	**[1] Soil**	**[2] Mogas**	**[3] F2**	**[4] F3**	**[5] F4**	**[6] Crude**
	Eisenia andrei	7-d mortality	Artificial soil	NV	NV	NV	NV	NV
	Eisenia andrei	14-d mortality	Artificial soil	NV	NV	NV	NV	NV
	O. folsomi		Clay loam	NV	1.99	4.01	NV	NV
	O. folsomi		Sandy loam	2.06	NV	NV	NV	NV
	O. folsomi		Artificial soil	2.06	NV	NV	NV	NV
NOEC (no-observed-effect concentration)	*Eisenia andrei*		Clay-loam	1000	0.35	0	0.5	NA
	Eisenia andrei		Clay-loam	1000	NA	NA	NA	NA
	Eisenia andrei		Sandy loam	500	750	NA	NA	NA
	Eisenia andrei		Sandy loam	NA	NA	NA	NA	NA
	Eisenia andrei		Artificial soil	1000	500	NA	NA	NA
	Eisenia andrei		Artificial soil	NA	NA	NA	NA	NA
	O. folsomi		Clay loam	NA	1	3	NA	2
	O. folsomi		Sandy loam	2000	NA	NA	NA	NA
	O. folsomi		Artificial soil	NA	NA	NA	NA	NA
LOEC (lowest-observed-effect concentration)	*Eisenia andrei*		Clay-loam	NA	0.50	0.50	1	NA
	Eisenia andrei		Clay-loam	NA	NA	NA	NA	NA
	Eisenia andrei		Sandy loam	1000	NA	NA	NA	NA
	Eisenia andrei		Sandy loam	NA	NA	NA	NA	NA
	Eisenia andrei		Artificial soil	1000	NA	NA	NA	NA
	Eisenia andrei		Artificial soil	NA	NA	NA	NA	NA
	O. folsomi		Clay loam	NA	2	4	NA	4
	O. folsomi		Sandy loam	3000	NA	NA	NA	NA

Table 5.1 Acute to chronic ratios for Invertebrate species (data courtesy of PTAC; ESG International Inc. 2003)								
Test Method	**Species**	**Parameter**	**[1] Soil**	**[2] Mogas**	**[3] F2**	**[4] F3**	**[5] F4**	**[6] Crude**
	O. folsomi		Artificial soil	NA	NA	NA	NA	NA
MATC (maximum acceptable toxicant concentration)	*Eisenia andrei*		Clay-loam	NV	0.42	NV	0.71	NV
	Eisenia andrei		Clay-loam	NV	NV	NV	NV	NV
	Eisenia andrei		Sandy loam	707.11	750	NV	NV	NV
	Eisenia andrei		Sandy loam	NV	NV	NV	NV	NV
	Eisenia andrei		Artificial soil	1000	500	NV	NV	NV
	Eisenia andrei		Artificial soil	NV	NV	NV	NV	NV
	O. folsomi		Clay loam	nv	1.41	3.46	NV	2.83
	O. folsomi		Sandy loam	2449.49	NV	NV	NV	NV
	O. folsomi		Artificial soil	NV	NV	NV	NV	NV
AF (application factor)	*Eisenia andrei*		Clay-loam	NV	0.41	NV	NV	NV
	Eisenia andrei		Clay-loam	NV	NV	NV	NV	NV
	Eisenia andrei		Sandy loam	1122.39	NV	NV	NV	NV
	Eisenia andrei		Sandy loam	NV	NV	NV	NV	NV
	Eisenia andrei		Artificial soil	812.35	420.17	NV	NV	NV
	Eisenia andrei		Artificial soil	NV	NV	NV	NV	NV
	O. folsomi		Clay loam	NV	0.48	0.58	NV	0.58
	O. folsomi		Sandy loam	584.74	NV	NV	NV	NV
	O. folsomi		Artificial soil	NV	NV	NV	NV	NV
ACR from Kenaga (1982) ACR/1/AF	*Eisenia andrei*		Clay-loam	NV	2.46	NV	NV	NV
	Eisenia andrei		Clay-loam	NV	NV	NV	NV	NV
	Eisenia andrei		Sandy loam	0	NV	NV	NV	NV

Table 5.1 Acute to chronic ratios for Invertebrate species (data courtesy of PTAC; ESG International Inc. 2003)

Test Method	Species	Parameter	[1] Soil	[2] Mogas	[3] F2	[4] F3	[5] F4	[6] Crude
	Eisenia andrei		Sandy loam	NV	NV	NV	NV	NV
	Eisenia andrei		Artificial soil	0	0	NV	NV	NV
	Eisenia andrei		Artificial soil	NV	NV	NV	NV	NV
	O. folsomi		Clay loam	NV	2.06	1.72	NV	1.72
	O. folsomi		Sandy loam	0	NV	NV	NV	NV
	O. folsomi		Artificial soil	NV	NV	NV	NV	NV
Extrpolation Factor 10xLC50	*Eisenia andrei*	7-d mortality	Clay loam	10.72	10.30	NV	NV	39.80
	Eisenia andrei	14-d mortality	Clay loam	NV	5.30	223.62	NV	NV
	Eisenia andrei	7-d mortality	Sandy loam	6.30	NV	NV	NV	NV
	Eisenia andrei	14-d mortality	Sand loam	NV	NV	NV	NV	NV
	Eisenia andrei	7-d mortality	Artificial soil	12.31	11.90	NV	NV	57.30
	Eisenia andrei	14-d mortality	Artificial soil	NV	11.50	NV	NV	NV
	O. folsomi	mortality	Clay loam	NV	29.20	59.69	NV	48.60
	O. folsomi	mortality	Sandy loam	41.89	NV	NV	NV	NV
	O. folsomi	mortality	Artificial soil	59.60	32.30	NV	NV	NV

NA no experiment was conducted with this soil type or species

NV no value because either the LC50 or IC50 could not be calculated

[1] Soil types were sandy loam, clay loam, and artificial soil

[2] Mogas was a surrogate for F1 of crude oil (units were mg mogas/g soil)

[3] F2 (>nC10-C16 of crude oil, mg F2/g soil)

[4] F3 (>nC16-C34 of crude oil, mg F3/g soil)

[5] F4 (>C34 of crude oil, mg F4/g soil)

Table 5.2 Acute to chronic ratios for plant species – root metrics (data courtesy of PTAC; ESG International Inc. 2003)

Method	Plant Name	Parameter	[1] Soil	[2] Mogas	[3] F2	[4] F3	[5] F4	[6] Crude
Acute LC50	Barley	Root length	Clay loam and sandy loam	2.22	2.77	58.2	7526.9	44
	Barley	Root length	Artificial soil	5.44	3.44	119.6	NA	27.61
	Corn	Root length	Clay loam and sandy loam	3.96	NA	NA	NA	62.04
	Corn	Root length	Artificial soil	2.70	NA	NA	NA	26.49
	Alfalfa	Root length	Clay loam and sandy loam	4.58	NA	10	70.41	5.18
	Alfalfa	Root length	Artificial soil	5.01	NA	NA	NA	1.05
	Northern wheatgrass	Root length	Clay loam	NA	NA	51.1	4441.4	23.19
	Northern wheatgrass	Root length	Artificial soil	NA	NA	121	NA	16.64
Chronic IC50	Barley	Root length	Clay loam and sandy loam	1.60	4.55	3.2	149.34	10.68
	Barley	Root length	Artificial soil	NA	NA	NA	NA	NA
	Corn	Root length	Clay loam	NA	NA	NA	NA	8.1
	Corn	Root length	Artificial soil	NA	NA	NA	NA	NA
	Alfalfa	Root length	Clay loam and sandy loam	3.90	1.86	6.3	29.94	30.77
	Alfalfa	Root length	Artificial soil	NA	NA	NA	NA	NA

Table 5.2 Acute to chronic ratios for plant species – root metrics (data courtesy of PTAC; ESG International Inc. 2003)

Method	Plant Name	Parameter	[1] Soil	[2] Mogas	[3] F2	[4] F3	[5] F4	[6] Crude
	Northern wheatgrass	Root length	Clay loam	NA	2.32	7.3	42.15	5.88
	Northern wheatgrass	Root length	Artificial soil	NA	NA	121	NA	NA
LC50/EC50	Barley	Root length	Clay loam	1.39	0.61	18.19	50.40	4.12
	Barley	Root length	Artificial soil	NV	NV	NV	NV	NV
	Corn	Root length	Clay loam	NV	NV	NV	NV	7.66
	Corn	Root length	Artificial soil	NV	NV	NV	NV	NV
	Alfalfa	Root length	Clay loam	1.17	NV	1.59	2.35	0.17
	Alfalfa	Root length	Artificial soil	NV	NV	NV	NV	NV
	Northern wheatgrass	Root length	Clay loam	NV	NV	7	105.37	3.94
	Northern wheatgrass	Root length	Artificial soil	NV	NV	NV	NV	NV
Extrapolation Factor 10x IC50	Barley	Root length	Clay loam/ sand loam	22.20	27.70	582	75269	440
	Barley	Root length	Artificial soil	54.40	34.40	1196	NV	276.10
	Corn	Root length	Clay loam/ sandy loam	39.63	NV	NV	NV	620.40
	Corn	Root length	Artificial soil	27	NV	NV	NV	264.90
	Alfalfa	Root length	Clay loam/ sandy loam	45.82	NV	100	704.10	51.80
	Alfalfa	Root length	Artificial soil	50.1	NV	NV	NV	10.5
	Northern wheatgrass	Root length	Clay loam/ sandy loam	NV	NV	511	44414	231.90

Table 5.2 Acute to chronic ratios for plant species – root metrics (data courtesy of PTAC; ESG International Inc. 2003)

Method	Plant Name	Parameter	[1] Soil	[2] Mogas	[3] F2	[4] F3	[5] F4	[6] Crude
	Northern wheatgrass	Root length	Artificial soil	NV	NV	1210	NV	166.40

NA no experiment was conducted with this soil type or species

NV no value because either the LC50 or IC50 could not be calculated

[1] Soil types were sandy loam, clay loam, and artificial soil

[2] Mogas was a surrogate for F1 of crude oil (units were mg mogas/g soil)

[3] F2 (>nC10-C16 of crude oil, mg F2/g soil)

[4] F3 (>nC16-C34 of crude oil, mg F3/g soil)

[5] F4 (>C34 of crude oil, mg F4/g soil)

Table 5.3 Acute to chronic ratios for plant species – shoot metrics (data courtesy of PTAC; ESG International Inc. 2003)								
Method	**Plant Name**	**Parameter**	**[1] Soil**	**[2] Mogas**	**[3] F2**	**[4] F3**	**[5] F4**	**[6] Crude**
Acute LC50	Barley	Shoot length	Clay loam and sandy loam	3.10	7.15	53.40	367.54	80.60
	Barley	Shoot length	Artificial soil	5	6.37	98.20	NA	Not toxic
	Corn	Shoot length	Clay loam and sandy loam	5.02	NA	NA	NA	116.50
	Corn	Shoot length	Artificial soil	4.65	NA	NA	NA	130.64
	Alfalfa	Shoot length	Clay loam and sandy loam	6.60	NA	51.90	69.42	10.51
	Alfalfa	Shoot length	Artificial soil	5.45	NA	NA	NA	149.05
	Northern wheatgrass	Shoot length	Clay loam/ sandy loam	NA	NA	42.1	962.9	26.12
	Northern wheatgrass	Shoot length	Artificial soil	NA	NA	81.9	NA	29.86
Chronic IC50	Barley	Shoot length	Clay loam and sandy loam	1.68	4.13	27.6	115.01	15.27
	Barley	Shoot length	Artificial soil	NA	NA	NA	NA	NA
	Corn	Shoot length	Clay loam and sandy loam	NA	NA	NA	NA	NA
	Corn	Shoot length	Artificial soil	NA	NA	NA	NA	NA
	Alfalfa	Shoot length	Clay loam and sandy loam	5.13	2.71	8.3	16.21	19.88
	Alfalfa	Shoot length	Artificial soil	NA	NA	NA	NA	NA
	Northern wheatgrass	Shoot length	Clay loam	NA	7.44	12.70	75.83	6.67

Table 5.3 Acute to chronic ratios for plant species – shoot metrics (data courtesy of PTAC; ESG International Inc. 2003)								
Method	**Plant Name**	**Parameter**	**[1] Soil**	**[2] Mogas**	**[3] F2**	**[4] F3**	**[5] F4**	**[6] Crude**
	Northern wheatgrass	Shoot length	Artificial soil	NA	NA	NA	NA	NA
LC50/EC50	Barley	Shoot length	Clay loam/ sandy loam	1.85	1.73	1.93	3.20	5.28
	Barley	Shoot length	Artificial soil	NV	NV	NV	NV	Not toxic
	Corn	Shoot length	Clay loam/ sandy loam	NV	NV	NV	NV	NV
	Corn	Shoot length	Artificial soil	NV	NV	NV	NV	NV
	Alfalfa	Shoot length	Clay loam/ sandy loam	1.29	NA	6.25	4.22	0.53
	Alfalfa	Shoot length	Artificial soil	NV	NV	NV	NV	NV
	Northern wheatgrass	Shoot length	Clay loam/ sandy loam	NV	NV	NV	NV	3.92
	Northern wheatgrass	Shoot length	Artificial soil	NV	NV	NV	NV	NV
Extrapolation Factor 10x IC50	Barley	Shoot length	Clay loam/ sandy loam	31.04	71.50	534	3675.40	806
	Barley	Shoot length	Artificial soil	49.97	63.70	982	NV	NV
	Corn	Shoot length	Clay loam/ sandy loam	50.2	NV	NV	NV	1165
	Corn	Shoot length	Artificial soil	46.5	NV	NV	NV	1306.40
	Alfalfa	Shoot length	Clay loam/ sandy loam	66	NV	519	684.20	105.10
	Alfalfa	Shoot length	Artificial soil	54.5	NV	NV	NV	1490.50

Table 5.3 Acute to chronic ratios for plant species – shoot metrics (data courtesy of PTAC; ESG International Inc. 2003)

Method	Plant Name	Parameter	[1] Soil	[2] Mogas	[3] F2	[4] F3	[5] F4	[6] Crude
	Northern wheatgrass	Shoot length	Clay loam/ sandy loam	NV	NV	421	9626	261.20
	Northern wheatgrass	Shoot length	Artificial soil	NV	NV	819	NV	298.60

NA no experiment was conducted with this soil type or species

NV no value because either the LC50 or IC50 could not be calculated

[1] Soil types were sandy loam, clay loam, and artificial soil

[2] Mogas was a surrogate for F1 of crude oil (units were mg mogas/g soil)

[3] F2 (>nC10-C16 of crude oil, mg F2/g soil)

[4] F3 (>nC16-C34 of crude oil, mg F3/g soil)

[5] F4 (>C34 of crude oil, mg F4/g soil)

Table 5.4 Summary of Acute to Chronic Ratio Results for Plants and Soil Invertebrates																
Test	**EC50/LC50**															
Species	***O. folsomi***			***E. andrei***		**Barley**	**Alfalfa**	**Northern wheatgrass**	**Barley**		**Corn**		**Alfalfa**		**Northern wheatgrass**	
Parameter Measured						**Shoot length**	**Shoot length**	**Shoot length**	**Root length**	**Root length**	**Root length**	**Root length**	**Root length**	**Root length**	**Root length**	**Root length**
Soil	SL	AS	RS	RS	RS	RS/SL	RS/SL	RS/SL	RS	AS	RS	AS	RS	AS	RS	AS
Mogas	2.06	2.06	n/v	n/v	n/v	1.85	1.29	n/v	1.39	n/v	n/v	n/v	1.17	n/v	n/v	n/v
F2	n/v	n/v	1.99	2.10	1.08	1.73	n/a	n/v	0.61	n/v	n/v	n/v	n/v	n/v	n/v	n/v
F3	n/v	n/v	4.01	n/v	28.82	1.93	6.25	n/v	18.19	n/v	n/v	n/v	1.59	n/v	7.00	n/v
F4	n/v	n/v	n/v	n/v	n/v	3.20	4.22	n/v	50.40	n/v	n/v	n/v	2.35	n/v	105.37	n/v
Crude	n/v	n/v	n/v	2.44	2.44	5.28	0.53	3.92	4.12	n/v	7.66	n/v	0.17	n/v	3.94	n/v

n/v no value calculated

Table 5.4 Summary of Acute to Chronic Ratio Results for Soil Invertebrates (continued)								
Test : Extrapolation Factor 10 x LC50								
Species	***O. folsomi***			***E. andrei***				
Soil	Sandy loam (SL)	Artificial (AS)	Clay loam (RS)	Clay loam (RS)	Clay loam (RS)	Sandy loam (SL)	Artificial (AS)	Artificial (AS)
Mogas	41.89	59.6	n/v	10.72		6.3	12.31	n/v
F2	n/v	32.30	29.20	10.30	5.30	n/v	11.90	11.50
F3	n/v	n/v	59.690	n/v	223.620	n/v	n/v	n/v
F4	n/v	n/v	n/v	n/v	n/v	n/v	n/v	n/v
Crude	n/v	n/v	48.600	39.800	n/v	n/v	57.300	n/v

Table 5.4 Summary of Acute to Chronic Ratio Results for Plant Species (continued)

Species	Barley	Barley	Corn		Alfalfa		Northern Wheatgrass		Barley	Barley	Corn	Corn	Alfalfa	Alfalfa	Northern wheatgrass	Northern wheatgrass
Parameter Measured	Shoot length	Shoot length	Shoot length	Shoot length	Shoot length	Shoot length	Shoot length	Shoot length	Root length	Root length	Root length	Root length	Root length	Root length	Root length	Root length
Soil	RS/SL	AS	RS/SL	AS	RS/SL	AS	RS/SL	AS	RS/SL	AS	RS/SL	AS	RS/SL	AS	RS/SL	AS
Mogas	31.04	49.97	50.2	46.5	66	54.5	n/v	n/v	22.2	54.4	39.6298	27	45.821	50.1	n/v	n/v
F2	71.5	63.7	n/v	n/v	n/v	n/v	n/v	n/v	27.7	34.4	n/v	n/v	n/v	n/v	n/v	n/v
F3	534	982	n/v	n/v	519	n/v	421	819	582	1196	n/v	n/v	100	n/v	511	1210
F4	3675.4	n/v	n/v	n/v	684.2	n/v	9629	n/v	75269	n/v	n/v	n/v	704.1	n/v	44414	n/v
Crude	806	n/v	1165	1306.4	105.1	1490.5	261.2	298.6	440	276.1	620.4	264.9	51.8	10.5	231.9	166.4

Table 5.4 Summary of Acute to Chronic Ratio Results for Soil Invertebrates (continued)

Test	Application Factor (AF) AF=MATC/LC50				
Species	*E. andrei*			*O. folsomi*	
Parameter Measured					
Soil	Clay loam (RS)	Sandy loam (SL)	Artificial (AS)	Clay loam (RS)	Sandy Loam (SL)
Mogas	n/v	1122.39	812.35	n/v	584.74
F2	0.41	n/v	420.17	0.48	n/v
F3	n/v	n/v	n/v	0.58	n/v
Crude	n/v	n/v	n/v	0.58	n/v

6 COLLECTION, HANDLING, AND STORAGE OF SOIL SAMPLES

6.1 Introduction

This section of the Guidance for Tier 2 report summarizes the sources of available guidance for the collection, handling, and storage of soil samples from contaminated sites that are destined for a toxicity assessment. The documents include guidance for the development of a sampling plan, inclusion of QA/QC methods, the application of different sampling design options, different methods for collecting soil samples, and the conditions for transportation and storage of soil samples. The guidance provided in these documents is relatively generic and not specific to PHC-contaminated site assessments; however, much of it can be directly adopted and applied to PHC-contaminated sites. Some of the critical issues are presented in more detail in the subsections below.

6.2 Existing Protocols, Guidelines or Guidance Documents

There are a number of soil sampling protocols and guidance documents available in the literature. Many have been generated by governmental and international agencies such as Environment Canada (EC), United States Environmental Protection Agency (U.S. EPA), American Standards of Testing and Materials (ASTM), International Standards Organization (ISO), etc. Recently a large study conducted by the Standards, Measurement and Testing Programme of the European Commission was completed, in which the comparability of the different strategies and guidelines for soil sampling and preparation of the member states for investigating soil contamination was empirically assessed. A list of some of these guidelines, standards and reference texts is provided in Subsection 6.7.

6.3 Developing a Sampling Plan

All soils are naturally variable, and their physico-chemical and biological properties change spatially (e.g., both horizontally and vertically) and temporally. It is estimated that up to one half of the variability between similar soils might occur within a distance of one metre (U.S. EPA, 1992). As a result, site soils are extremely variable, and any soil sampling study must take into consideration this inherent variation during the design of a soil sampling plan.

The most important component of a sampling plan, the development of which should be the first step in designing any soil sampling study, is the definition of the study objectives and goals. Typical goals include the identification of the type, concentration, toxicity, and distribution of the contaminants of concern. It is important that the individuals who collect, analyze and use the data work closely together during the entire planning process, since their different perspectives and experiences are important to defining data quality and quantity (Keith 1992). An integral part of defining the study goals in the sampling plan is the development of the data quality objectives (DQOs). Data quality objectives should be defined in light of the constraints needed to design a study, including a specification of the level of uncertainty that a data user is willing to accept in the decision. Data quality objectives determine the degree of total variability (uncertainty or error) that can be tolerated in the data from the entire study, i.e., from collecting and analyzing to data processing and reporting (CCME 1993). The DQO process is intended to give the decision maker data that meet a predetermined level of precision, representativeness, completeness and comparability. DQOs are not always quantitative, and might be restricted to elucidating decisions that will be made depending on the results of the sampling. Table 6.1 illustrates the sequential steps in the formulation of the DQOs.

Table 6.1. Steps in the Data Quality Objectives Process*

1. State the problem(s) to be resolved
2. Identify the decision(s) to be made
3. Identify inputs to the decision(s)
4. Narrow the boundaries of the study
5. Develop decision rule(s)
6. Develop uncertainty constraints
7. Optimize design for obtaining data

*from CCME 1993

As well as stating the study goals and the data quality objectives, a sampling plan provides detail on the location, type, and number of samples to be collected, the sampling protocols (including management of soil sampling waste), all quality control procedures, cleaning and decontamination of equipment, description of collection methods, the schedule of work tasks, and the personnel to perform each task (E-Edu. 2002). Separate quality assurance and safety plans should also be prepared and are usually attached to the sampling plan as appendices. Table 6.2 provides a sampling plan checklist that includes the issues and considerations for sampling contaminated sites. If a specific and detailed sampling protocol is included in the sampling plan, it will help to minimize the chance of errors or erroneous assumptions. Table 6.3 provides an example of a sampling protocol checklist.

Because most consultants and contract laboratories have, in their best professional judgement, optimized these practices through their standard operating procedures, the focus of the subsequent subsections is on sampling requirements unique to the assessment of site-soil toxicity to ecological receptors via the soil contact exposure pathway.

Table 6.2. Sampling Plan Checklist*

What are the program objectives?

What are the data quality objectives? (DQOs)

What will you do if your DQOs are not met (i.e., re-sample or revise DQOs?)

Summary of background information

Have arrangements been made to obtain samples from the sites?

Have alternate plans been prepared in case not all sites can be sampled?

Have personnel and equipment requirements, including subcontractors been addressed?

Is specialized sampling equipment needed and/or available?

Are samplers available and experienced in the type of sampling required?

What is the duration of sample collection?

Have all analytes been listed?

Has the level of detection (LOD) for each been specified?

Have methods been specified for each analyte?

What sample sizes are needed based on method and desired LOD?

List specific good laboratory practice, federal, provincial, or method QA/QC protocols required.

Are there percentages or required numbers and types of QC samples?

Are there specific instrument calibration or other special requirements?

What type of sampling approach will be used?

Geostatistical, control charts, hypothesis testing (classical statistics), etc.?

Will the data analysis methods meet your DQOs?

Is the sampling approach compatible with the data analysis methods?

Detailed descriptions of the exact location of each sampling point, including a map showing the exact location of each point and photographs (if available) documenting each location.

Soil sampling methods and equipment to be used

Composite sampling instructions, if necessary

Sample volumes/weight

How many samples are needed?

How many sample sites are there?

How many analytical (or biological) methods were specified?

How many test samples are needed for each method?

How many control site samples are needed?

What types of QC samples are needed?

Will the QC samples types meet your DQOs?

How many of each type of QC samples are needed?

Are these QC samples sufficient to meet your DQOs?

How many exploratory samples are needed?

How many supplementary samples will be taken?

- Number of samples = Test + Control + QC + Exploratory + Supplementary
- Test samples = Methods x Sample Sites x Samples per Site
- Control samples = Methods x Sample Sites x Samples per Site
- QC samples = Methods x Type of QC sample x % Needed to meet DQOs
- Exploratory samples = (Test samples + Control samples) x 5 to 15%

Table 6.2. Sampling Plan Checklist*

- Supplementary samples = (Test samples + Control samples) x 5 to 15%
Types, numbers and sizes of sample containers
Sample preservation instructions (no chemical preservatives can be used for soils undergoing ectoxicity assessments)
Sample collection documentation (e.g., sample container labels, field logs, field measurements)
Chain-of-custody procedures, as appropriate
Plans for transportation and conditions during transportation
Equipment decontamination procedures
Management of sampling wastes
Analytical methods
Required detection limits
Limits for precision
Holding times and conditions
Laboratory documentation requirements
Desired format of the final soil sampling results (e.g., contour maps of contaminant concentrations, contour maps of variation in confidence limits because of sampling density, aggregated data sets)

* from Keith 1992 and HW 1992

Table 6.3. Sampling Protocol Checklist*

What observations at sampling sites are to be recorded?
Has information concerning DQOs, analytical methods, LODs, etc. been included?
Have instructions for modifying protocols in case of problems been specified?
Has a list of all sampling equipment been prepared?
Does it include all sampling devices?
Does it include all sample containers?
Are the container compositions consistent with analytes?
Are the container sizes consistent with the amount of samples needed?
Does it include all preservation materials/chemicals?
Does it include materials for cleaning the equipment?
Does it include labels, tape, waterproof pens, and packaging materials?
Does it include chain-of-custody forms and sample seals?
Does it include chemical protective clothing or other safety equipment?
Are there instructions for cleaning equipment before and after sampling?
Are instructions for equipment calibration and/or use included?
Are instructions for cleaning or handling sample containers included?
Have instructions for each type of sample collection been prepared?
Are numbers of samples and sample sizes designated for each type?
Are any special sampling times or conditions needed?
Are numbers, types, and sizes of all QC samples included?
Are numbers, types, and sizes of exploratory and supplementary samples included?

Table 6.3. Sampling Protocol Checklist*

Are instructions for compositing samples needed?
Are instructions for field preparations or measurements included?
Have instructions for completing sample labels been included?
Have instructions for preserving each type of sample been included?
Do they include maximum holding times of samples?
Have instructions for packaging, transport, and storage been included?
Have instructions of chain-of-custody procedures been included?
Have safety plans been included?

* from Keith 1992

6.4 Background/Reference/Control Sites for Eco Soil Contact Testing

Background samples, or control site or matrix samples, are samples taken near the time and place of the sample of interest, except that they are not contaminated or contain only acceptably low (e.g., minimal) levels of contaminants. In ecotoxicity testing with soils, these samples also enable differentiation between the effects of the physico-chemical characteristics of the contaminated site soil and the presence of contamination on toxicity to test organisms. The soil characteristics should be as similar as possible to those of the contaminated soil. These samples are collected from either local reference sites (reference sites adjacent to, or very near, the test sample site) or area reference sites (reference sites in the same area, e.g., a city or county, as the sampling site). In both cases, care should be taken to insure that the reference sites are upwind of the prevailing winds at the sampling site, that travel between reference and sampling areas be kept to a minimum, and that the reference site samples be collected before the contaminated site samples (Keith 1992). The use of local reference sites is preferred; however, when an appropriate local reference site is not available, an area reference site is acceptable. Sample collection and QA/QC techniques at reference sites must be identical to those at sampling sites. The reference soil might be used as a diluent in the event that a contaminated site soil is to be proportionally diluted for use in a toxicity test to determine the degree of contamination that is associated with an adverse effect.

6.5 Sampling Soils with Volatile Organic Compounds for Eco-soil Contact Testing

Volatile organic compounds are frequently found in soils contaminated with complex PHC mixtures. The most important aspect of sampling soils with volatile organic compounds (VOCs) is the minimization of losses because of sample collection, handling, storage, and transportation. Negative bias (i.e., underestimation of VOC soil concentrations) is the most significant and difficult source of random and systematic error associated with collecting soils contaminated with VOCs. There are currently no standard procedures for sampling soils containing VOCs, and the selection of a particular sampling device is site-specific (E-Edu. 2002).

Several types of soil sampling devices that obtain intact cores or samples are available for use (e.g., soil punches, split-spoon samplers, Shelby tubes). The key characteristic of an effective sampler is that the core remains intact until removed from the sampler, and that the sampler is made of non-reactive materials such as stainless steel.

Once a sample is extracted from the ground, it is typically placed into a large container made of inert material. For a battery of terrestrial toxicity tests (including different species and methods) a relatively

large quantity of soil is required. Therefore, soil is usually placed into three 20-L, wide-mouthed buckets that are filled to minimize headspace and sealable lids are fastened tightly. Care should also be taken when filling containers to eliminate headspace. The headspace can result in desorption of the VOCs from the soil particles into the headspace and cause loss of VOCs when the container is opened in the laboratory. Once the soil is sampled, the sample containers are sealed and shipped directly to the laboratory. The use of plastic sample containers is not recommended because organic constituents in the liner can leach into the soil to cross-contaminate the sample. Alternatively, the diffusion coefficient of plastic materials might allow the diffusion of the volatiles that are small in size through these materials.

In the laboratory, further VOC losses can result during preparation of the soil sample for analysis. Therefore, precautions should be taken to both minimize and document these losses at different stages of test soil preparation. Subsamples of soil can be collected for analyses at intervals where manipulations might result in significant loss of VOCs from the soil. The use of 40-mL VOA vials that have modified caps that allow direct attachment to the laboratory purge-and-trap analytical device are ideal for collection of soil subsamples as they eliminate the VOC losses associated with the sample-analytical instrument transfer step (E-Edu. 2002). Also, VOC losses can be lessened by the use of the methanol immersion procedure. The methanol immersion procedure entails the transfer of the soil subsample into a glass jar containing a known volume of chromatographic-grade methanol. The methanol preserves the volatile components of the sample at the time of sample placement into the jar. Minimum VOC losses have been reported by using an undisturbed sample followed by immediate immersion into methanol compared to the normal procedures (E-Edu. 2002). Another way to minimize loss of VOCs while trying to collect sub-samples to send to the laboratory is to collect a number of small increments using a subcorer, rather than homogenizing the soil in a tray and obtaining subsamples with a stainless steel spatula (E-Edu. 2002).

Rigid plastic 20-L buckets can be closed tightly but cannot be sealed, and therefore are not suitable for shipping or storing soils with VOCs. There is also the potential for organic analytes in the bucket to adhere to the plastic bucket or its liner. Both concerns can be addressed by using 10 or 20-L lightweight metal buckets that are readily available, and not too expensive. They do, however, require specialized equipment to seal the bucket and then remove the lid.

Field storage and shipment of samples containing VOCs requires particular care. Samples should be stored and shipped in a cool (0 to 4^{o}C), dark container, away from materials containing VOCs such as hand lotion, tape, adhesives, vehicle exhaust, and ink from waterproof pens (E-Edu. 2002). Because of the short holding time for VOC-contaminated soils, it is highly recommended that samples be shipped by air, accompanied with a non-mercury maximum-minimum thermometer. If samples containing VOCs are to be stored for a significant period of time (e.g. archived) it is recommended that the methanol immersion procedure be used, since VOCs are highly soluble in methanol (E-Edu. 2002).

6.6 Quality Assurance and Quality Control (QA/QC)

Quality control (QC) denotes the procedures established and observed in field and laboratory to assure that the end result of sampling activities meets the intended data quality objectives. Quality assurance (QA) is the management system that is in place to assure that QC procedures are being performed correctly. The QA system usually entails an active auditing function that formalizes not only the QA process but also the findings of the QA audits being performed (E-Edu. 2002). The goal of QA/QC programs is to identify, measure, and control the errors associated with every component of a sampling study, including planning, sampling, analysis and reporting. Detailed QA/QC program and project plans should be a part of any soil sampling study, and these documents outline the QA/QC requirements for a specific project, as well as the commitment of the investigators to QA/QC activities.

Bias and precision are the two most commonly used parameters to assess measurement quality objectives (MQOs), and one objective of any sampling QA program is to provide the type and number of quality control samples necessary to control and minimized the effects of bias and precision in the sampling study (Keith 1992). The number of QA/QC samples that should be taken is best determined from statistical calculations based on the level of confidence estimated to be obtainable from a specific method used with a specific environmental matrix (E-Edu. 2002). However, these estimates are not readily available and default values are usually selected that relate to a percentage of the environmental test samples analyzed (E-Edu. 2002). One of the challenges associated with site soil assessments is the selection of an appropriate experimental control soil or experimental controls soils. We recommend that two types of experimental control soils be used for a toxicity assessment. The first experimental control soil should be a well-characterized soil (formulated or field-collected) for which there are performance criteria by which to measure the performance and health of the test organisms. The second experimental control soil should be selected to assess the influence of potentially confounding factors such as the physico-chemical characteristics of the test soil that are not related to the contamination.

6.7 Index of Guidance Documents

Alberta Environment. 1991. Draft subsurface remediation guideline for underground storage tanks. Alberta MUST project, Departments of Labour and Environment.

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APPENDIX A

APPROACHES TO ACUTE TO CHRONIC RATIOS

Appendix A - Approaches to Acute to Chronic Ratios						
Parameter	**Description**	**Method**	**Matrix**	**Author**	**Positives**	**Negatives**
ACR	LC50/LD50	Divide LC50 data by LC50 data to obtain a ratio	Aquatic	Various	Simple method Sufficient data usually exists	Requires sufficient data from both acute and chronic toxicity test as a basis for the predictions. May be inaccurate Variable
Application Factor (AF)	AF=MATC/LC50	Ratio of the MATC to LC50	Aquatic	Mount and Stephan, 1967	Simple method	Requires sufficient data from both acute and chronic toxicity test as a basis for the predictions. May be inaccurate Variable Have to calculate the NOEC and the LOEC
Inverse of AF	1/AF	The inverse of the application factor	Aquatic	Kenaga, 1982	Simple method Usefulness is questionable	Requires sufficient data from both acute and chronic toxicity test as a basis for the predictions. May be inaccurate Variable Have to calculate the NOEC and the LOEC
Linear Regression	linear regression of LC50 and LOEC(r)	LC50 of 24 and 48 hr invertebrate tests as the acute value and the LOEC(r) as the chronic endpoint (r=observed effect concentration at which the intrinsic rate of population increase (r) is affected). Analyzed using linear regression and a least-square approximation to establish a relationship between the LOEC(r) and the LC50.	Aquatic	Roex, 2000		

Appendix A - Approaches to Acute to Chronic Ratios						
Parameter	**Description**	**Method**	**Matrix**	**Author**	**Positives**	**Negatives**
Accelerated life testing		Uses a number of statistical tests such as regression, least squares estimates and asymptotic normality to create a user friendly computerized program.	Aquatic	Sun *et al.*, 1995	Produces relatively accurate results	Need a software programme Based on the following assumptions: 1) that the mode of action of the chemical is simple; 2) that the biological mechanisms for lethality are the same at high andlow doses; and, 3) that the model fits the data.
Multiple regression (probit)		Two basic models were formulated to represent the response surfaces of probit (percent) mortality as a function of toxicant concentration and acute exposure time. Requires that data quality be sufficient to permit estimates with small standard errors, so a minimum of two conditions is recommended. At least five concentrations or doses resulting in mortalities >10% and <90% over a fixed exposure time are desirable. Several observation times should be represented, and a minimum of four is proposed. The range in times of exposure should be adequate to permit estimation of the trend in lethality within a concentration.	Aquatic	Lee, 1995		Might not be applicable to acute invertebrate terrestrial tests already developed as the observation times in these tests are generally only two, which are usually taken at 7 days and 14 days. Requires use of a model for which some parameters have not been calculated. Requires specific software
Acute-prolonged ratio (APR)		Used fish and daphnid LC(EC)50 and prolonged NOEC data to calculate the acute-prolonged ratio (APR) for general chemicals and pesticides	Aquatic	Heger *et al.,* 1995	Obtains one APR ratio (100 for pesticides)	Over estimates NOEC for some substances

Appendix A - Approaches to Acute to Chronic Ratios						
Parameter	**Description**	**Method**	**Matrix**	**Author**	**Positives**	**Negatives**
Probabilistic Model	For literature derived values which were paired LC50	Acute value (LC50) was divided by the chronic value (not specified) Related ACRs to Kow values	Terrestrial literature derived values	Fuchsman, 1998		
Univariate and multiyvariate and partial least mean squares models	Used the physicochemical properties of eight hydrocarbon-contaminated soils to predict toxicity to earthworms (*Eisenia fetida*).	Related physicochemical properties of eight hydrocarbon-contaminated soils to predict toxicity to earthworms and plants. Calculated no-observed-effect and used models to to determine predictive pwers	Terrestrial	Wong *et al.,* 1999	Interesting attempt at modeling predictors	Requires the model developed in the paper Predictive powers were only powers of 42 and 29%, even for the data set for which it was developed Not applicable to this data set