MOBILIZATION OF HYDROPHOBIC CONTAMINANTS FROM SOIL IN A MODEL DIGESTIVE SYSTEM

by

Jaswinder Minhas
M.Sc., Punjab University, India, 1994
B.Sc., Guru Nanak Dev University, India, 1991

RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ENVIRONMENTAL TOXICOLOGY

In the Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

April 2004

APPROVAL

Name: Jaswinder Minhas

Degree: Master of Environmental Toxicology

Title of Project:

Mobilization of hydrophobic contaminants from soil in a model digestive system

Examining Committee:

Chair: Dr. F. Breden

Dr., M.M. Moore, Associate Professor, Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. C.J. Kennedy, Associate Professor
Department of Biological Sciences, S.F.U.

Dr. F.A.P.C. Gobas, Associate Professor
School of Resource and Environmental Management, S.F.U.

Dr. R.A. Nicholson, Associate Professor
Department of Biological Sciences, S.F.U.
Public Examiner

April 6, 2004
Date Approved

SIMON FRASER UNIVERSITY

Partial Copyright Licence

ABSTRACT

Soil ingestion is often considered the most important pathway of exposure to soil-bound contaminants, especially for children. The chemically extractable fraction of the contaminant is often greater than the bioavailable amount, i.e. the concentration that crosses the gastrointestinal (GI) membrane. Current methods to estimate bioavailability often use animal tests, which are not only expensive and cumbersome but also raise ethical concerns. The objective of this research was to measure mobilization of a soil-bound model hydrophobic organic contaminant, chrysene, in a cell-free digestive system using ethylene vinyl acetate (EVA) to mimic the intestinal epithelium.

^{14}C-chrysene contaminated soil in an EVA-coated vial was digested using synthetic digestive fluids (gastric and intestinal) at 37°C. The contaminant that partitioned into EVA represented the bioavailable fraction. In vitro digestion resulted in a mobilization of 53% of the soil-bound chrysene. Only 45% partitioned to EVA if digestive fluids were replaced with water, and only 28% when gastric fluids were not neutralized. The aqueous fraction contained a maximum of 4%, 3% or 1% of the chrysene, respectively. Six months ageing onto soil did not affect the extent of mobilization but resulted in ~2.5-fold less contaminant in the aqueous phase. This was consistent with the lack of other soil contaminants, e.g. non-aqueous phase liquid, and agreed with the kinetic data that showed a single compartment. Comparative experiments between EVA and Caco-2, a human intestinal cell line, showed similar partitioning kinetics, which indicates that EVA might be a good model for predicting bioavailability. However, EVA was found to have a 23-fold higher fugacity capacity than Caco-2 cells.

Neutralization of gastric fluid was found to be the most important factor increasing the mobilization of chrysene in this GI model. A possible explanation is that as the pH increased, humic/fulvic acids of the organic matter became deprotonated, and revealed negative charges that reduced their affinity for chrysene. The presence of protein in GI fluids also had a positive effect on mobilization, which did not depend on proteolytic activity. The model presented herein is a promising tool for studying the effect of ingestion matrix on mobilization of bound contaminants.

DEDICATION

This thesis is dedicated to my parents,
Harpal Singh and Rajinder Kaur Minhas.

ACKNOWLEDGEMENTS

First of all, I would like to sincerely thank my senior supervisor, Dr. Moore, for her valuable guidance with the project and support in every possible way, through out the course of my degree. I feel privileged to have had the opportunity to work with her. She is one of the finest people I have ever known.

Thanks to my committee members, Dr. Gobas and Dr. Kennedy for their insightful comments and helpful discussions during committee meetings and with thesis write-up. I would like to express my sincere appreciation to Mrs. Linda Pinto. I have no words to express my gratitude.

I would like to acknowledge all the members of the Moore Lab, especially, Luba Vasiluk. I also wish to thank Victoria Otton and Lizzane Meloche of Gobas Lab for always being there for help, in person as well as by email. Financial support from Natural Sciences and Engineering Research Council of Canada is greatly acknowledged.

Lastly, I would like to thank my family. I thank my dad for his inspiration and continually positive attitude. My parents not only gave me moral support but also helped in numerous other ways. I acknowledge the sacrifices they have made for me. I thank my husband, Harjit, for emotional and financial support. Last but not the least, I would like to thank my beautiful, six-year old daughter, Enika for her support, love, good behaviour and cooperation. I look forward to spending more time with her.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

As	Arsenic
BSA	Bovine Serum Albumin
CEPA	Canadian Environmental Protection Agency
DPM	Disintegrations Per Minute
EPA	Environmental Protection Agency
EVA	Ethylene Vinyl Acetate
GI	Gastro-intestinal
GIT	Gastro-intestinal Tract
HOC	Hydrophobic Organic Contaminants
PAHs	Polycyclic Aromatic Hydrocarbons
Pb	Lead
PBET	Physiologically Based Extraction Test
PBS	Phosphate Buffered Saline
PCB	Polychlorinated Biphenyl
SOM	Soil Organic Matter
TCDD	Tetrachlorodibenzo-dioxin
TCDF	Tetrachlorodibenzo-furan
TRIS	(Hydroxymethyl) aminomethane
WHO	World Health Organization

CHAPTER ONE:
GENERAL INTRODUCTION

Introduction

In Canada, it has been estimated that as much as 25% of the land area in major urban centres is potentially contaminated because of the previous industrial activities (De Sousa, 2001). Most of these contaminants are hydrophobic and comprise broad classes of chemicals that may persist in soils. Many of these organic pollutants in soil were introduced years or decades ago at a time when industry and the public were not adequately aware of the scope, magnitude, and importance of soil pollution. Soil ingestion can be a major route of human exposure to many immobile soil-borne contaminants, especially in young children (ages 1.5-3.5 years) due to their hand to mouth behaviour (Sheppard et al., 1995; Staneck et al., 1995). Therefore, children may represent a subpopulation at increased exposure risk. Ingestion rate is an important determinant of exposure but is not inherently a component of bioavailability.

At present, the amount of contaminant that is chemically extractable from the soil is considered the exposure dose (Pinto and Moore, 2000; Liste and Alexander, 2002). In recent years, hydrophobic partitioning (octanol-water partition coefficient, Kow) has been used as a predictor of bioavailability in studies of the environmental fate of hydrophobic organic chemicals. Because of their high K_{ows}, these chemicals partition to tissue to a greater extent than more polar compounds (Mackay, 1991). However, these hydrophobic organic contaminants (HOCs) also have a high affinity for the organic constituents of soil matrices and therefore tend to bind tightly to soil, particularly if they have been in contact with the soils for a considerable length of time (Fries, 1990; Luthy et al., 1997; Alexander and Alexander, 1999). In higher animals, the majority (>90%) of

absorption occurs in the small intestine (Duggan et al., 2002), although absorption of soil particles in the gastrointestinal tract is thought to be negligible (Norris et al., 1998) and the fraction of contaminant that is sorbed to soil is considered to be unavailable for absorption (Ruby et al., 1996). However, intestinal secretions and digestive enzymes could mobilize a proportion of the sorbed fraction during digestion and this mobilized fraction could ultimately be absorbed.

Some baseline risk assessments are based on extrapolations from data obtained from studies performed with pure chemicals. Others use the assumption that the oral bioavailability of contaminants from soil is equal to the oral bioavailability from matrices administered in toxicity studies, such as liquid or food matrix (Ruby et al., 1992; Freeman et al., 1992; Ruby et al., 1993; Freeman et al., 1993; Freeman et al., 1994; Freeman et al., 1996; Hack and Selenka, 1996; Ruby et al., 1996; Casteel et al., 1997; Ellickson et al., 2001). Differences in the oral bioavailability of contaminants bound to different matrices may result in incorrect estimate of exposure and risk and hence result in erroneous and costly assumptions.

Current methods to estimate bioavailability often use animal tests, which are neither feasible economically nor ethically for testing the 23,000 chemicals that are presently awaiting registration with the Canadian government (CEPA Act, 1999). Due to the existence of thousands of untested toxic chemicals as well as new compounds being isolated or developed at ever-increasing rates, a simple, accurate, reproducible and inexpensive in vitro model is needed urgently to screen compounds for their oral bioavailability. In this study, we evaluated the oral bioavailability of the polycyclic aromatic hydrocarbon (PAH), chrysene bound to soil, in an in vitro digestion model.

Rationale for Investigating Oral Bioavailability from Soil

One important aspect of risk assessment is the determination of contaminant exposure. In human health risk assessment, ingestion of soil is considered a major route of exposure to many soil-borne contaminants especially in children (Sheppard et al., 1995; Staneck et al., 1995; Paustenbach et al., 1997). Three specific pieces of information are needed for environmental safety assessments: the amount of soil ingested, the concentration of the contaminant on the ingested soil, and the bioavailability of the contaminant on the ingested soil (Sheppard, 1998). In general, risk assessment models may over-simplify the latter two parameters in two ways: 1) by assuming that the ingested soil has the same contaminant concentration as the bulk soil, and 2) by assuming that the contaminant is as bioavailable from the ingested soil as from all other ingestion sources. Sheppard (1998) showed that these assumptions could lead to errors of up to 20-fold. The assessment of the health risk to humans exposed to chemically contaminated soils is primarily based on results from animal studies performed with pure chemicals. However, physical and chemical parameters of soil such as particle size, percent organic carbon content, and percent clay affect the degree to which contaminants are adsorbed to soil and therefore the degree to which they are desorbed. An assumption of complete bioavailability of soil-bound contaminants may therefore overestimate related health risks.

Human exposure to PAHs is 88-98% connected with food (in 5% with food of plant origin) and smoked food products are an important source of PAHs in the diet (Kluska, 2003). Contaminated dust or soil may also be ingested together with insufficiently cleaned vegetables, wild mushrooms or fruits grown on contaminated ground. Furthermore, inhaled air-borne particles may be swallowed from the respiratory tract, thus additionally affecting the intake of contaminated material by gastrointestinal

tract. Soil particles that stick to objects (e.g. toys) or fingers when put into the mouth are ingested. This is of major concern in the case of small children as they play outside and end up ingesting significant amounts of soil due to their hand-to-mouth behaviour (Calabrese et al., 1996; Calabrese et al., 1997). Because their immune systems are still developing and their bodies still growing, children are at higher risk than adults when exposed to environmental contaminants in the soil. Several studies have been undertaken to estimate the amount of soil that is ingested by children (Calabrese et al., 1996; Calabrese et al., 1997; Davis and Waller, 1997; Sheppard, 1998). In addition to unintentional ingestion of soil due to their hand-to-mouth activities, some children have also been observed to ingest significantly large amounts (60 g) of soil intentionally (pica behaviour). Therefore, the oral bioavailability of ingested soil contaminants is a critical issue in assessing the potential health risk especially for children.

The fate of ingested soil-bound contaminants is shown in figure 1. The fraction of contaminant that is mobilized from the soil into the digestive juices, i.e., chyme, and is available for absorption during transit through the small intestine is defined as the 'bioaccessible' fraction (Ruby et al., 1999). This fraction represents the maximum amount of contaminant that is available for transport across the intestinal epithelium. Oomen et al. (2000) defined the oral bioavailable fraction of soil-borne contaminants as the contaminant fraction that reaches the systemic circulation. In the present study, the contaminant that is absorbed by the gastrointestinal membrane is considered as the bioavailable amount. The bioavailable fraction will generally be less than the bioaccessible fraction due to incomplete uptake of solubilized contaminant in the small intestine.

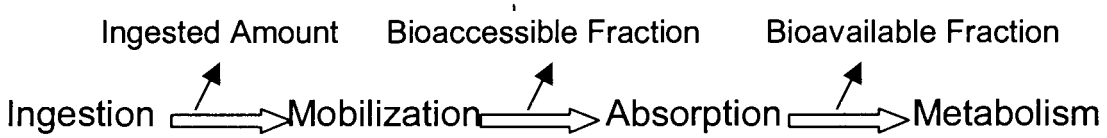


Figure 1: Fate of ingested contaminants

The contaminant that is absorbed by the GI membrane may be rapidly biotransformed and excreted in the intestinal lumen and/or transported to the liver for metabolism and then excreted via the kidneys before the systemic circulation is entered. This is referred to as first-pass effect (Sips et al., 2001). The fraction of contaminant that passes through the liver without being metabolised will be transported to tissues via the systemic circulation.

Since the solubilization of hydrophobic compounds in digestive juices is a prerequisite for their absorption, it has been proposed that the amount of compound mobilized in the absorptive compartments of the gastrointestinal tract, namely the stomach and the small intestine, is a good estimate of the bioavailability of the substances in question (Hack and Selenka, 1996) i.e., the bioaccessible fraction = bioavailable fraction.

The contaminants that can become sorbed to soil and therefore be ingested along with soil are usually hydrophobic in nature. For example, most PAHs are highly lipid soluble and thus are readily absorbed from the gastrointestinal tract of mammals (Cerniglia, 1984); however, PAHs also tend to be strongly sorbed to soil particles. PAHs with high K_{OW} have a high affinity for the organic constituents of soil-matrices and tend to bind tightly to soil (Luthy et al., 1997).

In general, the role of the gut is to extract nutrients from the ingested material. Gut fluids are very protein-rich (Mayer et al., 1997), and large globular proteins offer a hydrophobic interior environment that can solubilize HOCs, like PAHs (Voparil and Mayer, 2000). Rigorous mixing, extreme pH shifts and enzymatic attack in the gut create non-steady-state conditions that may promote extraction of bound contaminants. Nonetheless, the soil will remain a competitive sink for these strongly sorbed contaminants. As a result, the presence of soil in the diet will generally decrease the bioavailability of many contaminants. Indeed, soil and related materials such as clay are sometimes added to animal feeds to decrease the toxicity or bioavailability of hydrophobic materials (Stephenson et al., 1992). But, to what extent do the gastrointestinal fluids extract soil-bound PAHs? Does the soil matrix affect the extractability of PAHs? Are they as bioavailable as the same contaminants ingested with food or water?

In vitro studies (Ruby et al., 1992; Ruby et al., 1993; Hack and Selenka, 1996; Ruby et al., 1996; Ellickson et al., 2001) and studies in experimental animals (Freeman et al., 1992; Dieter et al., 1993; Freeman et al., 1993; Freeman et al., 1994; Freeman et al., 1996; Ruby et al., 1996; Casteel et al., 1997; Van Schooten et al., 1997; Ellickson et al., 2001) and humans (Maddaloni et al., 1998) suggest that oral bioavailability of contaminants from soil can be significantly lower than from matrices such as water or food. These differences in bioavailability of contaminants ingested from different matrices may lead to significant errors in risk estimation. Standard methods of contaminant analysis are not intended to quantify the bioavailable fraction and the total amount of contaminant extracted often is much greater than the amount of contaminant that is actually bioaccumulated (Kelsey and Alexander, 1997).

Both in vivo and in vitro studies may be carried out to study absorption and bioavailability. However, the complexity of the gastrointestinal tract, and of the pre- and post-absorptive kinetics hampers the collection of accurate and reliable data. In vivo studies have the drawback of dealing with a complex system in which it is difficult to determine the relative importance of different factors. It would be not only be expensive and cumbersome but also time-consuming to assess the oral bioavailability of every soil type and every contaminated site with in vivo studies. In vitro methods have been used to estimate bioavailability of minerals and other hydrophilic compounds for at least 50 years but no suitable in vitro model exist for hydrophobic compounds. For example, the European Standard for Safety of Toys provides an extraction test to evaluate the bioaccessiblity of eight metals from children's toys. The European method involves extraction of the particular metal (toy material reduced to <500 μm in size, at a liquid-to-solid ratio of 50:1 in pH 1.5 (HCl) fluid at 37±2°C for 2h. This method has been in use since 1994 by the 18 member countries of the Comite European de Normalization (CEN) to regulate the safety of toys. In vitro testing would reduce the need for lab animals as well as be more ethically acceptable to the public. In contrast to in vivo studies, an in vitro model facilitates manipulations and makes investigation of different variables feasible. Hence, there is a need for an in vitro digestion model based on human physiology as a simple, inexpensive and reproducible tool to investigate the mobilization or bioaccessibility of soil contaminants.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous toxic chemicals that have accumulated in the environment from a variety of anthropogenic activities. There are more than 100 different PAHs. They generally occur as complex mixtures (for example, as part of combustion products such as soot), rather than single

compounds. PAHs are produced by high-temperature industrial processes, such as petroleum refining, coke production, wood preservation and synthetic oil and gas production (Hurst et al., 1996). Some PAHs (e.g. naphthalene and phenanthrene) have also been used in the synthesis of different organic compounds, such as pesticides, detergents, dyes, plastic, and mothballs (Samanta et al., 2002). A wide variety of PAHs are found in the environment as a result of incomplete combustion of organic matter, emission sources, automobile exhausts, power plants, forest fires, agricultural burning, volcanic eruptions, domestic matter (e.g. tobacco smoke and residential wood or coal combustion) and also in food. These compounds may also be released into the environment through the disposal of coal tar and other coal processing wastes, petroleum sludge, asphalt, creosote, chemical wastes and soot (Laha and Luthy, 1991).

Physical and Chemical Properties of PAHs

PAH compounds are non-polar hydrophobic molecules comprising two or more fused benzene rings in linear, angular or cluster arrangements. PAHs can be grouped according to their molecular weights: low molecular weight, 128-178 g/mol (2-3 rings); medium molecular weight, 202 g/mol (4 rings, fluoranthene and pyrene) and high molecular weight, 228-278 g/mol (4-6 rings) (Table 1). Low molecular weight PAHs are relatively volatile, soluble and more degradable than the higher molecular weight compounds. Biotransformation of higher molecular weight PAHs is less well understood, but it is generally accepted that these compounds are more resistant to microbial degradation and may persist in contaminated environments. Table 1 outlines the physical-chemical properties of some three-, four-, and six-ring PAHs.

As pure chemicals, PAHs generally exist as colourless, white or pale yellow-green solids. They can have a faint pleasant odour. Generally, PAH solubility and volatility decreases and hydrophobicity increases with an increase in the number of

fused benzene rings. Their aqueous solubilities range from ~3 mg/L for naphthalene to <0.001 mg/L for perylene (Laha and Luthy, 1991). Because of low aqueous solubility and volatility, PAHs tend to partition onto hydrophobic adsorbents especially organic matter (Chantal et al., 2000). The role of soil mineral matter in sorption is thought to be relatively insignificant (Laha and Luthy, 1991; Mader et al., 1997). These properties make PAHs persistent and limit their biological transformation, which is believed to be primary removal process from soil systems (Guha and Jaffe, 1996b). PAHs mostly exist at contaminated sites either as non-aqueous phase liquid (NAPL) or sorbed onto soil (Guha and Jaffe, 1996a).

Table 1: Structure and physico-chemical properties of some three-, four-, five- and six-ring polycyclic aromatic hydrocarbons (Juhasz and Naidu, 2000; Mrozik et al., 2003)

PAH	No. of Rings	Mol. Wt.	MP (°C)	BP (°C)	Sol (mg/L)	Log K_{ow}	VP (torr)
Phenanthrene	3	178.20	101	340	1.29	4.46	6.8×10^{-4}
Anthracene	3	178.20	216	340	0.7	4.45	2.0×10^{-4}
Fluoranthene	4	202.26	111	250	0.262	5.33	6.0×10^{-6}
Benz[a]anthracene	4	228.29	158	400	0.014	5.61	5.0×10^{-9}
Pyrene	4	202.26	149	360	0.145	5.32	6.8×10^{-7}
Chrysene	4	228.29	255	488	0.003	5.61	6.3×10^{-7}
Benzo[a]pyrene	5	252.30	179	496	0.0038	6.04	5.0×10^{-7}
Dibenz[a,h]anthracene	5	278.36	262	524	0.0005	5.97	1.0×10^{-10}
Benzo[g,h,I]perylene	6	276.34	222	>500	0.0003	7.23	1.0×10^{-10}
Indeno[1,2,3-c,d]pyrene	6	276.34	163	536	0.062	7.66	1.0×10^{-10}

Mol. Wt molecular weight
MP melting point
BP boiling point
Sol aqueous solubility
Log K_{ow} logarithm of the octanol:water partitioning coefficient
VP vapor pressure

Toxicity of Polycyclic Aromatic Hydrocarbons

Contamination of soils by organic pollutants such as PAHs is of crucial environmental concern due to their toxic, mutagenic, teratogenic and carcinogenic characteristics. PAHs are essentially devoid of polar and ionisable functional groups and would therefore be expected to dissolve readily in and cross the lipoprotein membranes of mammalian cells. Furthermore, the fact that isolated cells and tissues metabolise PAHs by means of intracellular enzymes, and that some of these metabolites react with intracellular constituents suggest that uptake across cellular membranes occurs readily (WHO, 2000). Generally, the most injurious PAHs for humans are those compounds that contain 4-6 condensed benzene rings because of their carcinogenic potential. In contrast, 1,2, or 3 ringed PAHs have been found to be non-carcinogenic, although phenanthrene is known to be a human skin photosensitizer, mild allergen and an inducer of sister chromatid exchanges (Mrozik et al., 2003).

Little is known about the adverse health effects of short-term (acute) and intermediate duration (typically defined as less than one year) exposure to PAHs. Acute skin exposure can cause skin irritation and lesions, and long-term dermal exposure can cause chronic dermatitis, an abnormal thickening of the skin (called hyperkeratosis), and potentially skin cancer (ATSDR, 1995). Short-term exposure can cause temporary effects on the respiratory system such as chest pains and difficulty breathing, while long-term inhalation exposure can lead to lung cancer (ATSDR, 1995). Exposure to PAHs may lead to alterations in cellular physiology by disrupting genetic expression in cells of chronically exposed animals (Buckpitt and Frankin, 1989; Safe, 1990). At the cellular level, PAH exposure may lead to cytochrome P-450 induction and increased macromolecular adduct formation, altered biochemical functions, and oxidative stress. These molecular abnormalities may be evidenced at the tissue level as cell death,

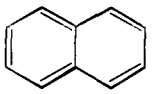
necrosis, malignancy, immune system dysfunction, decreased fecundity, fetotoxicity or developmental abnormalities (Safe, 1990).

Besides their mutagenic and carcinogenic effects, PAH exert many other toxicological effects in experimental animals, the most significant being bone marrow toxicity (Nebert et al., 1980) and reproductive toxicity (Legraverend et al., 1984). Serious effects are also produced in the progeny of exposed mice, such as testicular atrophy and interstitial cell tumours, immunosupression and tumour induction (Urso and Gengozian, 1980). The carcinogenic PAHs as a group have an immunosuppressive effect (White et al., 1985). While the carcinogenic potential of PAHs has been investigated in numerous studies, very little is known about the potential of these chemicals to produce pathological effects on the nervous system. Some PAHs may be distributed to the brain and are associated with effects such as inhibition of enzymes involved in the metabolism of neurotransmitters and impairment in nervous functions. Although the concentrations of BaP in brain tissue are much lower than in liver or kidney, their clearance from the brain is apparently much slower than from other tissues (Tang et al., 2003). Therefore, long-term low-level exposure to BaP may be a potential neurotoxic hazard. Tang et al. (2003) suggested that PAHs are capable of producing damage to neural cells only at concentrations near their aqueous solubility limits.

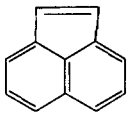
It has been well known for over 50 years that emissions from incomplete combustion contain carcinogenic PAHs, like benzo(a)pyrene. Cancer is the health effect of greatest concern with respect to PAHs. Animal and human studies have shown that long-term PAH exposure can cause lung cancer (from inhalation of PAHs), The US Environmental Protection Agency has determined that several PAHs, including BaP, are probable human carcinogens. PAHs exert their mutagenic and carcinogenic activity through biotransformation to chemically reactive intermediates, which bind covalently to

DNA. Extensive and systematic studies on the tumorigenicity of individual PAH metabolites in animals have led to the conclusion that vicinal or so called bay-region diol epoxides are the ultimate mutagenic and carcinogenic species of PAHs, although not necessarily the only ones (WHO, 2000). Many PAHs produce tumours in experimental animals. BaP has been the most studied PAH and has shown carcinogenicity after oral administration, intraperitoneal injection, subcutaneous injection, and inhalation or after direct application into the lungs or onto the skin of experimental animals (IARC, 1983). Thus, PAHs include a group of priority organic pollutants of critical environmental and public health concern due to chronic health effects (carcinogenicity), microbial recalcitrance, and low removal efficiencies in traditional treatment processes (Hurst et al., 1996). PAHs can sorb to organic-rich soils and sediments and accumulate in fish and other aquatic organisms, and may be transferred to humans through seafood consumption (Mrozik et al., 2003). PAHs in the environment are problematic not only because they are a human health risk, but also because they may alter native ecological communities (Shuttleworth and Cerniglia, 1995). Many PAHs are considered to be environmental pollutants that can have detrimental effects on the flora and fauna of affected habitats resulting in the uptake and accumulation of toxic chemicals in food chains and, in some instances in serious health problems and/or genetic defects in humans. Consequently, the US Environmental Protection Agency has listed 16 PAHs as priority pollutants for remediation (Samanta et al., 2002; Shuttleworth and Cerniglia, 1995) (Figure 2).

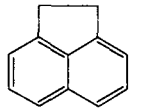
PRIORITY PAHs



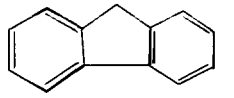
Naphthalene



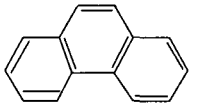
Acenaphthylene



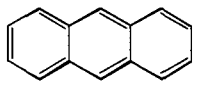
Acenaphthene



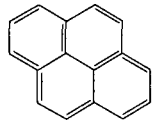
Fluorene



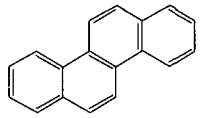
Phenanthrene



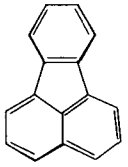
Anthracene



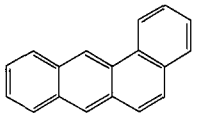
Pyrene



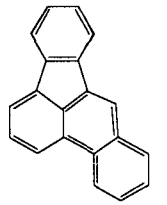
Chrysene



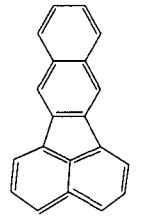
Fluoranthene



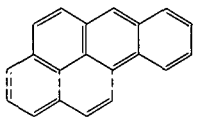
Benz(a)anthracene



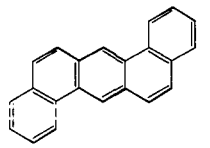
Benzo(b)fluoranthene



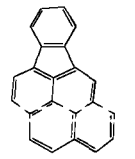
Benzo(k)fluoranthene



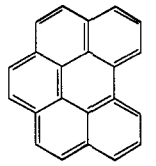
Benzo(a)pyrene



Dibenz(a,h)anthracene



Indeno 1,2,3-c,d)pyrene



Benzo(g,h,i)perylene

Figure 2: Priority PAHs designated by the US Environmental Protection Agency as Class B2 Carcinogens. Class B2 indicates sufficient evidence of carcinogenicity in animals but inadequate or no evidence in humans.

PAHs and Soil

Composition of Soil

Various types of soils and sediments are commonly referred to as geosorbents or simply sorbents. The geosorbents consist of structurally and/or chemically different constituents that interact differently with hydrophobic organic chemicals (HOCs) in terms of binding energies and rates of associated sorption processes. The schematic in Figure 3 shows conceptual geosorbent domains. First is the mineral domain with surface reactivity attributable to: (1) exposed external mineral surfaces at the particle scale and surfaces within macropores; (2) interlayer surfaces of swelling clays at nanometer scale; and (3) the surfaces within mesopores and micropores of inorganic mineral matrices. Second domain is the soft and hard carbon soil organic components at the nanometer scale of the geosorbent. Third domain includes adherent or entrapped non-aqueous phase liquids (NAPLs), e.g., tars, oils and solvents. Combustion residue (e.g. chars, soot and ashes) is another type of organic matter; that might act like hard carbon. Natural organic matter may not be accessible to HOCs because of encapsulation. Combustion residues and NAPLS that are entrapped or adherent to the geosorbent can also function as sorbents for HOCs.

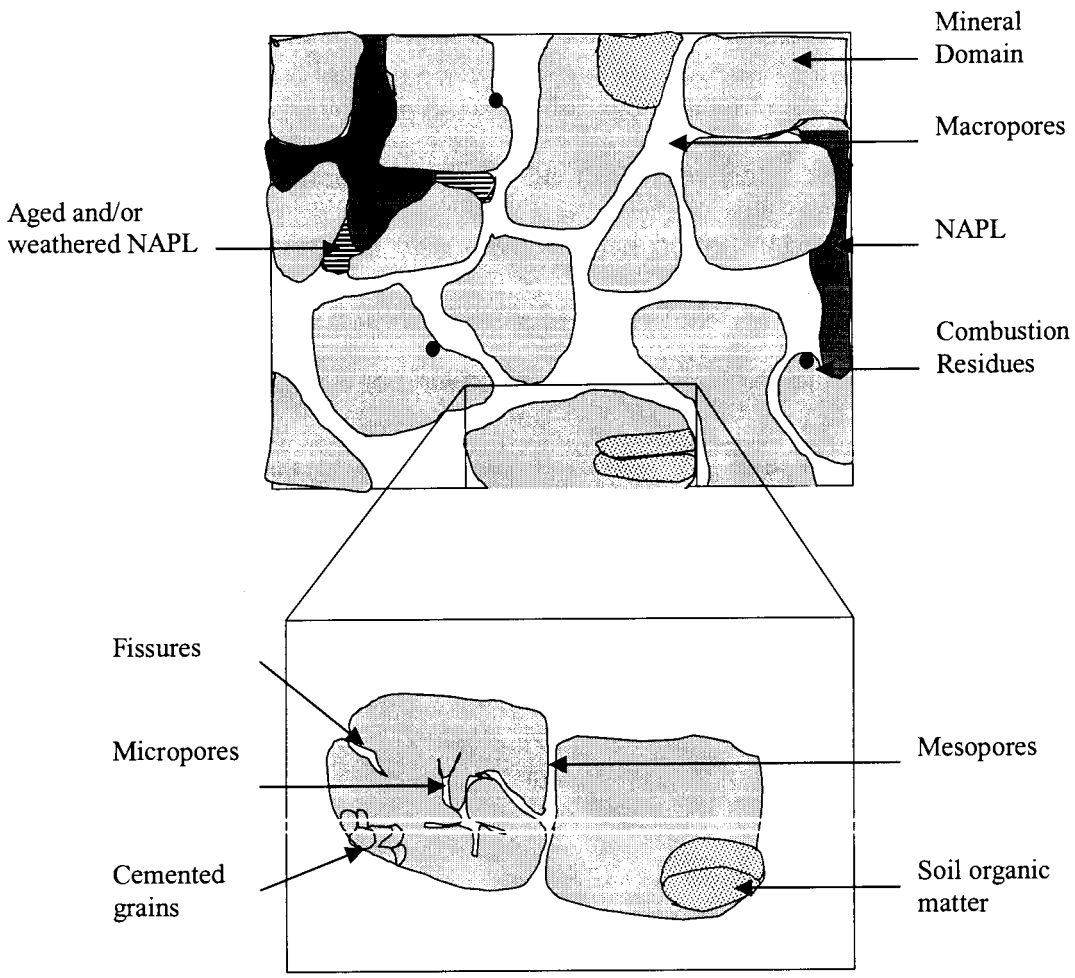


Figure 3: Conceptual model of different sorbent domains in a soil.

The Soil Structure and Aggregate Size

The soil structure consists of aggregates of mineral grains that are clustered together with natural organic matter. Silt and sand particles are known to be coated with clay-sized particles (<2 μm) that are cemented to each other and to the larger particles by organic matter, mineral oxides and carbonates. The coating of clay-sized particles

can shield the organic matter from equilibrium with the bulk, which makes the soil organic matter domain a strong sorption site for HOCs (Danielsson, 2000). The coating can be thin in some places such as in and along the fissures and pits associated with the primary particle. The void volume between the aggregates forms macropores (>50 nm). In the saturated zone, these pores will be filled with water and constitute the bulk solution. The soil aggregates also have internal pores that are usually divided into meso- (pore diameter 2-50 nm) and micro- (pore diameter <2 nm) pores (See Figure 3). The micropores will influence sorbate sorption by the proximity of two solid surfaces.

The four classes of soil are sand, coarse silt, fine silt, and clay (Table 2). Chemical analysis showed that low and high molecular weight PAHs were much more concentrated in the fine soil fraction (fine silt and clay) and were present at very low concentration in the larger aggregate size fractions (sand) (Amellal et al., 2001). Among silt aggregate fractions the PAH contents were lower in the coarse silt than in the fine silt (Amellal et al., 2001).

Table 2: Soil particle groups and their sizes

Particle Name	Particle Size (μm)
Sand	2000 – 50
Coarse Silt	50 – 20
Fine Silt	20 – 2
Clay	2 - 0

Soil Contamination

A contaminated site is generally defined as a property that has soil, groundwater, or surface water containing contaminants at levels that exceed those considered safe by regulators (De Sousa, 2001). Since the early 1980s, the contaminated sites have been considered a major problem because of the increasing presence of contaminated industrial and commercial properties in urban cores. About 25% of the land area in major urban centres in Canada is estimated to be potentially contaminated because of the previous industrial activities (De Sousa, 2001), excluding everyday emissions and industrial refuse dumping.

Soil contamination can occur when solid or liquid hazardous substances are mixed with the naturally occurring soil. Usually, contaminants in the soil are physically or chemically attached to soil particles, or, if they are not attached, are trapped in the small spaces between soil particles. Contaminated sites are the legacy of a long period of industrialization involving production and handling of hazardous substances and dumping of wastes. The expansion of industries and subsequent increase in amount of industrial wastes has led to environmental pollution in all the industrialized countries.

Soil contamination results when hazardous substances are either spilled or buried directly in the soil or migrate to the soil from a spill that has occurred elsewhere. For example, soil can become contaminated when small particles containing hazardous substances are released from a smokestack and are deposited on the surrounding soil as they fall out of the air. An alternative source of soil contamination is water that washes contamination from an area containing hazardous substances and deposits the contamination in the soil as it flows over or through it.

PAHs in Soil

Carcinogenic PAHs are found in all surface soils though the levels are much higher in urbanized areas. Typical concentrations in forest soils range from 5-100 μg/kg. Substantial amounts of PAHs are transferred to forest soil from vegetative litter because the compounds are adsorbed from air onto organic matter such as leaves and needles. Rural soil contains carcinogenic PAHs at levels of 10-100 μg/kg originating mainly from atmospheric fallout (WHO, 2000). For both forest and rural soil, values as high as 1000 μg/kg may occasionally be found (WHO, 2000). Metropolitan areas have higher PAH concentrations than forest and agricultural areas because of the many sources of fossil fuel combustion. The majority of urban soil concentrations fall in the 600-3000 μg/kg range; PAH are often distributed unevenly among various soil pools. Higher values near areas of heavy transportation and industrialization are probable. Values in the order of 1000-3000 μg/kg are regarded as the upper range; however, levels as high as 8000 – 336,000 μg/kg have also been reported for road dust (WHO, 2000). The PAH levels found at field contaminated sites not only vary from site to site, but are often much higher than what is generally used in laboratory bioavailability studies.

There is considerable information about PAH concentrations in soils of the temperate regions of the northern hemisphere. Much less information is available on PAHs in soils from tropical, southern hemispheric and polar zones (Wilcke, 2000). There is evidence that climate substantially influences PAH patterns in soils because soil temperature and soil moisture influence PAH decomposition and volatilisation. However, up to now, little is known about the climatic effect on the PAH patterns on a global scale (Wilcke, 2000).

Exposure – Soil Ingestion

Direct consumption of soil; i.e. geophagy, is much more common than many people suspect (Sheppard, 1998). Recently, the role of geophagy in contaminant transfer has been highlighted because it can be the dominant pathway for ingestion of many environmentally immobile soil-borne contaminants. Geophagy describes both purposeful and inadvertent ingestion of soil.

In general, between 50 and 200 mg soil per day is ingested by children (Calabrese et al., 1996; Calabrese et al., 1997; Calabrese et al., 1999; Davis and Waller, 1990). Besides unintentional ingestion of soil due to their hand-to-mouth behaviour, some children are also known to deliberately ingest soil i.e. pica behaviour (profound soil ingestion). Children have been observed to ingest up to 25-60g soils during a single day (Calabrese et al., 1997). Sheppard (1998) has also estimated that the amount ingested purposefully by people with pica behaviour can be large; up to 50g/day (Sheppard, 1998).

Amounts of soil ingested inadvertently are much smaller and very difficult to quantify. Both children and adults ingest soil along with food, although children are likely to ingest more because of their hand-to-mouth behavior. Furthermore, studies of soil loads commonly found on hands resulted in estimates of 0.06-3.5 mg/cm^2 skin surface (Sheppard, 1998). A useful benchmark is that at soil loads above 1 mg/cm^2, the hands are visibly soiled and would likely be cleaned before hand-to-mouth activities such as eating. At soil loads below this, the hands appear clean and hygiene might not be as routine. U.S. Environmental Protection Agency (U.S. EPA) assumes that 95% of children ingest up to 200 mg of soil per day (U.S. EPA, 1996) when estimating risks from soil contaminants under a residential land use scenario and in setting risk-based cleanup goals (Calabrese et al., 1997). A soil ingestion rate of 50-80 mg/day is used in Canada

for risk assessments (CEPA, 1994; Health Canada,1995; and CCME, 1996 and 2000). Other countries use similar values for risk assessments.

It has been estimated that the soil loads for leafy vegetables after normal washing are 20 mg soil/kg dry weight, and for fruits 2 mg soil/kg dry weight (Sheppard and Evenden, 1995). PAHs can penetrate food indirectly from air or water as well as directly during smoking. A permissible amount of PAHs in smoked products in western countries is 1µg/kg (Kluska, 2003). The content of PAHs in fruits and vegetables depend on the degree of pollution in the environment and on the area of contact. For example, apples contain usually 0.2-0.5 µg/kg, tomatoes 0.2 µg/kg, spinach 6.6 µg/kg, cabbage 20.4 µg/kg (Kluska, 2003). The majority of hydrocarbons are deposited on the skin of fruits and vegetables (Bodzek et al., 1998). Studies of the total diet cited in Phillips (1999) show that the majority of PAH contributions come from cereals, oils, fats, fruits, and vegetables, while the contributions from meat, fish, milk, and beverages are minor. In addition, regarding the contribution from oils and fats, evidence in the literature indicates that concentrations of PAHs are significantly higher in margarine than in butter and significantly higher in synthetic creams than in real cream because of processing of PAHs in the animals before production of the dairy product (Dennis et al., 1991; Phillips, 2000).

Effect of Soil Matrix on Mobilization of Soil-Bound Contaminants

Soils are the primary sink of organic compounds in the terrestrial environment. For instance, soils have been estimated to carry 93% of the polychlorinated biphenyl (PCB), 94% of the polycyclic aromatic hydrocarbon (PAH) and 95% of the polychlorinated dibenzo-p-dioxin and dibenzofuran (PCDD/F) burden in the environment of the United Kingdom (Hippelein and McLachlan, 1998). When test animals ingest contaminants in a soil matrix, generally less absorption occurs and fewer toxic effects

are observed than when they ingest the same contaminant in a food or liquid matrix (Dieter et al., 1993; Casteel et al., 1997; Van Schooten et al., 1997; Freeman et al., 1992; Freeman et al., 1993; Freeman et al., 1994; Freeman et al., 1995). The difference can be ascribed to a lower oral bioavailability, resulting from a lower bioaccessibility of contaminants in soil than from contaminants in food or liquid. Gobas et al., (1993 and 1999) also suggested that dietary uptake rates could differ substantially depending on the matrix in which the chemical substance is administered.

Many studies have shown that exposure risk assessment models that assume complete bioavailability of soil-bound PAHs probably overestimate the bioavailable dose. For example, Van Schooten et al. (1997) found that absorption of pyrene and BaP was reduced when administered with soil, compared to pure forms; 0.5 versus 0.2% and 1 versus 0.3% respectively. Koganiti and others (1998) found that PAHs from a manufactured gas plant residue are only 76% available from soil ingested by mice. These estimates were made based on DNA adduct formation and urinary excretion of the primary metabolite, 1-hydroxypyrene (1-OHP). Incorporation of coal tar into the soil resulted in decreased availability, which was demonstrated by a reduction in 1-OHP elimination and generally lower concentrations of high molecular weight PAHs in the livers (Reeves et al., 2001). Similarly, a study with rats fed coal tar spiked onto soil or an inert carrier showed a protective effect of soil, demonstrating the ability of soil to limit bioavailability (Bordelon et al., 2000).

Freeman et al. used New Zealand white rabbits (1993) and female cynomolgus monkeys (1996) to measure the bioavailability of arsenic in contaminated soils from the site of a former copper smelter. Using urinary excretion data, they found that arsenic in soil was only 30% bioavailable to rabbits, while aqueous arsenic was 2-fold more bioavailable. In the primate studies, the results were equally dramatic, 70% being

available from water, but only 14% from soil. Soil ingestion resulted in the bulk of the arsenic being eliminated in the faeces. Similar results were found when soil-bound arsenic was fed to dogs, with only 7% excreted in the urine (Groen et al., 1993).

The above examples illustrate the necessity of accounting for matrix effects when performing an environmental assessment of a contaminant. This can be achieved by introducing a 'relative bioavailability factor' into the calculations. The physiologically based in vitro digestion models appear to be a promising tool for estimating the effects of matrix on bioaccessibility from ingestion and thus on oral bioavailability.

Gastrointestinal System

PAHs are largely absorbed in the gastrointestinal tract of mammals (Cerniglia, 1984; Hack and Selenka, 1996), and subsequently metabolized and excreted as metabolites in the urine and faeces. Van Schooten et al. (1997) found that a large amount (>90%) of the oral dose is absorbed and/or metabolised by rats, indicating that the bioavailability is high after oral intake of pure PAH. Knowledge about functioning of the gastro-intestinal tract is a prerequisite for understanding of the oral bioavailability of soil-borne contaminants. Hence, the following provides some basic information on the physiology of the gastro-intestinal tract.

Physiology of Gastro-Intestinal Tract

Gastrointestinal physiology is the study of the many normal functions of the organs that comprise the gastrointestinal (GI) tract. The organs include the oesophagus, stomach, liver, pancreas, small intestine and colon. The gastrointestinal tract is ultimately responsible for the fate of swallowed food and involves a number of processes essential for the conversion of food into a form that can be utilized by the rest of the body and for the elimination of waste material. Food is chewed in the mouth to smaller

pieces and homogenized with saliva. The salivary enzyme α-amylase initiates the degradation of starch. Cells of the gastric mucosa secrete solutions of hydrochloric acid in remarkably high concentrations into the lumen of the stomach. A highly acidic gastric juice is required to activate pepsin, a proteolytic enzyme, which is responsible for dissolution of ingested food into soluble chyme.

Pancreas produces an alkaline solution that neutralizes the gastric acid and contains a wide array of enzymes, which participate actively in the breakdown of carbohydrates, fat, and protein ingested in our food. Pancreatic enzymes comprise three classes, based upon the foodstuff digested. The amylytic class has amylase, which hydrolyses starch to maltose, maltotriose and dextrins. The lipolytic class of pancreatic enzymes consists of a lipase and phospolipases. Pancreatic lipase hydrolyses triglycerides to free fatty acids, monoglyceride and glycerol. Its action is facilitated by bile salt emulsification of fat droplets into much smaller particles, micelles, that have a much greater total surface area for lipase to act upon. The proteolytic class of pancreatic enzymes consist of trypsin, chymotrypsin and carboxypolypeptidase. Trypsin and chymotrypsin cleave peptide bonds within the protein molecule and carboxypolypeptidase cleaves from the ends of the protein molecule. Pancreatic enzymatic activity accounts for most of the digestion of carbohydrates, fats, and proteins into their respective products: mono- and disaccharides, fatty acids and monoglycerides, dipeptides and amino acids. The more minor enzymes of pancreatic juice digest phospholipids and nucleotides. The secretion of the liver is called bile and consists of an alkaline solution containing bicarbonate and bile salts. Bile is stored in the gallbladder and released into the duodenum at mealtime where the bile salts emulsify fat.

These actions result in the presentation of simpler molecules to the small intestine for absorption. Another vital function of the system is the transport of small

molecules from the lumen of the small intestine into the circulation. The small intestine merges into the large intestine. The role of the large intestine is primarily to reabsorb water and to store unabsorbed material, while further metabolism of remaining food components can take place due to microbial activity.

Small Intestinal Anatomy

The small intestine is an important part of the digestive system as greater than 90% of absorption occurs here (Duggan et al., 2002). Most nutrients are absorbed in the duodenum and jejunum; however, other substances are absorbed in the terminal ileum (e.g. bile salts). The small intestinal epithelium is composed of large number of small, finger-like projections called villi, which extend about 1mm into the lumen. Each villus has blood and lymphatic capillaries, through which compounds are transported to the rest of the body. The surface of each villus is composed of heterogeneous population of cells, which include enterocytes or absorptive cells, mucus secreting goblet cells, endocrine cells and several more types of cells which take up nutrients from the lumen and transport them into blood (Creamer, 1974). The enterocyte is the most common cell and is predominantly responsible for intestinal absorption. The luminal membrane of each enterocyte contains microvilli, each about 1μm long and 0.1μm in diameter. The architecture of the intestine is designed to increase its surface area. The microvilli, villi, and large ridges in the intestinal tissue attribute to a magnification of the total surface area of the small intestine, of up to 200m^2 for adults; a 600-fold increase. This large surface area facilitates mass transfer from the intestinal lumen to enterocytes and thereby to the blood and lymph flow (Mansbach et al., 2001).

Intestinal Absorption Routes

The permeability of the GI mucosa governs the passive transport of substances into and out of the lumen. Transport of a molecule across the cellular membranes can take place by passive diffusion or facilitated diffusion (processes that do not require energy), or by a specific carrier mediated active diffusion (process requiring energy). The force driving passive transport of solutes is the electrochemical gradient and the force driving passive transport of water is the osmotic gradient across the GI mucosa. Compounds can be absorbed across the intestinal epithelium in two ways, either through the cells i.e. the transcellular route or between the cell-cell junction, i.e. the paracellular route. Transport across healthy intestinal epithelium by the paracellular route is minimal due to the presence of the tight junctions between the cells (Mansbach et al., 2001). For passive flux of a molecule to occur, it must have the correct physicochemical properties (e.g., small, uncharged and hydrophobic) to cross both the apical and basolateral membranes, which are lipophilic, and diffuse through the cytoplasm, an aqueous environment separating the two membranes. Hydrophobic compounds such as PAHs are mainly transported across the intestinal epithelium via passive diffusion (Leib and Stein, 1986).

Two types of mediated transport, facilitated diffusion and active transport, also play an important role in transporting substances across the GI membrane. Specific membrane carriers can facilitate the movement of otherwise slowly transported molecules, e.g. the sugar fructose is somewhat too large to permeate the porous protein channels of the intestinal microvillous membrane. By combining with the specific membrane carrier, the fructose-protein complex can diffuse or be shuttled from the lumen to the cell interior. Substances are also actively transported against their electrochemical gradient into and out of the lumen. This movement requires attachment

of the substance to a membrane carrier protein with the expenditure of metabolic energy, generally the hydrolysis of ATP. The process of pinocytosis (membrane engulfment of the solute) can absorb minute amounts of undigested protein intact into intestinal cells, although pinocytosis of compounds is minimal in adult small intestine and is therefore not a probable mechanism for absorption of contaminants (Norris et al., 1998).

Fugacity

The fugacity approach can be used to predict the behaviour of toxic compounds (Mackay, 1979). Fugacity calculations are used to predict the static and dynamic behaviour of toxic substances within an organism and in the environment. The environment comprises several compartments, e.g. the atmosphere, a lake, sediment, soil, or biota; some are in contact (for example, lake-atmosphere) and others are not (for example, atmosphere-aquatic sediment). If it is assumed that each compartment is well mixed, i.e. homogenous, and sufficient time has elapsed so that all compartments are in equilibrium, then thermodynamic calculations can be used to predict the nature of the contaminant partitioning. It is recognized that these assumptions are sometimes invalid because of in- and outflows of a physical, chemical or biological nature, but interestingly they tend to be most valid for the persistent substances that often are of greatest toxicological concern (Mackay, 1979).

When compartments are not in equilibrium, thermodynamic equation can still be used to determine the likely direction of flow of a chemical as it moves toward equilbrium in a system. The thermodynamic concept of chemical potential describes passive diffusion of chemicals within and between phases (Mackay, 1991). Within a phase or compartment, mass diffusion occurs because of concentration differences whereas between phases quite different concentrations are usually reached at equilibrium.

In 1901, G.N. Lewis introduced a simpler and more convenient criterion for equilibrium between phases; fugacity. , The term fugacity comes from the Latin root *fugere*, describing a "fleeing" or "escaping tendency" that a substance exerts from any given phase and is assigned the symbol f. Fugacity is equivalent to chemical activity or chemical potential and a difference in fugacity provides a driving force for net passive chemical transport from high to low fugacity (Mackay, 1982). In the atmosphere, fugacity of a compound of interest is usually equal to the partial pressure of the substance; therefore, fugacity has units of pressure (Pascals). Equilibrium is achieved between two phase when the "escaping tendency" from one phase matches with escaping tendency from the other phase. At equilibrium there may be transfer or exchange of solute between the two phases but the net rate of exchange is zero. For example, oxygen in water at 0.3 mol/m^3 (10 ppm) and in air at 8 mol/m^3 exerts the same fugacity or escaping tendency of 0.2 atm; thus equilibrium is established and there is no net movement of oxygen between air and water.

To investigate the environmental fate and toxicity of organic chemicals, it is often preferable to measure the chemical's fugacity rather than its concentration (Wilcockson and Gobas, 2001). The reason for expressing the model in terms of fugacity is that net passive transport of a chemical between different media occurs in response to the fugacity, not concentration, difference between the media (Gobas et al., 1999; Wilcockson and Gobas, 2001). Although fugacity is proportional to concentration, it is important not to confuse fugacity with diffusion as concentration gradient is the sole determinant of diffusion only within a single matrix type. In the environment, it is common to have chemical movement between compartments with vastly different physical properties (Mackay and Paterson, 1981) and therefore fugacity gradients exist.

A high fugacity or active concentration means that more substance is available for movement across membranes and therefore, may exhibit greater bioavailability.

To understand fugacity on a conceptual basis, consider a system with two adjacent compartments: oil and water containing an equal concentration of a PAH in each compartment. Although the concentration gradient would be zero, a fugacity gradient exists which causes movement of PAH from the water to the oil compartment. This movement happens because oil and water exhibits different ability to 'hold on to' the hydrophobic PAH contaminant. Thus, fugacity (f) is a function of both concentration (C) and absorbing capacity of the matrix, referred to as fugacity capacity, (Z).

$$C = fZ$$

Where C is concentration (mol/m^3); f is fugacity (Pa); and Z is fugacity capacity ($mol.m^3/Pa$).

In organisms, hydrophobic organics tend to partition into lipid phases because Z of lipids is much greater than the Z of other compartments (aqueous, protein etc). If there are two phases, then equilibrium of a substance will be reached when the fugacities are equal, that is:

$$f_1 = f_2$$

Thus:

$$C_1/Z_1 = C_2/Z_2$$

Or

$$C_1/C_2 = Z_1/Z_2 = K_{12}$$

The dimensionless partition coefficient controlling the distribution of the substance between the two phases (K_{12}) is merely the ratio of the fugacity capacities (Mackay and Paterson, 1981).

Fugacity capacity (Z) quantifies the capacity of the phase to hold a chemical. At a given fugacity, if Z is low, then even a small amount of substance will exhibit a tendency to leave that phase. Toxic substances thus tend to accumulate in phases where Z is high, or high concentrations can be reached without creating high fugacities (Mackay and Paterson, 1981). For example, the Z for oxygen in water at room temperature is 1.5 mol/ m^3. atm and in air is 40 mol/ m^3.atm, a ratio of about 27. Oxygen thus adopts a concentration in air 27 times that in water. Therefore, if we can determine Z of a substance for each environmental phase, we can calculate how the substance will partition among the various compartments.

The fugacity approach is a useful tool, which can contribute to a better understanding of the fate of toxic substances. If concentration data are available for a pollutant in several phases, presumably as a result of a monitoring program and if the respective Z values are known, the concentration data can be converted to fugacities and the fugacity levels can be compared (Mackay, 1979).

Gastro-Intestinal Digestion and Fugacity

The main transport mechanism for GI absorption of hydrophobic compounds is passive diffusion (Leib and Stein, 1986). Hydrophobic organic contaminants (HOCs) readily pass through biological membranes due to their lipophilicity. Food absorption and digestion can act as "fugacity pump" by raising the fugacity or activity of the chemical in the gastro-intestinal tract (GIT) compared to the consumed food (Gobas et al., 1993). As food is digested, the fugacity capacity tends to decrease leading to an

increase in the fugacity of contaminant which is largely due to the absorption of lipids and possibly other organic matter from the food in the GIT during digestion. If chemical absorption is at a low rate or if the food is absorbed but the chemical is not absorbed, then food absorption will result in a higher concentration and fugacity. Food absorption is expected to magnify the chemical concentration in the food, hence raising the chemical fugacity in the GIT above that in the food (Gobas et al., 1988).

The chemical properties of a substance are also known to play an important role in partitioning to various matrices. Organisms often rapidly eliminate polar substances with low Kow, and accumulation cannot occur despite an effective uptake from the digestive system. If the diet is poorly digested fugacity of a chemical may be raised whereas it may be dropped if the diet is more easily digested (Gobas et al., 1999; Weber and Lanno, 2001). Weber and Lanno (2001) observed absorption of a constant proportion of ^{3}H-BaP regardless of the concentration in the incubation solution, which supports the theory that uptake of dietary lipophilic contaminants is fugacity dependent. They also observed reduced BaP absorption when excess lipids in the form of monoglycerides and fatty acids were added, which increased the fugacity capacity of the incubation solution and facilitated the retention of BaP in the incubation medium. Generally, the fugacity of HOCs is expected to increase during digestion. Several studies have suggested that digestive fluid extraction is a fugacity-driven partitioning process and the proportion of HOCs solubilized by digestive fluid were dependent on K_{ow} of the compounds (Mayer et al., 1996; Miller et al., 1995 and Weston et al., 1998a).

Approaches to Measure Bioavailability

Considerable evidence now exists to show that the vigorous extraction techniques that are currently used to estimate the concentration of organic pollutants in soil appreciably overestimate the levels that are actually bioavailable (Alexander, 2000).

It is evident that not all of the compounds removed from soil by these extraction procedures are bioavailable to be assimilated or have a biological effect. This has been demonstrated by studies with dieldrin and DDT on insects (Robertson and Alexander, 1998), by the availability of DDT, DDE and DDD and dieldrin to earthworms (Morrison et al., 2000), by the biodegradability of 1,2-dibromoethane (Steinberg et al, 1987), by the genotoxicity of several carcinogenic PAHs (Alexander and Alexander, 1999), and by the availability of PAHs to microorganisms (Weissenfels et al., 1992; Erickson et al, 1993; Tang et al., 1998). Because the percentage of an organic toxicant that is bioavailable depends on the properties of the particular compound, the soil (White et al., 1997; Robertson and Alexander, 1998), the persistence time (Chung and Alexander, 1998), and probably the organism (Kelsey et al., 1997; Alexander, 2000), no single percentage availability can be established. This indicates the need for an assay for toxicants in soils that estimates the bioavailable fraction rather than the total concentration.

A number of chemical and physical procedures have been proposed for assessing the bioavailability of organic compounds in soils, and the results of those assays show correlations with bioavailability of the test compounds to invertebrate animals and microorganisms (Cornelissen et al., 1997; Kelsey et al, 1997; Tang et al., 1999). Chemical methods that have been proposed include: pore water analysis (Mayer et al., 2000), solid-phase extractants such as C18 membranes (Tang et al., 1999; Leslie et al,. 2002; Ramos et al., 1998) and Tenax beads (Steinberg et al., 1987), organic solvents such as tetrahydrofuran (Tang et al., 1999) and alcohols (Kelsey et al., 1997; Liste and Alexander, 2002), high temperature desorption and desorption kinetics (Cornelissen et al, 1997, 1998) and equilibrium partitioning (Ma et al., 1998). Furthermore, use of artificial lipophilic material (lipid containing semi-permeable membrane device or solvent filled dialysis membrane) to estimate bioaccumulation and

bioavailability of HOC in natural waters has been proposed (Sodergren, 1987; Huckins et al., 1993).

The two biomimetic approaches that have commonly been used to estimate the partitioning of contaminants between the pore water and the organism are solid phase micro-extraction (SPME) and C18-disks. Both biomimetic approaches assume that the freely dissolved concentration will represent the bioavailable fraction. However, for substances that may be biotransformed in the organism, these methods overestimate the concentration in the biota, whereas for organisms that have additional routes of uptake (than exclusively via pore water), the biomimetic methods underestimate the concentration in the organism.

Thin-Film (EVA) Solid-Phase Extraction

Wilcockson and Gobas (2001) demonstrated that the thin film solid phase extraction technique could be applied to investigate the fugacity of semi volatile and poorly volatile organic chemicals in air, water, sediments, and soils; and may provide a better measure of the bioavailability of contaminants in different media than the total concentration. They suggested that, it would be beneficial to use solid phases that exhibit quick absorption kinetics, resulting in chemical equilibria between the medium of interest and the solid phase after short application times. Fugacities in the environmental medium of interest can be measured indirectly using this technique. When the chemical substance in the medium of interest reaches equilibrium with the solid phase (ethylene vinyl acetate, EVA), the fugacities in the two media of would be equal. The fugacity of the chemical in the medium of interest can then be found from the concentration in the solid phase if the fugacity capacity is known.

Because the fugacity capacity of the EVA films is independent of the medium to which the films are applied, differences in chemical concentrations in the films are a direct measure of the difference in chemical fugacities between the media or sample to which the films are applied. When films of varying thickness were equilibrated with air, the amount of PCB accumulated increased with increasing thickness of the EVA, indicating that uptake was by absorption into the entire polymer matrix (Harner et al., 2000). Mayer et al. (2000) also showed that organic compounds are not adsorbed, but absorbed into the polydimethylsiloxane (PDMS) layer so that neither saturation nor competition between compounds will occur in the PDMS coating in aqueous solutions.

Oral bioavailability has commonly been measured using one or more of four different approaches. Toxicity is in part a function of bioavailability, but toxicity tests are complicated by a host of physiological and biochemical factors (e.g., detoxification mechanisms) that often make them unsuitable for comparative bioavailability measurements (Lee, 1991). The steady-state body burden, when expressed as ratio between the contaminant in the tissue and the surrounding media (i.e., sediment or water) is another measure of bioavailability, although they also are a function of contaminant biotransformation rates. In recent years, toxicokinetic measurements, specifically uptake clearance, have been advocated as an absolute measure of bioavailability (Landrum, 1989; Lee, 1991). This parameter represents the ratio of contaminant concentration in tissue normalized to the sediment concentration. Absorption efficiency, as determined by direct measurement or indirect estimation of contaminant loss between ingested material and faeces can be used as a measure of bioavailability, however, for many deposit feeding organisms, this approach was complicated, and sometimes compromised by particle-selective feeding behaviour (Lee, 1990).

The synthetic digestive fluid approach has the simplicity of a chemical extraction method and provides more environmental realism than is achieved by conventional chemical methods. Most digestive models are static gastrointestinal models, which simulate transit through the human digestive tract by sequential exposure of the soil to simulated mouth, gastric, and small intestinal conditions. Dynamic gastrointestinal models mimic gradual transit of ingested compounds through the digestive tract. More complex models can simulate more aspects of human physiology, but simple models are easier to use and allow simultaneous examination of a larger number of samples. The simplest approach to design an in vitro digestion model is the stomach models where mobilization of the contaminants from soil under gastric pH conditions is simulated (Oomen et al., 2000). Because >90% absorption occurs in the intestines (Duggan et al., 2002), stomach models may yield bioaccessibility values that are not representative for the site of absorption. Stomach models would give bioaccessibility values correctly only if gastric pH conditions were responsible for determining bioaccessibility.

Various studies have determined the oral bioavailability of contaminants from soil (Ruby et al., 1992; Ruby et al., 1993; Gasser et al., 1996; Hack and Selenka, 1996; Ruby et al., 1996; Van Schooten et al., 1997; Hamel et al., 1998; Maddaloni et al., 1998; Hamel et al., 1999; Ellickson et al., 2001). However, most of these studies were conducted using specific soil samples. As soil characteristics may affect oral bioavailability it is difficult to extrapolate the result of such studies to soil samples in general. Alternatively, bioaccessibility was investigated in order to obtain information on the influence of the soil matrix on oral bioavailability. In recent years, the number of studies on bioaccessibility has increased. Ruby et al. (1992) were one of the first to report on bioaccessibility of lead from soil using a model with simulated gastric conditions. The dissolved lead concentration in the stomach would provide an

overestimation of soluble lead, since complexation and precipitation is expected in the intestine where pH values are higher. A new approach to assessment of the bioavailability of ingested, particle-associated contaminants that employs the digestive fluid of deposit feeders to solubilize contaminants has recently been proposed (Mayer et al., 1996; Weston and Mayer, 1998). Weston and Mayer (1998) incubated contaminated sediments with the digestive fluid of a deposit-feeding organism in vitro and quantified the amount of solubilized contaminant. However, none of these studies represent human physiology.

For the past decade, physiologically-based tests have been investigated. Rotard et al. (1995) proposed an in vitro digestion model using synthetically-prepared digestive juices. Unlike previous models (Ruby et al., 1992; Gasser et al., 1996) this model covered the entire route from mouth to small intestine. Another sophisticated model was introduced by Minekus et al. (1995). This model employed gradual transit from one compartment to another and defined bioaccessibility as the fraction of test compound that diffused through hollow fibre membranes. This model by Minekus et al. (1995) was not validated for soil matrices, but was focussed on matrices like food and pharmaceuticals. Another physiologically-based extraction test (PBET) was introduced again by Ruby et al. (1996). This model was designed to simulate heavy metal uptake by the gastrointestinal tract of a child 1.5-3.5 years old, as this young age group is believed to be at the greatest risk of contaminant exposure from accidental soil ingestion. The PBET has been used to mimic fasting conditions, which produce the most soluble heavy metal concentrations due to low gastric pH values, and hence, the most conservative conditions for accessing bioaccessibility. Ruby et al. (1996) validated the in vitro results with animal models (rats, rabbits, and monkeys). The relevance of this validation is questionable since it is not clear whether these animal models

represent the human gastro-intestinal tract adequately. Major interspecies differences have been reported when comparison of gastrointestinal tract of humans, rats, rabbits and monkeys was done (Ruby et al., 1996).

At present there are no standard methods to determine bioaccessibility or bioavailability. As they become validated and more widely employed, in vitro digestion models will likely become an important tool available to the researcher conducting risk assessment of polluted sites, and especially for determining the urgency of remediation.

Purpose of the Study

The overall aim of this project was to develop a tool for estimating the oral bioavailability of hydrophobic contaminants from soil, especially 125-250 μm size soil particles as this fraction of soil adhere more effectively to surfaces or hands than coarse particles. The specific aims of this project were:

- To establish a model digestive tract using synthetic gastric and intestinal fluids based on known pH values, components and human transit time for fasting conditions in children. Ethylene vinyl acetate (EVA), a model intestinal sorptive surface was employed.
- To compare EVA to the human intestinal epithelium, Caco-2 cell line.
- To measure the mobilization of the PAH, chrysene, a hydrophobic chemical, and glyphosate, a hydrophilic herbicide, from soil and to determine the distribution of these contaminants among different compartments of the model digestive system.
- To determine the effects of ageing of the contaminant onto soil on the partitioning of the contaminant into various compartments.

- To do a kinetic analysis of the data generated to determine the rate constants for partitioning into various compartments, and the percent bioavailability in vitro.
- To calculate the fugacity of the contaminant under various digestive conditions and to compare the fugacity capacities of different phases of the model.

CHAPTER TWO:
MATERIALS AND METHODS

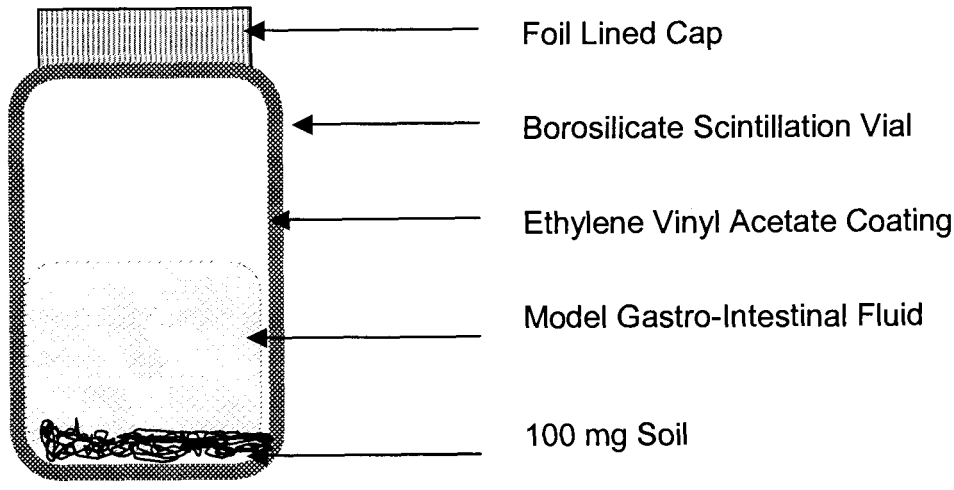
Experimental Approach

The Ethylene Vinyl Acetate (EVA) model introduced by Wilcockson and Gobas (2001) and composition of artificial digestive fluids from Hack and Selenka (1996) and Ruby et al. (1993) served as the starting point of this in vitro gastrointestinal model. The composition of digestive fluids, temperature, and transit times were based on human physiology. The chrysene contamination values used in our experiments were based on concentrations found in locally contaminated sites (Launen et al., 1995). Figure 4 is a schematic of our gastro-intestinal model. Briefly, soils were contaminated with ^{14}C-contaminant 3 days before the experiment. The spiked soils were incubated in the EVA-coated glass scintillation vials with the model gastric fluid followed by intestinal fluid (composition of fluids is given below). The amount of ^{14}C was monitored over time in the aqueous compartment and the EVA film. The schematic of the experimental design is shown in Figure 5.

Gastrointestinal Compartments

The in vitro digestive model employed in this study simulated the stomach and the small intestine as these are the two compartments of the digestive tract that are most important in determining mobilized fraction of a contaminant. Most of the digestion and more than 90% absorption takes place in the small intestine (Duggan et al., 2002). The intestinal epithelium was mimicked by the EVA coating of the vial, and thus the contaminant fraction that was mobilized from the soil into the digestive juices and absorbed by EVA film represented the bioavailable or mobilized fraction.

Figure 4: Gastro-intestinal digestion model



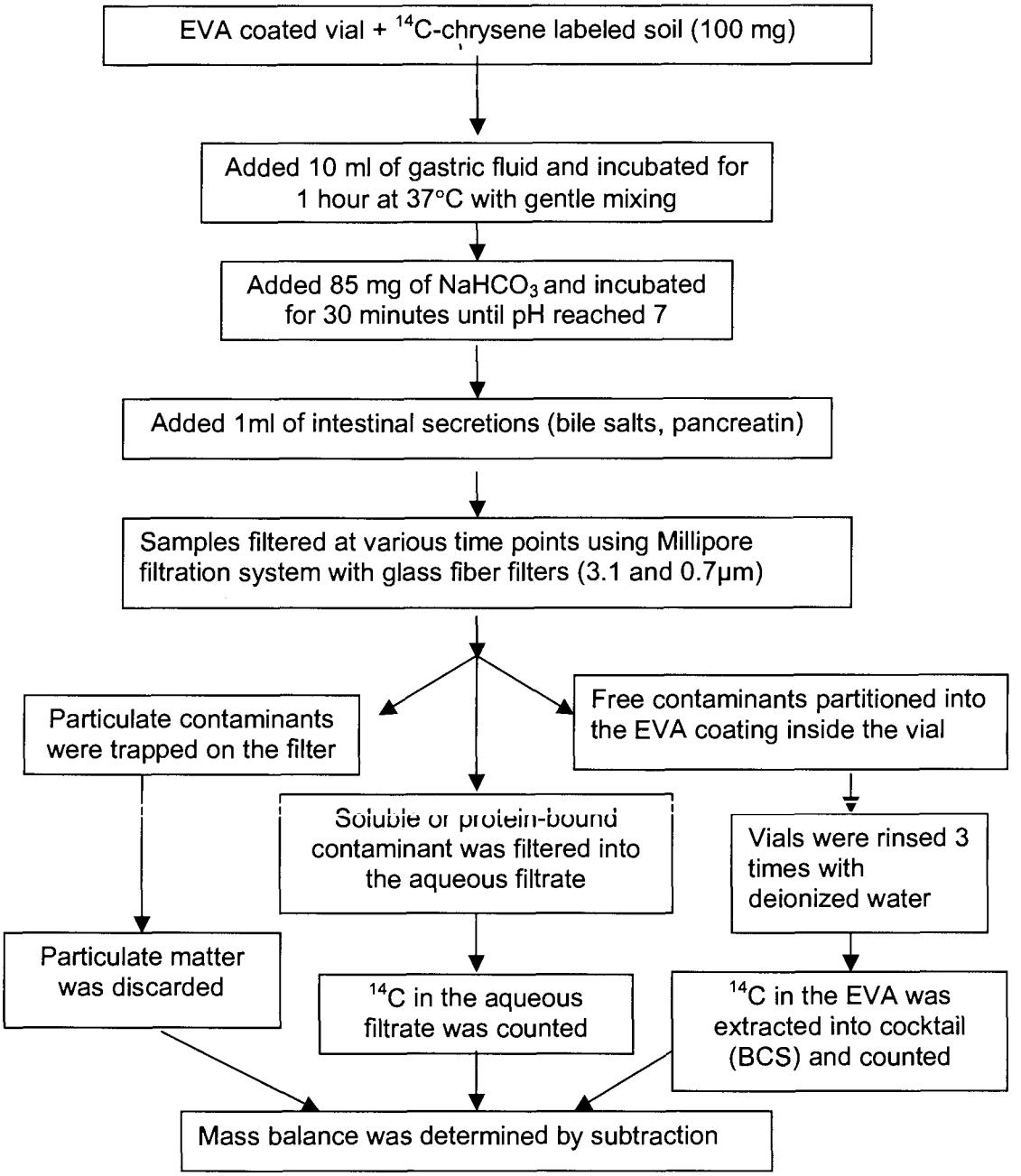


Figure 5: Schematic of experimental procedure for studying partitioning and mobilization of contaminants from soil into artificial digestive system

Model Gastric and Intestinal Fluid Composition

The composition of synthetic gastric and intestinal fluids was taken from studies done by Ruby et al. (1993), Hack and Selenka (1996) and Rodriguez and Basta (1999). Synthetic gastric fluid was prepared by dissolving 1.7 mM sodium citrate (Analar, BDH Inc. Toronto, Canada), 2.8 mM malic acid, 4.84 mM lactic acid syrup (both from Sigma Chemicals Co. St. Louis, MO, USA.) and 8.32 mM glacial acetic acid (Anachemia Canada Inc. Van., Canada) in deionized water. The solution was adjusted to pH 2.5 with 0.18 M hydrochloric acid (HCl) and autoclaved in 200 ml aliquots. To one 200 ml bottle of gastric fluid 0.13 mM pepsin (Sigma Chemicals Co. St. Louis, MO, USA) was added just prior to use (Hack and Selenka, 1996).

Transition from the stomach to the small intestine was modelled by neutralizing the pH of the solution to pH 7 by adding 1.01 mM sodium bicarbonate ($NaHCO_3$). Once neutralized, 1ml of freshly prepared synthetic intestinal secretion was added. Intestinal fluid (10 ml) contained 40 mg bile salts, 3 mg pancreatin (EM Science, Darmstadt, Germany), 10 mM calcium dichloride dihydrate, 40 mM Tris (Hydroxymethyl) aminomethane, pH 7 (Caledon Laboratories Ltd. Ont. Canada) and 4 ml of deionized water.

Solid to Fluid Ratio

In our model, the soil-to-fluid ratio was 0.1g soil : 10 ml gastric fluid (1:100) for first 1.5 hours and 0.1g soil : 11 ml intestinal fluid (1:110) subsequently. Soil-to-fluid ratio in the range of 1:100 to 1:5000 (g:ml) is assumed to cover all physiological conditions in vivo (Hamel et al., 1998). This ratio was selected to give bioaccessibility values representative of the in vivo situation. The absolute volumes of the digestive juices were scaled for practical purposes to fit 20 ml vials.

Thin Film Preparation

EVA was used to coat the inside of the vials. Various grades of EVA are available which contain 2-50% vinyl acetate (VA). We used pelleted Elvax 40W (gift of Dupont Canada Inc, Ontario) which contained 40% VA and had a density of 0.965 g/ml. This grade of EVA is known to have longer polymer chain, reduced hardness and increased solvent solubility than EVA with lower vinyl concentrations. Furthermore, high VA polymers have better resistance to aqueous and high salt environments. The product is designed for commercial use, and was not pure; it contained butylated hydroxytoluene to improve viscosity properties during processing and an amide wax to allow pellet flow.

Glass, 20 ml scintillation vials were washed with laboratory grade detergent, rinsed with deionized water and then with acetone. Dry vials were rinsed with dichloromethane (DCM) and 250 µl of 0.6% EVA in DCM and 5 µl of 2% dichlorodimethylsilane (Silane)(Aldrich Chemical Co., WI, USA) solution in hexane was added to each vial. (Without silane addition the EVA film pulled away from the glass vial after a few hours of incubation.) The vials were then manually rolled until all DCM evaporated leaving a thin film over the entire inner surface of the vial. Vials were left uncapped to allow drying. The inside surface area of the vial was 3412.5 mm^2 and the thickness of the coating was estimated to be an average of 0.45 µm.

Model Compounds

Chrysene

Chrysene ($C_{18}H_{12}$) is a hydrophobic organic contaminant with a log Kow of 5.8. It is a model for high molecular weight PAHs like benzo[a]pyrene. It occurs ubiquitously and in about the same concentration as benzo[a]pyrene in products of incomplete

combustion (IARC, 1983). In addition, chrysene is present in higher concentrations than most of the other polynuclear aromatic hydrocarbons in fossil fuels such as crude oil and lignite. It is formed when gasoline, garbage, or any animal or plant material burns, therefore, it is usually found in smoke, soot and in creosote, a chemical used to preserve wood (Luster-Teasley et al., 2002). There is no commercial production or known use for this compound. The general population may be exposed to chrysene in anthropogenic combustion emissions, from dust particles carried to water, soil and crops, cigarette smoke, and coal/fire cooked food. As a pure substance it is white powder. Chrysene is identified as a weak carcinogen that promotes skin, liver and lung carcinomas (Luster-Teasley et al., 2002). For the structure of chrysene see Figure 6.

Glyphosate

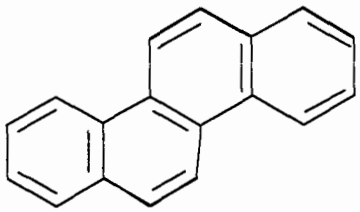
Glyphosate [N-(phosphonomethyl)-glycine] ($C_3H_8NO_5P$), is a polar compound with log Kow of - 3.2 . Glyphosate was chosen as a model polar compound to test our model. It is a weak organic acid consisting of deprotonated acid of glyphosate and a cation, e.g. isopropylamine salt or trimethylsulfonium salt (Daruich et al., 2001). Pure glyphosate is a colorless crystalline compound at room temperature. The high polarity of glyphosate molecule makes it practically insoluble in organic solvents but it is completely soluble in water. It is readily degraded over time by soil microbes into natural substances such as carbon dioxide and phosphonic acid (Williams et al., 2000). It is moderately persistent in soil, with an estimated average half-life of 47 days. It is strongly adsorbed to most soils, even those with lower organic and clay content (Williams et al., 2000).

Glyphosate is the active ingredient in Roundup® herbicide. It is a broad spectrum, post-emergent, non-selective systemic herbicide that is used in both agricultural and non-agricultural areas all over the world. It is used to control grasses,

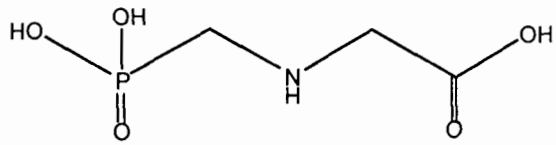
herbaceous plants including deep-rooted perennial weeds, broad-leaved weeds, shrubs, and some conifers (WHO, 1994). It is absorbed by foliage and translocated through the plant. It acts by inhibiting the pathway for biosynthesis of essential amino acids (phenylanine, tyrosine and tryptophan), which reduce the production of protein in the plant, and inhibits plant growth. This pathway is only present in plants and micro-organisms; thus glyphosate is considered non-toxic to mammals (Adam et al., 1997; Williams et al., 2000). Animal studies have shown that glyphosate is not carcinogenic, mutagenic or teratogenic (WHO, 1994).

Radioisotopes

To produce a test system with the highest sensitivity, radiolabeled chrysene and glyphosate were used to study the kinetics of partitioning. ^{14}C-glyphosate was obtained from Sigma Aldrich Canada (specific activity: 7.4 mCi/mmol). ^{14}C-chrysene was obtained from Chemsyn Science Laboratories, Lenexa, Kansas (specific activity: 46.8 mCi/mmol). All radioactive samples were counted in Biodegradable Counting Scintillant (Amersham Canada, Oakville, ON) on a Beckman LS 6500 multi-purpose liquid scintillation counter with automatic quench correction (Beckman, Fullerton, CA, USA).



Chrysene



Glyphosate

Figure 6: The structures of chrysene and glyphosate

Table 3: A comparison of the physico-chemical properties of chrysene and glyphosate (Mackay et al., 1992)

Properties	Chrysene	Glyphosate
Molecular Weight	228.3	169.09
Melting Point (°C)	256	100
Boiling Point (°C)	488	n.a.
Water Solubility (mg/L)	0.0015	12000
Vapor Pressure (Pascals at 25°C)	5.70 E-07	1.31E-05
Henry's Law Constant (Pam^3/mol)	0.106	5.7E-11
Log Kow	5.81	-3.2
Half Life in soil (days)	5.5 - 387	47

Soil Matrix: Characterization of Model Soil

The model soil was obtained from Burnaby Mountain at SFU and was collected on May 10, 2001. Topsoil was collected at 0-5 cm and the subsoil was from 1-20 cm deep. The organic content of the topsoil was 38.1% and its pH in water was 3.4. The organic content of the subsoil was 15.2% and its pH in water was 3.8. Soils were stored in the dark at 4°C. Soils were fractionated into various particles sizes by Oana Gheorghiu using different sizes of sieves that fit into a mechanical shaker. In this study 125-250 µm soil fraction of sub soil was employed as it is more likely to adhere to the skin and be transferred by hand-to-mouth activity of children (Rodriguez et al., 1999). The particles of the test soil in this study were of a size that optimised dissolution rate and the extent of absorption and were representative of the reported soil particle size values for human exposure (Oomen et al., 2003).

Labelling Soil with ^{14}C-chrysene and ^{14}C-glyphosate

100 mg of oven-dried soil was put into 20 ml borosilicate scintillation vials. 40 µl of toluene containing approximately 100,000 dpm of ^{14}C labelled-chrysene and 1.78 µg of cold chrysene was pipetted onto soil contained in each vial. For glyphosate experiments, 40 µl of methanol and water solution containing approximately 100,000 dpm of ^{14}C labelled-glyphosate and 1 µg of cold glyphosate was pipetted onto the soil. The solvent was allowed to evaporate at ambient temperature. The soils were kept at room temperature for 3 days after spiking.

Mixing

Mixing was accomplished by using a rotator in a hybridization oven (Hybaid Midi Model) at 37°C. This rotator was designed to hold 30 vials horizontally and rotated in cyclic manner (6 rotations/minute) enabling the contents of the vial to rotate inside the

vials in such a way that digestive contents of the vial made contact with the entire inner coated surface of the vial. For experiments with Caco-2 cells and comparative EVA coated coverslips, mixing was done on an orbital shaker at 100 rpm.

Digestion Procedure

For each experimental sample, 10 ml of gastric fluid was added to an EVA coated vial and incubated with 100 mg of soil contaminated with ^{14}C labelled compound for 1 hour at 37°C with gentle rotation in the hybridization oven. Transition from the stomach to the small intestine was modelled by neutralizing the pH of the solution to pH 7 by adding 85 mg sodium bicarbonate ($NaHCO_3$) to the vial with soil and gastric fluid. Once neutralized, 1ml of intestinal secretion was added. Sampling was done at various time points by sampling 3 vials during 48-72 hours of incubation.

^{14}C - Measurement of the Soil, Aqueous and EVA Compartments

Contents of tripiicate sampies (mixture of contaminated soii and gastrointestinai fluids) were filtered through a Millipore filtration system using 3.1 µm and 0.7 µm pore size glass microfiber filters (Ahlstrom, PA, USA). A vacuum pump was used to draw fluids into side arm flasks. Aqueous filtrate (2 ml) was sampled for each of the triplicate samples. BCS cocktail was added to the aqueous samples and was counted in the liquid scintillation counter. Deionized water was used to rinse the coated vials. BCS cocktail (10ml) was added to the rinsed EVA-coated vial that was agitated on their sides on shaker to extract the ^{14}C bound to EVA into the cocktail. The Millipore apparatus was washed with soapy water and then rinsed with acetone between filtrations. For some experiments, filters were transferred to labelled pieces of aluminium foil and extracted to obtain a mass balance. Attempts were made to extract the remaining ^{14}C bound to soil residues by both solvent extraction and high temperature oxidation and trapping of $^{14}CO_2$

(Harvey Biological Oxidizer). However, 100% recovery was not possible because the glass fiber filters melted at the high temperature required to release $^{14}CO_2$, thereby trapping soil within and reducing the efficiency of ^{14}C-release. Solvent extraction was found to be variable in efficiency and therefore unreliable. Therefore, the soil-bound portion of chrysene or glyphosate reported here was determined by subtraction.

Effect of altering the composition of the gastrointestinal components on chrysene partitioning

Effects of Gastro-Intestinal Constituents

Experiments were conducted to see the effect of altering the gastrointestinal fluid composition on chrysene partitioning. The procedures used were the same as described in previous sections except that one of the gastro-intestinal constituents was withheld in each experiment. A series of experiments were done in which no pepsin, boiled pepsin, no intestinal secretions or no sodium bicarbonate were added to the gastrointestinal fluid. Experiments were also done with water only (no gastro-intestinal component).

Effects of aging ^{14}C contaminant onto soil

Experiments were conducted with ^{14}C-chrysene contaminated soil that had been aged for two different periods: 6 months and 1 year. The same procedure as described in earlier sections was followed.

Determination of the volume of organic matter in experimental soil and organic matter digested during digestion process

Falcon tubes (15), each containing 400 mg soil were incubated with gastro-intestinal fluids at 37°C for 48 hours. Tubes with digested soil solutions were centrifuged at 3000g for 7 minutes and the supernatant was decanted. The soil was resuspended in

30 ml deionised water and centrifuged (twice) at 3000g at room temperature. Soil pellets were obtained by aspirating the remaining fluid and drying in the oven at 60°C for a week. Soils from groups of five falcon tubes were collected in three oven-dried pre-weighed crucibles and weighed. The volume of the dried digested soil was determined by displacement. Soils were again dried in oven and then ashed in muffle furnace (Thermolyne, 48000 Muffle furnace) at 350°C for one hour and 550°C for 16 hours. The ashed soils in crucibles were weighed again and the volume of inorganic soil was determined by displacement. The difference between the volume of oven dried soil and volume of ashed soil gave the volume of organic matter. The difference between the volume of original oven dried soil and volume of oven dried digested soil gave the volume of digested organic matter.

Comparing EVA with Cultured Human Intestinal Cells (Caco-2)

Coverslips were rinsed with 40 µl of DCM and then coated with 40 µl of EVA solution, which contained 0.6% EVA and 0.04% silane. Custom made borosilicate mini beakers were placed in tissue culture plates with 12 wells (Becton Dickinson and Company, Franklin Lakes, NJ, USA). Sterlized, acid-washed glass beads, 3 mm in size (Fisher Scientific) were added to each of the dishes. ^{14}C-Chrysene solution in 100% ethanol was added to phosphate buffered saline (PBS) containing 0.05% Tween 20 to give ~100,000 dpm/ml and 1 ml of this solution was added to each of the mini dishes. PBS-tween was used instead of PBS as tween enhances solubilization and reduces the time taken to equilibrate. EVA coated coverslips were placed upside down in the dishes containing contaminated PBS solution because the hydrophobic surface of coated coverslips prevented complete wetting of the surface if left facing up. The tissue culture plates holding custom made mini beakers were put on a Fisher Scientific orbital shaker at 70 rpm in incubator at 37°C. At specific time intervals duplicate samples were

removed. EVA coated coverslips were rinsed in deionized water, extracted in BCS and ^{14}C bound to EVA was counted.

Caco-2 Cells

The Caco-2 epithelial cell line, derived from a human colorectal adenocarcinoma, has become an established in vitro tool for studies related to intestinal cell function and differentiation. When cultured on semi-permeable membranes, Caco-2 cells differentiate into a highly functionalized epithelial barrier with remarkable morphological and biochemical similarity to the mature intestinal columnar epithelial cells such as enterocytes or mucus cells. The membrane transport properties of novel compounds can thereby be assessed using these differentiated cell monolayers. The apparent permeability coefficients obtained from Caco-2 cell transport studies have been shown to correlate to human intestinal absorption (Irvine et al., 1999; Artursson, 1990).

The Caco-2 cell line, undergoes in culture a process of spontaneous differentiation leading, in two to three weeks after confluency, to the formation of a monolayer of highly polarized cells, joined by functional tight junctions with well developed and organized microvilli on the apical membrane and expressing many enzyme activities (disaccharidases and peptidases) and transport proteins typical of the small intestinal absorptive enterocyte. In recent years, Caco-2 cells have widely been used to study trans-epithelial transport and cytotoxicity of different nutrients and xenobiotics (Ismail et al., 1999; Oomen et al., 2001; Hilgers et al., 2003). We used the Caco-2 epithelial cell line to estimate uptake of contaminants and to compare its fugacity capacity with that of EVA. Our model did not include transport studies.

Cell Culture

Caco-2 cells were grown and maintained by Luba Vasiluk, a doctoral student in the Moore Laboratory.

The human colorectal carcinoma cell line Caco-2 was obtained from American Type Culture Collection (ATCC catalog No. HTB-37). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with high glucose (4.5 g/l) supplemented with 10% fetal bovine serum, 1% non-essential amino acids, penicillin (25 IU/ml) and streptomycin (100 µg/ml) at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. Cells were used between passage 30-90. For absorption/partitioning experiments, cells (1-2 x 10^5 cells/well) were seeded on glass cover slips in a 24-well plate. Cells used were incubated for three weeks and used between 21 and 26 days after seeding.

Cell Fixation

Cover slips with cells were fixed in 4% (w/v) paraformaldehyde in PBS, pH 7.4 for 30 min, at room temperature. The growth medium was aspirated and 300 µl of 4% paraformaldehyde in PBS at 37°C and was added to each well. Cells were allowed to fix for 30 minutes at room temperature and the paraformaldehyde solution was aspirated. Cells were then gently washed with PBS using a squirt bottle and the liquid was aspirated. Caco-2 cells were kept covered in PBS until used for the experiment.

^{14}C-Chrysene Partitioning to Caco-2 Cells

Acid washed mini glass beakers were placed in tissue 12-well plates. Glass beads were added to each of the dishes to support the coverslips. Chrysene solution (approximately 100,000 dpm) in 100% ethanol was added to PBS-tween and 1ml of this solution was added to each of the beakers. Coverslips with fixed Caco-2 cells were

placed in the dishes containing the PBS-chrysene solution. The tissue culture plates with mini dishes were put on a Fisher Scientific orbital shaker at 70 rpm in an incubator at 37°C. At specific time intervals, duplicate samples (coverslips with cells) were removed. At the end of the experiment, coverslips with fixed Caco-2 cells were rinsed in deionized water, extracted in BCS and ^{14}C bound to cells was counted in a scintillation counter.

Determination of Lipid Content and Volume of Lipid in Caco-2 Cells

This section of research was performed by Linda Pinto and Luba Vasiluk of Moore Lab.

Cell Culture

Caco-2 cells were seeded into 5 tissue culture flasks with an area of 75 cm^2 each and grown in DMEM (Dulbecco's minimal essential medium, Gibco) + 10% fetal bovine serum (Gibco) in a 5% CO_2 atmosphere. The media over the cells was changed 3 times per week. Cells were grown for 10 days and were confluent at time of harvest.

Harvest

The medium was removed, the cells washed once with PBS, which was removed and then 2 ml/flask of 0.25% trypsin (in EDTA) was added. The cells were incubated 20 minutes at 37° to allow the trypsin to dissolve the intercellular proteins. Fresh medium was added to stop enzymatic digestion, the cells were pipetted vigorously to complete the breakup, then centrifuged 400g for 4 min.

The pellet was washed once with PBS and then transferred to 2 pre-weighed microcentrifuge tubes. The tubes were spun down briefly and liquid pipetted off. The total volume of cells was estimated and the cells were dried by lyophilization.

Lipid Extraction

Lipids can be extracted using a buffer/solvent mixture which forms one layer during the extraction, but is then modified to form two liquid layers, so that the lipid is dissolved in the organic solvent. This method uses: methanol: chloroform: phosphate buffered saline 2:1:0.8 (10:5:4) or in miniature: 500 µl : 250 µl : 200 µl. Following is the method for about 100 mg wet weight cells.

Microcentrifuge tubes and glass vials were dried to constant weight, and accurate weights were obtained on analytical balance. The cells were transferred to a pre-weighed microcentrifuge tube, lyophilized and re-weighed to get the dry weight of the cells. The pellet was resuspend in 200 µl PBS. This step took a few hours, but the cells could not be resuspended at all in the extraction buffer. Methanol (500 µl) and chloroform (250 µl) were added, the tube was vortexed and allowed to extract for 2 - 3 hours, vortexing from time to time. The mixture was centrifuged to separate solids from extraction buffer and the supernatant was transferred to a 15-ml Falcon tube. 100 µl PBS, 250 µl methanol and 125 µl chloroform was added to the pellet and was vortexed and centrifuged. Supernatant from this step was added to the supernatant from previous step. The pellet was discarded. To combined supernatants, 900 µl PBS and 900 µl chloroform was added and then vortexed or centrifuged to form two layers. Bottom (chloroform) layer was removed to pre-weighed glass vial and was extracted twice using 1-ml chloroform each time. Combined chloroform layers were dried under nitrogen at 40°C and the vial was reweighed.

Measurement of the Volume of Lipid.

If the relationship between the weight of the lipids and the volume of the lipids can be approximated, the volume is calculated by measuring the weight. The density of phospholipids is 1.0 (Dr. Cornell, personal communication) and that they constitute about 2/3 of the lipid in a non-adipose cell. The final 1/3 should be fatty acids, which according to Dr. Cornell has a density (in humans) of 0.95 or ~1.0. We therefore assumed that within our ability to extract and weigh the lipid, 1 mg lipid = 1 μl of lipid. Percent lipid is calculated as (mg lipid/mg dry cells) x 100.

Experiments with Glyphosate

Experiments were conducted with glyphosate to study its partitioning and kinetics using model digestive system. The same procedures described in previous sections were followed.

Fugacity Calculations

Fugacity Capacity of EVA

$$K_{oa} = K_{ow} / K_{aw} \quad OR \quad K_{oa} = K_{ow} \times RT / H \qquad (1)$$

Because, $K_{aw} = Z_a / Z_w$ or H / RT as $Z_a = 1/RT$ and $Z_w = 1/H$

Where K_{oa} is octanol-air partitioning coefficient, K_{ow} is octanol-air partitioning coefficent, K_{aw} is air-water partitioning coefficient, Z_a is fugacity capacity of air, Z_w is fugacity capacity of water, H is Henry's law constant, T is temperature and R is the gas constant.

$K_{oa} = C_o / C_a$ at equilibrium and $C = f Z$

Where C_o is concentration in octanol, C_a is concentration in air, C is concentration, f is fugacity and Z is fugacity capacity.

Therefore, $K_{oa} = f_o Z_o / f_a Z_a$

Where f_o is fugacity in octanol, f_a is fugacity in air, Z_o is fugacity capacity in octanol and Z_a is fugacity capacity in air. At equilibrium, fugacities are equal, therefore,

$f_a = f_o$ OR $K_{oa} = Z_o / Z_a$

$Z_a = 1 / RT$ where R is the gas constant and T is temperature

So, $Z_o = K_{oa} / RT$ (2)

$Z_e = K_{oa} \times 3.8 / RT$ (Otton, 2003 by personal communication) (3)

By substituting the value of K_{oa} from Equation 1 in Equation 3

$Z_e = K_{ow} \times 3.8 / H$ (4)

Once the fugacity capacity (Z) of EVA is known and concentration (C) in EVA is known from experiments, the fugacity (f) at equilibrium can be calculated. Because fugacity is equal in all compartments at equilibrium, the fugacity in the EVA compartment will be equal to fugacity in all the other compartments. The concentration of contaminant in each compartment is known from experiments, the fugacity capacity (Z) can be calculated by dividing concentration in a particular medium (which is calculated by dividing number of moles by volume) by fugacity at equilibrium.

Fugacity Capacity of Biota (Caco-2 cells)

Z biota = øL x K_{ow} / H (Mackay, 1991)

Where øL is fraction of lipid in the cells, K_{ow} is octanol-air partitioning coefficient and H is Henry's Law constant

Data Handling and Statistical Analysis

The uptake of chemicals in EVA and Caco-2 cells was plotted. The mobilized fraction of chemical was presented as percentage of contaminant that was added to the soil at the beginning of the experiment. The mobilized fraction was calculated as shown below:

$$\text{Mobilized Fraction (\%)} = \frac{\text{contaminant determined in the gastrointestinal fluid} \times 100}{\text{contaminant present in the soil before digestion}}$$

Fugacity calculations were done using the formulas given in above section. The raw data (sampling times and concentrations) were fitted using a one-compartment model assuming first-order kinetics. The one compartment model was fit to desorption data using nonlinear regression technique with software designed to minimize the sum of squared residuals (Systat 8.0, SPSS Inc., 1998).

$$C = A (1 - \exp(-Kt))$$

Where C is the concentration in the thin film (mol/m^3), t is time (hours) and K is the rate constant ($hour^{-1}$) for uptake by EVA or desorption from soil.

Data was analyzed by one-way analysis of variance (ANOVA) test, followed by Tukey's multiple comparison procedure using JMP version 4.0. For each comparison, significant differences were identified at $p \leq 0.05$.

CHAPTER THREE:
RESULTS

Partitioning of Chrysene into Various Compartments of Digestive System

The objective of this research was to develop a method to measure the potential of a soil-bound hydrophobic contaminant to desorb into the gastrointestinal fluids and/or into the intestinal epithelium.

^{14}C-chrysene-contaminated soil was digested using in vitro digestion model. The in vitro test system consisted of a two-step model comprising an acidic gastric phase followed by a neutral or slightly alkaline intestinal phase. As a result of incubation with gastrointestinal fluids at 37°C, chrysene partitioned into the EVA coating of the vials (mimicking intestinal epithelium) and into the aqueous phase (gastrointestinal fluids). The extent of the mobilization of chrysene from the soil by means of synthetic digestive juices was compared with the total amount of the contaminant added to the soil sample. The fraction of the mobilized contaminant is given in percentage terms. Time course mobilization/partitioning experiments subjected to complete digestion process were repeated three times and each experiment was carried out in triplicate (Figures 7-9).

At the start of the experiment, all the chrysene was bound to the soil. The concentration in the soil dropped as the experiment progressed; and increased in the other compartments, namely, the aqueous compartment, and the EVA film. At time zero, no chrysene was detected in the gastric fluid, but within a few seconds ^{14}C-chrysene moved from the soil into the gastric fluid (Figures 7-9).

The first two experiments (Figure 7 and 8) were carried out for 24 hours. The uptake of chrysene by EVA continued for the entire duration of the experiment and did not reach equilibrium (Figure 7). Although the initial uptake into the EVA film was rapid, it slowed after 2 hours. In contrast, chrysene rapidly (3 hours) approached equilibrium in the aqueous phase at approximately 15 percent of added chrysene (Figure 7). After 8 hours, the concentration of chrysene started decreasing in the aqueous phase whereas uptake by the EVA film continued to rise. After 24 hours, 34% of ^{14}C chrysene had partitioned into the EVA film.

In the second experiment (Figure 8), the aqueous phase reached equilibrium after only 2 hours, and showed a more limited capacity to hold chrysene. Uptake into EVA continued and equilibrium was not reached even after 24 hours. A maximum of 2.5% of ^{14}C chrysene partitioned in the aqueous phase and 25% in the EVA phase.

The duration of third experiment was increased to 48 hours to attempt to reach equilibrium. As before, the aqueous phase reached equilibrium rapidly (after 2 hours). Uptake into the EVA film was rapid for first 8 hours and then partitioning occured at slower rate reaching a constant value after 32 hours. A maximum of 4% of chrysene partitioned into gastrointestinal fluids whereas 48% partitioned into the EVA film (Figure 9).

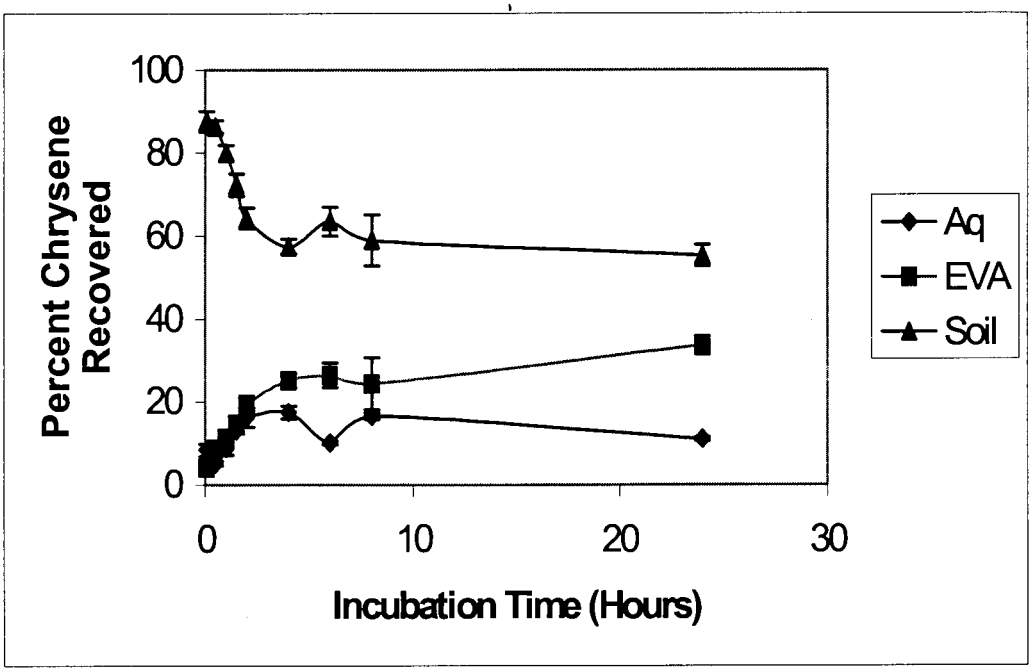


Figure 7: Partitioning of ^{14}C-chrysene from soil into aqueous (Aq) and EVA compartments of the in vitro digestive model when incubated for 24 hours at 37°C (Experiment 1).

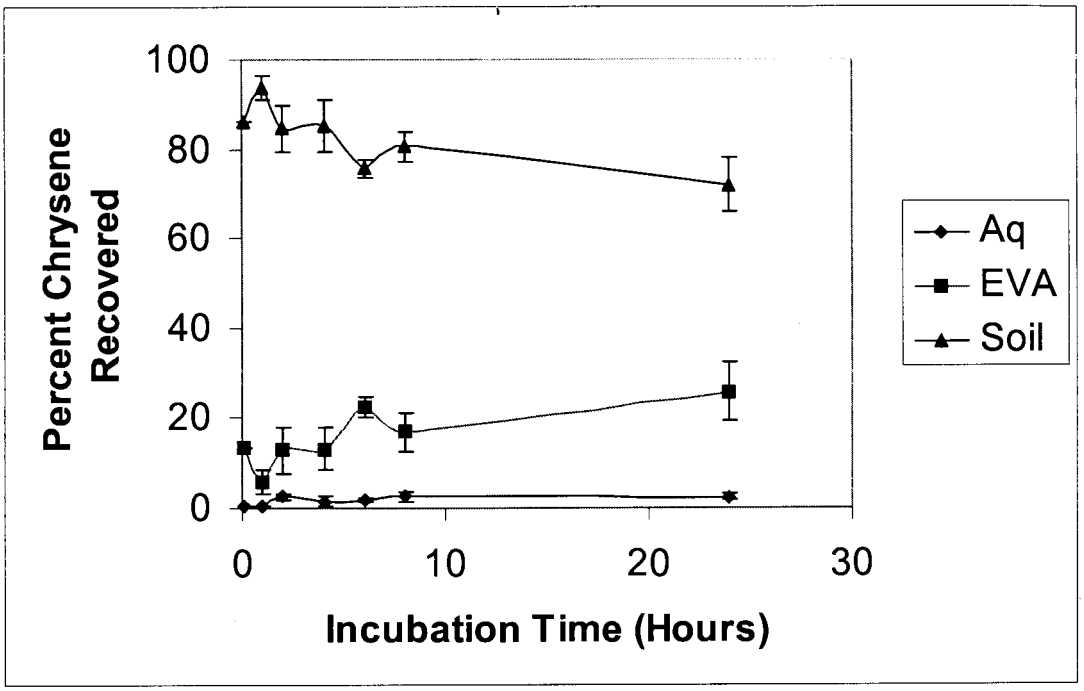


Figure 8: Partitioning of ^{14}C-chrysene from soil into aqueous (Aq) and EVA compartments of the in vitro digestive model when incubated for 24 hours at 37°C (Experiment 2).

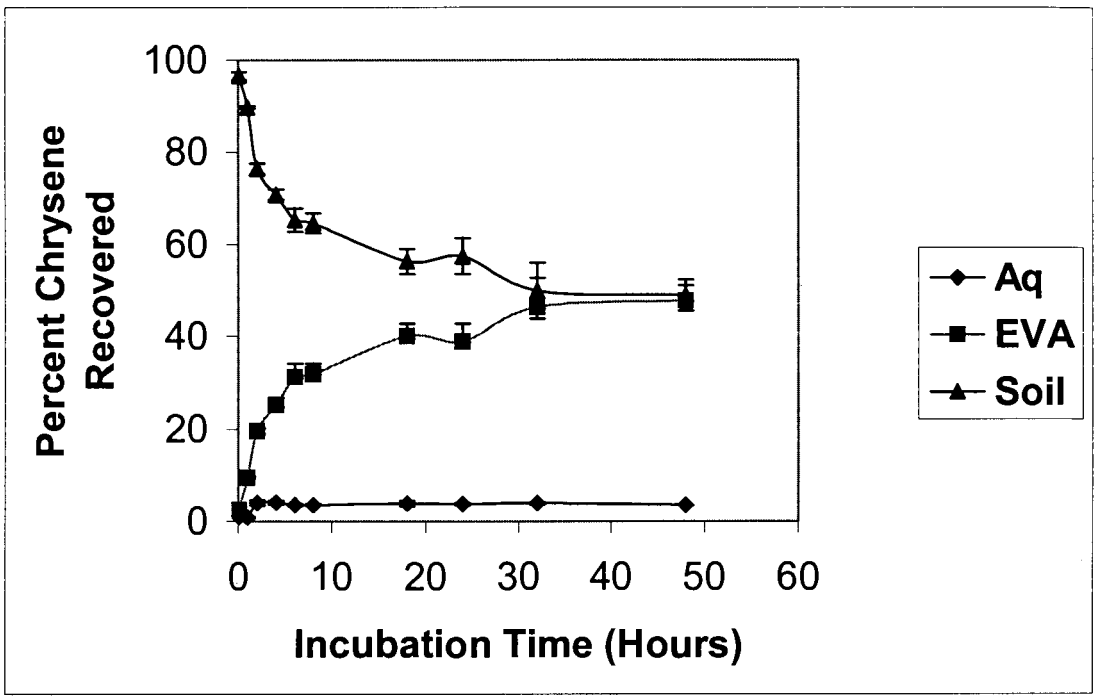


Figure 9: Partitioning of ^{14}C-chrysene from soil into aqueous (Aq) and EVA compartments of the in vitro digestive model when incubated for 48 hours at 37°C (Experiment 3).

Volume of Organic Matter in the Soil after 48 Hours of Digestion

To calculate the fugacity capacity of the soil, the concentration of chrysene in the soil at equilibrium was needed. Because hydrophobic compounds are known to bind exclusively to organic matter of the soil (Luthy et al., 1997; Alexander and Alexander, 1999), the volume of organic matter present in the soil is more relevant than the volume of whole soil. Using the volume of whole soil for fugacity calculations would underestimate the fugacity capacity of the soil. Soil that had been digested for 48 hours was heated in muffle furnace to burn off the organic matter. The difference in the volume of whole digested soil and burnt soil gave the volume of organic matter: 1.20E-08 ± 3.18E-10 m^3/100 mg dry soil.

The Fugacity Capacities of Different Compartments of the Model Digestive System

Fugacity capacity (Z) is the absorbing or holding capacity of a medium for a compound (Mackay, 1979). At equilibrium, fugacity (f), which is the escaping tendency, is same in all phases or compartments of a system (Mackay and Paterson, 1981). The fugacity and the fugacity capacity were calculated for each experiment that reached equilibrium.

The concentration of chrysene in the soil and its fugacity dropped as the experiment progressed, until a equilibrium was reached (no net movement of chemical). Because gastric fluid is aqueous and chrysene (log K_{ow} = 5.8) is hydrophobic, digestive fluid is expected to have a small Z value. This means that within a few seconds chrysene would move from the digestive fluid and it would move further into the EVA film as fugacity would be high in the aqueous phase. As the experiment progressed, this aqueous compartment reached equilibrium quickly (after 2 hours).

EVA has approximately 3.8 times more sorptive capacity to absorb hydrophobic compounds like chrysene, i.e., Z_{eva} is expected to be high (Otton, 2004, personal communication). At the beginning of the experiment, f was low because the concentration in EVA was low, but as the concentration increased the fugacity increased until a steady state was reached. At steady state, there was no further net movement of chemical from or to any compartment; therefore fugacity in each compartment of the system is assumed to be equal.

The usual way to measure fugacity is to measure the concentration of chemical in the air above a sealed system (Wilcockson and Gobas, 2001). Air has a known fugacity capacity - the molar capacity for all chemicals is identical. Because chrysene has a low vapour pressure (6.3×10^{-7} torr at 20°C), it was very difficult to measure the low concentrations in the air even using radiolabeled chrysene. Recent work by V.Otton, 2003 in Gobas lab (personal communication) suggest that K_{ea} (eva-air partitioning coefficient) is 3.8 times the K_{oa} (octanol-air partitioning coefficient), although earlier Kea was found to be twice the K_{oa} (Wilcockson and Gobas, 2001; Harner et al., 2003). Fugacity capacity (Z) of EVA was calculated using literature values (K_{ow} - octanol-air partitioning coefficient, H - Henry's law constant).

The fugacity and fugacity capacity for all the matrices involved in a system can only be calculated if the system reaches equilibrium, as it is a state of equal fugacity. Therefore, fugacity and fugacity capacity were calculated for experiment 3 only. The fugacity capacity of EVA film was calculated to be ~7 - fold higher than the soil (organic matter) and ~10^5 - fold higher than the aqueous phase (gastrointestinal fluids) using the data from experiment 3 (Table 4). The fugacity at equilibrium was 9.45E-08 Pascals.

Table 4: Comparison of fugacity capacities of EVA, aqueous and soil compartments of the model digestive system from Experiment 3.

Compartment	Concentration (C) (mol/m^3)	Fugacity Capacity (Z) ($mol/m^3.Pa$)	Fugacity (f) (Pa)
EVA	2.65	2.80E+07	9.45E-08
Aqueous	2.95E-05	313	9.45E-08
Soil	0.35	3.67+E06	9.45E-08

Effect of Altering the Composition of Gastrointestinal Fluids on Partitioning of ^{14}C-Chrysene

Experiments were conducted to determine the effect of different gastrointestinal constituents on partitioning of chrysene into various phases of the in vitro digestion model. Table 5 shows the composition of the gastrointestinal fluid and Table 6 shows the percentage of chrysene that partitioned into EVA and aqueous phase when chrysene-contaminated soil was digested for 48 hours, according to the protocol in Material and Methods (Chapter 2).

Experiments in which manipulations to the digestive fluids were done are compared with complete experiments that were run simultaneously (Experiments 4-5) and not with Experiments 1-3, which were also complete (had all digestive fluid components) experiments. The reason for this is that these sets of experiments (1-3 and 4-5) were done about 6 months apart, different batch of digestive fluids had been prepared, and if the soil did undergo any change (e.g. moisture level) then all the experiments would be affected equally. In experiments 4-5 (complete gastrointestinal fluids) 4 - 4.5% of chrysene partitioned into the aqueous phase and 54% into the EVA phase after 48 hours of digestion.

Experiments were also done in which contaminated soil was incubated with water only at 37°C (Experiments 6-7). No gastric or intestinal components were added. The amount of chrysene in the aqueous fraction was reduced by 37% as compared to the complete experiments (Table 6). 44% of chrysene partitioned into the EVA film. The amount of chrysene that partitioned into the EVA film and the aqueous phase was significantly different from that which was observed in complete experiments (Experiments 4-5) at $p \leq 0.05$.

Experiments were conducted without the addition of intestinal fluid (Experiments 8-9) to determine whether bile salts, pancreatin, TRIS, and calcium chloride had any role in enhancing mobilization of chrysene from soil. No significant difference was observed in the percentage of chrysene that partitioned into the aqueous and EVA compartments of the digestive model compared to the complete experiments (Table 6) implying that binding properties of chrysene and soil are not affected by these components.

We investigated whether the proteolytic ability of pepsin influenced partitioning by eliminating pepsin from the digestion process. Pepsin was substituted with Bovine Serum Albumin (BSA) to maintain the same protein concentration but eliminate the enzymatic activity of pepsin (Experiment 10). We found that 6% of chrysene had partitioned into the aqueous and 46% into the EVA film, suggesting that BSA increased the capacity of the aqueous phase for chrysene and decreased the amount of chrysene partitioning into EVA significantly ($p \leq 0.05$) compared with complete experiments. When both pepsin and intestinal constituents were eliminated and pepsin was not substituted with BSA (Experiment 11) the amount of chrysene that partitioned into the aqueous phase and into EVA phase did not change significantly compared to the complete experiments (Experiments 4-5) suggesting that pepsin and intestinal constituents did not play a significant role in enhancing the mobilization of chrysene from soil.

To determine whether neutralization of gastric fluid altered chrysene desorption, we conducted experiments in which we withheld sodium bicarbonate but added the remainder of the gastrointestinal fluid components. A significant effect was found when intestinal contents were not neutralized. Only 1% of chrysene partitioned into the aqueous compartment and 28% chrysene was found in the EVA compartment (Table 6).

65% reduction in chrysene in the aqueous compartment and 50% reduction in the EVA fraction was observed compared to the complete experiments (Experiments 4-5).

To determine whether the pH or the absence of $NaHCO_3$ itself caused the observed reduction in chrysene mobilization, $NaHCO_3$ was substituted with Tris. Comparing the 'complete' fluid (i.e., + $NaHCO_3$) with the substitution indicated that Tris could substitute for $NaHCO_3$, i.e., the pH shift from ~2.5 to 7 upon addition of base was primarily responsible for mobilizing ^{14}C–chrysene from soil into intestinal fluid. Experiment 14 ($NaHCO_3$ substitute) was compared with Experiment 15 (complete) and not with experiments 4-5 because experiments 14 and 15 were conducted at the same time and both showed similar results, although experiment 15 resulted in different amount of chrysene in the aqueous phase compared to the previous complete experiments (Experiments 4-5).

As seen in Table 7, the fugacity capacity of the aqueous compartment was affected when the composition of the gastrointestinal constituents was altered. In general, the fugacity capacity of the soil did not vary significantly among the different experiments. Wilcockson and Gobas (2001) have suggested that the fugacity capacity of EVA films is independent of the medium to which they are applied and thus the difference in chemical concentration in EVA films is a direct measure of the difference in chemical fugacities of the media or the sample to which it is applied. The amount and thickness of EVA were kept constant and same chemical was used in all the experiments, therefore, fugacity capacity of EVA for chrysene (2.8E+07 $mol/m^3.Pa$), should not change with changes in gastrointestinal components. Compared to complete experiments (Experiments 4-5) the fugacity capacity of the aqueous fraction was significantly reduced in experiments in which water was used instead of digestive fluids (Experiments 12-13) and also when gastric fluid was not neutralized (Experiments12-

13). Less chrysene was mobilized from the soil resulting in less partitioning into aqueous and EVA compartments of the model. The fugacity capacity of the aqueous compartment was similar when the complete experiment was run simultaneously with an experiment in which $NaHCO_3$ was substituted with Tris (Experiments 14-15) to enable neutralization. The fugacity capacity of the aqueous phase in experiments with pepsin substitute (Experiment 10) increased significantly and experiments in which no pepsin or no intestinal components (Experiment 11) were added no significant change was observed compared to the complete experiments (Experiments 4-5).

Because fugacity is proportional to concentration, we should see higher fugacity in experiments that resulted in higher concentrations in EVA. However, such comparisons could only be possible if every experiment had started with exactly same concentration of chrysene. With radiolabeled compounds it is very difficult to start with the same number of dpm each time as micro pipetting errors and concentrated solutions can result in significant differences in the total counts.

Table 5: Variable gastrointestinal compositions implied in Experiments 1-16 to study the effect of altered gastrointestinal composition on partitioning of ^{14}C-chrysene from soil into the aqueous (gastrointestinal) and EVA compartments in a model digestive system. Digestion was carried out for 48 hours. The concentration of each component given below is per 11ml of fluid.

Gastro-Intestinal Components		Concentration	Experiments						
			1-5, 15	6-7[a]	8-9, 16	10	11	12-13	14
"Gastric Fluid"	Sodium citrate	1.54 mM	**X**	–	X	X	X	X	X
	Malic acid	2.54 mM	**X**	–	X	X	X	X	X
	Lactic acid	4.40 mM	**X**	–	X	X	X	X	X
	Glacial acetic acid	7.56 mM	**X**	–	X	X	X	X	X
	Pepsin	0.006 mM	**X**	–	X	–	–	X	X
	Pepsin substitute[b]	0.003 mM	–	–	–	X	–	–	–
Neutralization	NaHCO3	0.91 mM	**X**	–	X	X	X	–	–
	Bicarb substitute[c]	14.18 mM	–	–	–	–	–	–	X
"Intestinal Fluid"	Tris	0.003 mM	**X**	–	–	X	–	X	X
	Calcium chloride	0.90 mM	**X**	–	–	X	–	X	X
	Pancreatin	0.027 mg/11ml	**X**	–	–	X	–	X	X
	Bile salts	0.36 mg/11ml	**X**	–	–	X	X	X	X

X = Added to the fluid and **X** bold means that experiment contained all constituents
– = Absent a = water only, no gastro-intestinal constituents
b = pepsin substitute was Bovine Serum Albumin
c = $NaHCO_3$ substitute wasTris-hydroxymethyl-amino methane

Table 6: The effect of gastrointestinal fluid composition on partitioning of ^{14}C-chrysene from soil into the aqueous (gastrointestinal) and EVA compartments in a model digestive system. Digestion was carried out for 48 hours. Values are presented as percentage of initial ^{14}C-chrysene bound to soil that partitioned into the aqueous and EVA phase of the in vitro digestive model.

Variation Experiments		Percentage Chrysene Recovered	
Experiment	Description	Aqueous	EVA
4	Complete	4.51 ± 0.2	53.58 ± 3.9
5	Complete	4.01 ± 0.2	53.58 ± 3.0
6	Water Only	2.54 ± 0.2*	44.43 ± 4.6*
7	Water Only	2.93 ± 0.3*	44.5 ± 4.3*
8	No Intestinal Additions	4.32 ± 0.6	48.38 ± 4.2
9	No Intestinal Additions	3.53 ± 0.2	45.59 ± 1.0
10	Pepsin Substitute	6.03 ± 0.4*	46.3 ± 3.0*
11	No pepsin and no intestinal additions	5.44 ± 0.3	47.38 ± 0.4
12	No Sodium Bicarbonate	1.07 ± 0.2*	28.27 ± 3.0*
13	No Sodium Bicarbonate	1.23 ± 0.3*	28.27 ± 4.3*
14[a]	Sodium Bicarbonate Substitute	2.62 ± 0.3	51.97 ± 2.8
15[a]	Complete	2.56 ± 0.5	52.56 ± 4.8

* significantly different from Experiment 4-5 at $p \leq 0.05$. [a] Experiments 14 and 15 were compared to each other. For details on the precise composition in each experiment, refer to Table 5.

Table 7: The effect of altering the composition of the gastrointestinal fluids on the fugacity and fugacity capacity of the aqueous and soil compartments. The *f* and Z values were calculated from the mean of triplicate values. Fugacity capacity of EVA remains constant (2.8E+07 mol/m^3.Pa).

Experiment	Variation Experiments Description	Fugacity (*f*) (Pa)	Fugacity Capacity (Z) (mol/m^3.Pa)	
			Aqueous	Soil
4	Complete	9.85E-08	322	2.75E+06
5	Complete	1.12E-07	363	4.47 E+06
6	Water Only	8.36E-08	218*	4.18 E+06
7	Water Only	8.26E-08	252*	4.13 E+06
8	No Intestinal Additions	9.03E-08	342	3.43 E+06
9	No Intestinal Additions	8.49E-08	296	3.90 E+06
10	Pepsin Substitute	9.61E-08	497*	3.50 E+06
11	No Pepsin and No Intestinal Additions	8.84E-08	439	3.49 E+06
12	No Sodium Bicarbonate	9.83E-08	77*	3.07 E+06
13	No Sodium Bicarbonate	9.40E-08	132*	3.93 E+06
14[a]	Sodium Bicarbonate Substitute	1.12E-07	193	3.06 E+06
15[a]	Complete	1.13E-07	186	2.99 E+06

* significantly different from Experiment 4-5 at $p \leq 0.05$ [a] Experiments 14 and 15 were compared to each other. For details on the precise composition in each experiment, refer to Table 5.

Effect of Ageing Chrysene onto Soil on its Partitioning to EVA During In Vitro Digestion Process (72 hours)

Experiments were conducted with chrysene-contaminated soils that were aged for different periods to see if ageing affects desorption or mobilization of contaminant from soil into digestive fluids and EVA film. ^{14}C-chrysene (2µg/100mg soil)) was added to the soil and the soil was aged in the dark at room temperature for 6 months and 12 months.

For 6-month aged soil (Figure 10), a significant reduction in the chrysene concentration in aqueous phase was found. After 2 hours of incubation the aqueous phase reached equilibrium and a maximum of 1.5% partitioned into the aqueous phase after 72 hours. Rapid uptake by EVA was observed for first two hours and then slow uptake continued for rest of the duration of experiment. A maximum of 44% of added chrysene partitioned into the EVA film.

For 12-month aged soil (Figure 11) 3.9% of chrysene partitioned into the aqueous phase, which is similar to the values obtained with non-aged soil. Equilibrium was reached at which point 45% of added chrysene was found in the EVA film.

Fugacity Values

Fugacity of 8.23E-08 Pa was calculated at equilibrium for 6 months aged soil experiment and 8.78E-08 for 12 months aged soil experiment which is not significantly different from complete experiments (Table 8). As before, EVA had 6 to 7-fold higher fugacity capacity than soil, which was aged for 6-months or 12 months. EVA's fugacity capacity was approximately 2 x 10^5 fold and 10^5 fold higher than the fugacity capacity of the aqueous phase for 6-months and 12-months aged soil experiments, respectively. Fugacity capacity of aqueous fraction was significantly lower than complete and 12 months aged soil experiment (Table 8).

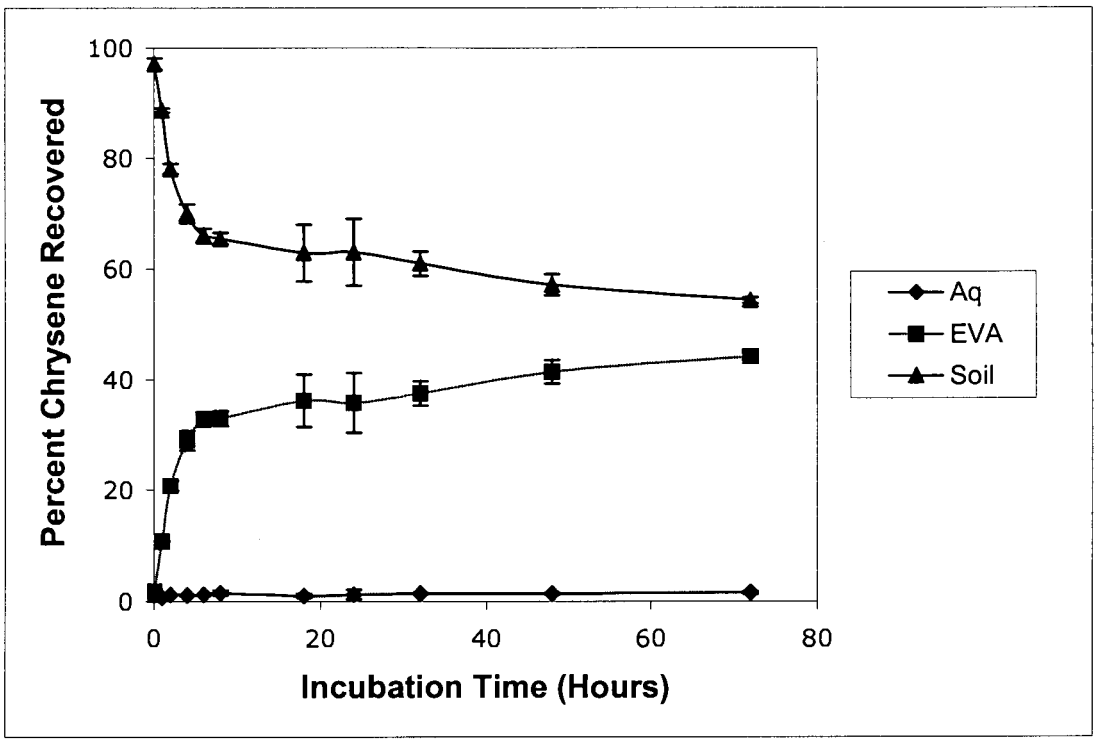


Figure 10: Partitioning of aged ^{14}C-chrysene from soil into the aqueous (gastrointestinal) and EVA compartments of the in vitro digestive model when incubated for 72 hours at 37°C. ^{14}C-Chrysene was applied to the soil as described in Chapter 2 and aged for 6 months in the dark at room temperature prior to the experiment.

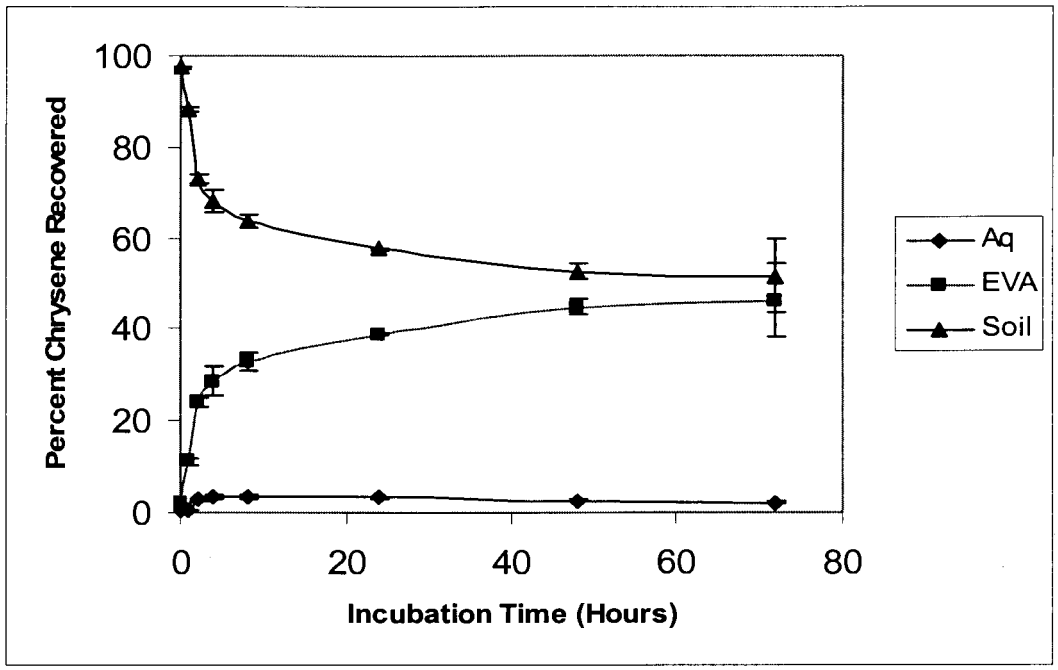


Figure 11: Partitioning of aged ^{14}C-chrysene from soil into aqueous and EVA compartments of the in vitro digestive model when incubated for 72 hours at 37°C. ^{14}C-Chrysene was applied to the soil as described in Chapter 2 and aged for 12 months in the dark at room temperature prior to the experiment.

Table 8: Comparison of fugacity capacities of EVA, aqueous and soil compartments of the model digestive system. Chrysene was aged onto soil for 6 months and 12 months.

Ageing	Compartment	Concentration (C) (mol/m^3)	Fugacity Capacity (Z) ($mol/m^3.Pa$)	Fugacity (f) (Pa)
Non-Aged (3days)	EVA	2.65	2.80E+07	9.45E-08
	Aqueous	2.95E-05	313	9.45E-08
	Soil	0.35	3.67E+06	9.45E-08
6 – Months	EVA	2.31	2.80E+07	8.23E-08
	Aqueous	1.05E-05	128	8.23E-08
	Soil	0.38	4.56E+06	8.23E-08
12 – Months	EVA	2.46	2.80E+07	8.78E-08
	Aqueous	2.49E-05	284	8.78E-08
	Soil	0.35	4.02E+06	8.78E-08

Partitioning of ^{14}C-Glyphosate from Soil During In Vitro Digestion (8 -72 hours)

Glyphosate is a polar compound with low K_{ow} of –3.2 (Mackay et al., 1992). Glyphosate is known to bind to soil (Williams et al., 2000) and it was used as a model polar compound to test the model gastrointestinal system.

Partitioning of ^{14}C-glyphosate from soil to aqueous (gastrointestinal phase) and EVA film of the model digestive system was studied to test the in vitro digestion model. Experiments with glyphosate were conducted twice in triplicate. The first experiment was carried out for 72 hours and second experiment focussed on partitioning during the first 8 hours.

In the first glyphosate experiment (Figure 12), ^{14}C-glyphosate in the aqueous phase reached equilibrium after two hours and a maximum of 78% of the added glyphosate was found in the digestive fluids. Less than 1% of added glyphosate partitioned into EVA during 72 hours of incubation with gastrointestinal fluid at 37°C. The maximum amount of glyphosate that partitioned into EVA film at 72 hours was 0.15%.

In the second experiment, more samples were analysed at earlier time points but the results were very similar to those found in the first experiment. The aqueous phase reached equilibrium (Figure 14) within1.5 hours and 78% of the ^{14}C-glyphosate moved into the digestive fluid. The EVA film had a maximum of 0.14% of glyphosate. ~20% of the soil bound glyphosate was not mobilized even after 72 hours.

Fugacity Values

Soil was found to have 30-fold and 39-fold higher fugacity capacity than EVA when experiment with glyphosate contaminated soils were conducted for 72 and 8 hours

respectively. The aqueous phase had 7000-fold and 9000-fold higher capacity than EVA as expected because of the polar nature of glyphosate.

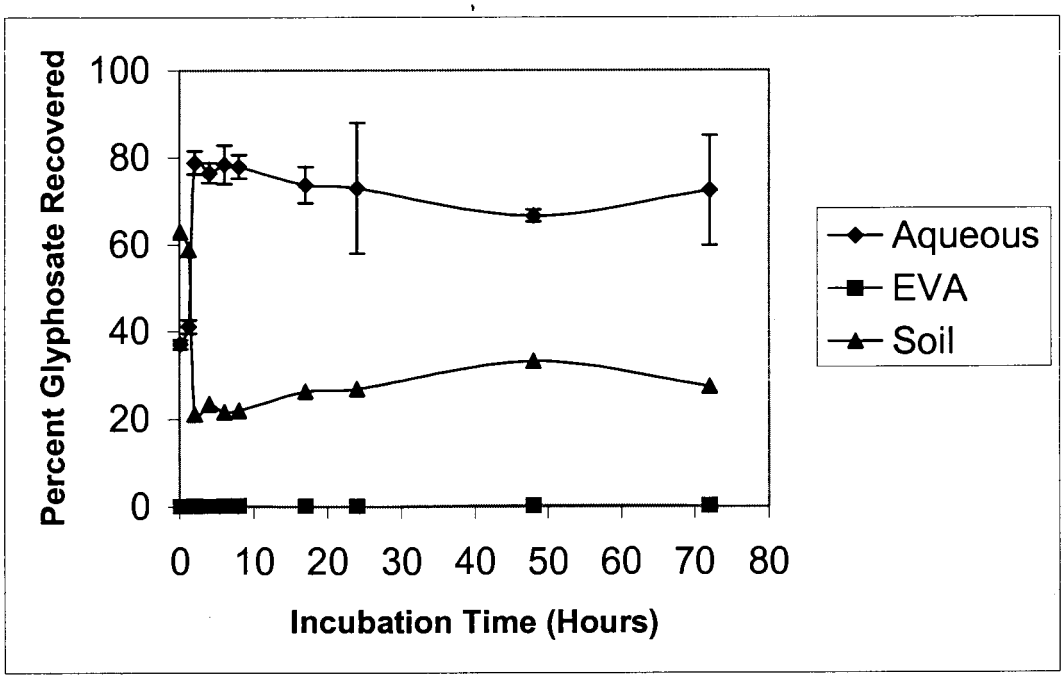


Figure 12: Partitioning of ^{14}C-Glyphosate from soil into aqueous and EVA compartments in an in vitro model digestive system when incubated for 72 hours at 37°C (Glyphosate experiment 1)

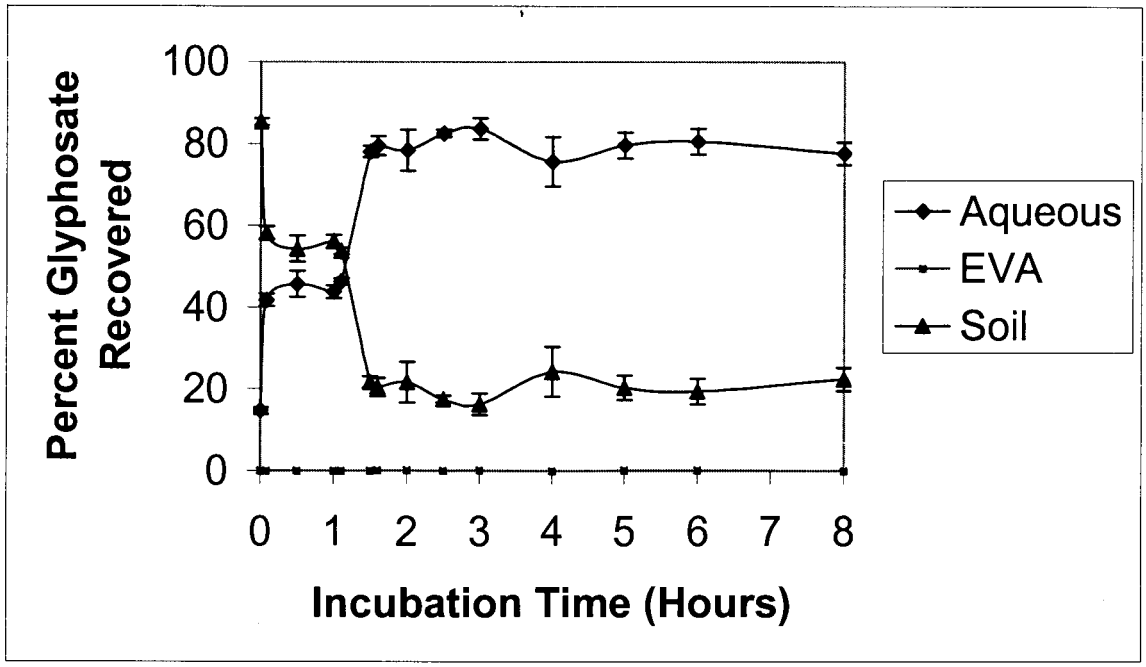


Figure 13: Partitioning of ^{14}C-Glyphosate from soil into aqueous and EVA compartments of an in vitro model digestive system when incubated for 8 hours at 37°C (Glyphosate experiment 2).

Table 9: Comparison of fugacity capacities of EVA, aqueous and soil compartments of the model digestive system when partitioning of glyphosate was studied for 72 hours (Experiment 1) and 8 hours (Experiment 2).

Experiment	Compartment	Concentration (C) (mol/m^3)	Fugacity Capacity (Z) ($mol/m^3.Pa$)	Fugacity (f) (Pa)
1	EVA	2.45E-05	1.16E+04	2.12E-09
	Aqueous	7.40E-04	3.49E+05	2.12E-09
	Soil	1.81E-01	8.54E+07	2.12E-09
2	EVA	2.10E-05	1.16E+04	1.82E-09
	Aqueous	8.27E-04	4.54E+05	1.82E-09
	Soil	1.92E-01	1.05E+08	1.82E-09

Comparing the Fugacity Capacity of EVA and a Human Intestinal Cell line, Caco-2 Cells

The EVA film has been used as a model of the gastrointestinal epithelium to predict uptake in vivo. The purpose of the following experiments was to compare the fugacity capacity of the EVA film with a cultured human enterocyte like cell line, Caco-2. Experiments with EVA and Caco-2 cells were conducted twice and each experiment was conducted in duplicate.

Partitioning of ^{14}C Chrysene

In the following experiments we used coverslips instead of vials because it was not possible to culture cells along the walls of the vials. We used PBS instead of digestive fluid because cells cannot withstand low gastric pH. PBS with 0.05% Tween 20 was used to reduce the time to reach equilibrium.

We conducted experiments with EVA coated coverslips which were incubated in chrysene dissolved in PBS-tween. In the first experiment equilibrium was reached after 2 hours and in the second experiment equilibrium was reached after 4 hours (Figure 14). The maximum uptake of mobilized chrysene by EVA was 37% and 33% in the two experiments, respectively. The reason for difference in the time to reach equilibrium might be the difference in the coating itself. It was difficult to determine whether the EVA coated the coverslip uniformally or whether the entire volume of EVA solution remained on the surface. It is a possible that the coating on the coverslip in the first experiment was thinner than the second; a thicker coating would take more time to reach equilibrium. Stock solution of EVA could have evaporated.

In both of the Caco-2 cell experiments both experiments, equilibrium was reached after 6 hours, although the extent of uptake by the cell line showed significant difference, 19% versus 48%, respectively (Figure 15). This difference could be due to

difference in the density of the cells; more cells would provide more lipid content per coverslip resulting in higher uptake in the second experiment. Cells used in the second experiment were 3 days older than the ones used in the first experiment.

Lipid Content and Volume of Lipid in Caco-2 Cells

The volume of lipid in Caco-2 cells grown on the coverslips was determined experimentally for fugacity calculations. In order to do this, cells from 5-75 cm^2 flasks were harvested and dried. From the 33.37 mg cell pellet, 5.61 mg lipid was extracted assuming that Caco-2 cells grown on coverslips (113.1 mm^2) contained the same % lipid, we determined that lipid per coverslip was 0.036 µl or 3.63E-11 m^3.

Fugacity Values

EVA was calculated to have 23 fold higher fugacity capacity than human intestinal epithelial cell line, Caco-2 (Table 10). Experiments done with EVA coated coverslips showed similar concentrations and fugacity values whereas first experiment with epithelial cells had 3-fold lower fugacity than second experiment and therefore 3-fold lower concentration than second experiment. Fugacity is directly proportional to concentration; therefore as concentration increases, fugacity increases. Higher seeding density, cell maturity and lipid content could be responsible for higher concentrations and hence higher fugacity in the second cell experiment.

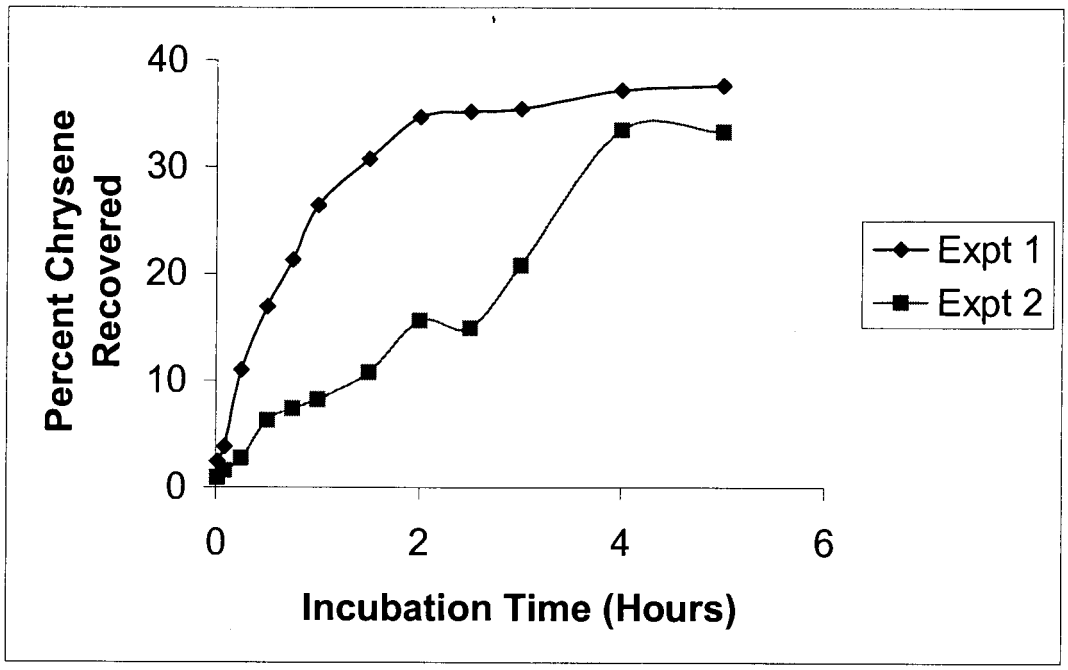


Figure 14: Partitioning of ^{14}C-chrysene from chrysene solution in PBS-tween into EVA film on the coverslip when incubated for 6 hours at 37°C.

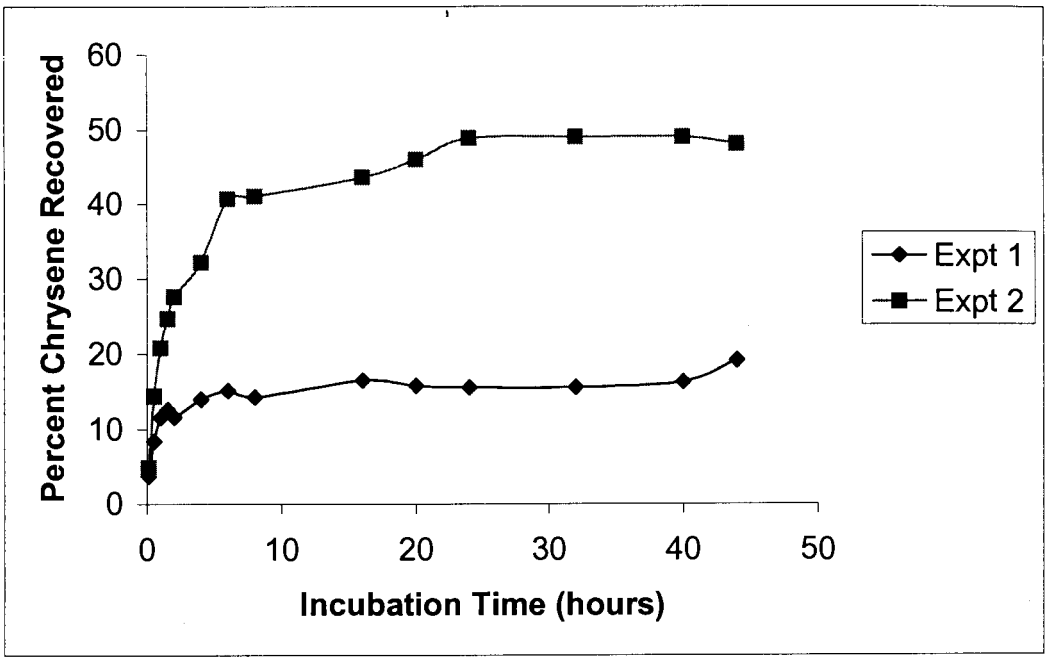


Figure 15: Partitioning of ^{14}C-chrysene from chrysene solution in PBS-tween into Caco-2 monolayer on the coverslip when incubated for 44 hours at 37°C.

Table 10: Comparison of the fugacity capacity of EVA with Caco-2 cells after incubation for 6-44 hours with ^{14}C-chrysene solution in PBS-tween at 37°C. Values were calculated using mean of duplicates.

Compartment	Concentration (C) (mol/m^3)	Fugacity Capacity (Z) (mol/m^3.Pa)	Fugacity (f) (Pa)
EVA (Experiment 1)	11.43	2.80E+07	4.08E-07
EVA (Experiment 2)	15.13	2.80E+07	5.40E-07
Caco-2 Cells (Experiment 1)	34.36	1.22E+06	2.81E-05
Caco-2 Cells (Experiment 2)	108.28	1.22E+06	8.85E-05

Kinetics of Uptake of Chrysene

The one compartment model was fit to desorption data using a nonlinear regression technique with software designed to minimize the sum of squared residuals. The complete experiments (Experiments 1-3) and 6-12 months aged soil experiments (Table 11) were found to have rate constants that did not differ significantly at $p \leq 0.05$.

Comparative EVA and Caco-2 cell experiments also did not have significantly different rate constants. One reason is that only two experiments were done for each (EVA and cells) and there is too much variation observed between the two experiments. EVA experiment 1 has higher rate constant than experiment 2 (Table 11), which is probably due to difference in the coatings in the two experiments. Similarly, the cell experiments resulted in different extents of mobilization due to differences in the cell density, age and lipid content. Rate constants describing partitioning to EVA in experiments that used chrysene solution in PBS + 0.05% Tween 20 were significantly higher than rate constants from soil experiments (Table 11).

Table 11: Rate constants for uptake of ^{14}C-chrysene by EVA and Caco-2 monolayers derived from a one compartment model.

Description	Aqueous Compartment	Rate Constant 95% Confidence Interval	R-Squared Value
Complete Expt-1	Gastrointestinal Fluid	0.49 + 0.20	0.94
Complete Expt-2		0.27 + 0.31	0.75
Complete Expt-3		0.24 + 0.17	0.76
6-month Aging		0.31 + 0.09	0.98
12-month Aging		0.39 + 0.20	0.71
EVA Coverslip Expt -1	PBS + 0.05% Tween 20	1.20 + 0.10	0.99
EVA-Coverslip Expt-2		0.66 + 0.15	0.95
Cells-Coverslip-Expt-1		1.08 + 0.40	0.94
Cells-Coverslip Expt-2		0.44 + 0.12	0.96

CHAPTER FOUR:
GENERAL DISCUSSION

Introduction

Massive relocation of natural materials to different areas of the ecosystem has taken place during the past several decades as a result of human activities, thus exposing living systems to different HOCs. Since the industrial revolution, human activities have created enormous amounts of chemicals that are eventually released into the environment, either deliberately for agricultural and industrial purposes or accidentally by mishandling of chemicals and spills. Surveys have shown that there are about 50,000 contaminated sites in the U.S. alone (Errampalli et al., 1997), and it is estimated that 25% of the land area may be contaminated in Canada (De Sousa, 2001).

The focus of this research was on mobilization of HOCs after oral exposure as this route is typically assumed to provide the greatest exposure for soil-borne contaminants. The ultimate goal of this research was to reduce the uncertainty in exposure estimates by determining the factors that control mobilization/bioaccessibility, and therefore bioavailability. Due to the rapid increase in the number of compounds that are being synthesized as well as the vast number of existing toxic chemicals, more accurate methods are required to estimate exposure for use in human health risk assessments. Given the expense (a factor of 500-1000), time constraints and ethical concerns associated with in vivo studies, a rapid, reproducible, and inexpensive in vitro method for predicting the oral bioavailability of chemicals is needed. It is important to emphasize here that the model used in our study is not intended to replace in vivo work but rather to complement such studies.

Total extraction of PAH from contaminated material by means of organic solvents may considerably overestimate the amount of contaminants contributing to the general health risks caused by ingested polluted materials. These methods of chemical analysis are not intended to quantify the bioavailable fraction and the total amount of the contaminant extracted often has little relationship to the amount of contaminant that is actually bioaccumulated (Kelsey and Alexander, 1997). Although there have been some attempts to develop weaker chemical extractions for metals to better quantify the bioavailable contaminant fraction, the validity of these methods remains uncertain, and no comparable partial extraction methods exist for HOCs that are capable of distinguishing between the bioavailable and nonbioavailable fraction of these compounds.

Several studies have measured the oral bioavailability of contaminants, especially metals. These studies incorporated various leaching and dissolution techniques (Ruby et al., 1996; Davis et al., 1993), studies of soil type and structure (Davis et al., 1994), human feeding studies (Maddaloni et al., 1998) and animal experiments (Freeman et al., 1992, 1993, 1995, 1996; Groen et al., 1994). Studies have also been performed to compare gastrointestinal dissolution techniques with in vivo animal models (swine model – Casteel et al., 1997, Rodriguez et al., 1999; mouse model – Sheppard et al., 1995, Freeman et al., 1996; rat, rabbit, and monkey models – Freeman et al. 1993, 1995, 1996, Ruby et al., 1996). Some of these studies found good correlation between in vitro and in vivo results (Davis et al., 1994; Ruby et al., 1996; Rodriguez et al., 1999; Ellickson et al., 2001). The gastrointestinal incubation approach is more relevant to bioavailability and is, in essence, a chemical extraction with a biologically relevant extractant. To evaluate the extent of the absorption of HOCs by intestinal epithelium following ingestion of soil-borne contaminants, we developed an in

vitro test system that simulated the transition of contaminants from the soil into the synthetic digestive juices by means of gastrointestinal model. The test system simulated the influence of the acidic environment of the stomach (gastric model) followed by the neutral or slightly alkaline environment of the small intestine (intestinal model) and furthermore, uptake by intestinal epithelium which was mimicked by an EVA (plastic) coating.

Mobilization of Soil-Bound Glyphosate Using In Vitro Digestion

Glyphosate is a widely used herbicide in agricultural and residential settings, which does not bioaccumulate in living cells due to its polar nature (Ghassemi et al., 1982). No information is available on the absorption of technical grade glyphosate into human intestinal epithelium; however, several studies have been conducted in rats to investigate the fate of orally administered glyphosate. Glyphosate has a phosphoric acid moiety and exists as a charged species at the physiologic pHs found in the intestinal lumen.

In the present study, we used glyphosate to determine the behaviour of a polar compound in our in vitro model and to compare it with chrysene, a hydrophobic compound. Over a period of 72 hours digestion, we found that less than 1% of the added glyphosate was taken up by EVA from glyphosate-contaminated soil and ~80% was found in the aqueous fraction. This was expected due to the highly polar nature of glyphosate (log K_{ow} = -3.2). In contrast, ~50% of recovered ^{14}C-chrysene partitioned into EVA and ~4% was found in the aqueous phase at equilibrium (48 hours). Williams et al. (2001) demonstrated that glyphosate and its metabolite AMPA (amino methyl phosphonic acid) are poorly absorbed. Only 15-36% of orally administered material given repeatedly, or as a single dose to Sprague-Dawley rats, was absorbed. Brewster et al. (1991) found that 35-40% of the administered dose was absorbed from the GI

tract, and urine and faeces were equally important routes of elimination. Nearly 100% of the body burden of radioactivity was present as unmetabolized parent glyphosate; however, only 7.7% of radioactivity was found to be associated with small intestinal tissue in another study after 3 hours of administration (NTP, 1992). Other studies conducted in non-rodent species such as rabbits, goats and chickens have also shown that the absorption, and resulting tissue levels were low (Williams et al. 2001).

We found that a significant proportion (~20%) of the added glyphosate remained bound to soil after 72 hours of incubation with digestive fluids. Brewster et al. (1991) suggested that the phosphoric moiety of the glyphosate reversibly binds to calcium ions in the bone matrix thereby slowing down elimination of glyphosate from this tissue. This type of binding is also known to occur with glyphosate and soils (Sprankle et al., 1975) and may explain the persistence of soil-bound glyphosate in our study. Ederbach and Douglas (1991) and Ederbach (1999) found that less than 50% of the amount of glyphosate applied was recovered from all soils 24 hour after the addition of glyphosate except for one soil from which 66% was recovered. These four different soils were loamy sand, grey clay, silty clay and loam. At two different temperatures (10 and 25°C) a marked loss in the amount of extractable glyphosate was observed immediately after addition of the herbicide to soil, and this rapid loss of glyphosate was ascribed to adsorption of the herbicide into a non-extractable form. Greater sorption occurred at higher temperatures (Ederbach, 1999). Glyphosate is readily bound to many soils and clay minerals; and sorption of glyphosate can be expected in the presence of available phosphate binding sites, the presence of iron and aluminium (oxides and hydroxides), and appropriate combinations of clay and organic matter (WHO, 1994). The above studies found higher estimates of bioavailability compared to our study because glyphosate used in these studies was not soil-bound and soil is known to reduce

bioavailability. Another explanation for higher absorption by GI tissue in these studies could be that glyphosate was not absorbed but adsorbed to the mucus lining of the GI epithelium. Our in vitro model measured <1% bioavailability which correlates with another study done in our lab that found ~0.5% of applied glyphosate bound to Caco-2 monolayers (unpublished data). This suggests that in vitro model used in this study is a promising technique that could be used to assess bioavailable amounts of soil-bound contaminants.

Mobilization of Soil-Bound Chrysene Using In Vitro Digestion

Several studies have shown that gastrointestinal fluids may effectively mobilize certain organic contaminants. Weston and Mayer (1998) suggested that the measurement of solubilization potential alone might serve as a good approximation of bioavailability (or bioaccessibility) even when the absorptive step is not included in the digestive fluid extraction procedure. We developed an in vitro test system for examining the mobilization of pollutants from contaminated material into digestive juice by means of a standardized artificial gastro-intestinal model. We also added a model epithelium in the form of a thin plastic film (EVA). The temperature, the continuous motion of the gastro-intestinal tract, pH-values, and the concentration of digestive enzymes, bile salts and other components were held as close as possible to physiological conditions. We did not investigate the effects of other dietary constituents, e.g. fats, although it has been shown in many studies that presence of alimentary components have enhanced the mobilization in the gastrointestinal tract. As milk fat and milk protein play an important role in human nutrition, whole or skim milk powder is routinely added to the test suspensions in order to simulate dietary effects (Hack and Selenka, 1996; Ruby et al., 1999; Oomen et al., 2000).

We conducted complete digestion experiments in which contaminated soil was subjected to all steps of the digestion process. The extent of the mobilization of ^{14}C-chrysene from contaminated soil by means of the synthetic digestive juices was compared with the total amount of the contaminant that was added to the sample. After 24 hours, 34% and 25% of ^{14}C-chrysene was mobilized in the first two experiments whereas 48% was mobilized when the experimental duration was increased up to 48 hours. As well as analysing the uptake by EVA film, which was considered the bioavailable fraction of chrysene, we measured the bioaccessible concentration in the aqueous phase (gastrointestinal phase) through which chrysene moved from the soil matrix into the EVA film. We observed that the aqueous phase reached equilibrium very quickly, as expected, (within 2 hours), and had limited capacity for chrysene (4%) due to its hydrophobic nature.

Our in vitro results agree with in vivo data from Weston and Mayer (1998). These authors found a general agreement in magnitude between the proportions of PAH solubilized in digestive fluid and the proportion absorbed during gut passage by a deposit feeder (*Arenicola brasiliensis*) when they used PAH contaminated sandy sediments. Absorption efficiencies of PAHs during gut passage were 27–35% for benzo(a)pyrene and 12-50% for phenanthrene, similar to the extent of in vitro solubilization by digestive fluids from the same sediment (13-42% for benzo(a)pyrene and 22-49% for phenanthrene). This study showed that although the absorption with respect to the bulk sediment was less than 50%, with respect to the solubilized fraction it approached 100%. Kukkonen and Landrum (1995) showed estimates of benzo(a)pyrene absorption efficiencies ranging from 0-26% by annelid, *Lumbriculus variegates*, and 45-60% by amphipod (*Diporeia sp.)*. Fries (1989) showed availability of

different radiolabeled PCB after a single oral dose to rats of artificially contaminated soil ranged from 67% to 95%, depending on the compound examined (Fries et al., 1989).

Cornelissen et al. (1998) determined that up to 55% of the sediment bound PAH was mobilized using tenax extraction method. Tenax is a porous polymer that is used for desorption studies due to its high sorptive capacity and its rapid solute absorption from water. In another study, the maximum extent of desorption of native compounds (aged since 1950s) was approximately 0.30% of the soil-bound material whereas the maximum extent of desorption for ^{14}C-labeled chrysene and phenanthrene, spiked for 1-28 days prior to the experiment, was in the order of 2.5-12% of soil-bound material (Carmichael et al., 1997). They did not use any sorptive material and desorption was measured by analysing water. Kan et al. (1994) sorbed naphthalene and phenanthrene to sediment with 0.27% natural organic matter. Successive desorption steps into water usually lasting 1-7 days released less than 40% of the chemical even from samples sorbed for only 1 day.

In contrast, other studies showed that the absorption of organic contaminants from ingested soils or from other particulate material might be low. Weber and Lanno (2001) observed ~4% absorption of ^{3}H-BaP into the isolated channel catfish (*Ictalurus punctatus*) intestine segments from the incubation solution regardless of the concentration in the incubation solution. Hack and Selenka (1996) investigated PAH-contaminated soil samples and observed that only 0.8% of the total contaminant present in the soil sample was mobilized in the gastric fluid and 6% in the gastro-intestinal fluid. When dry milk was added, 9% mobilized in the gastric and 23% mobilized in the gastrointestinal fluid, which illustrated the influence of alimentary components on mobilization in the gastro-intestinal tract. The results of this study revealed that in many

cases only a minor portion of the contaminants in polluted material such as soil or the solid matrices were bioavailable.

Soil Organic Matter

Sorption and desorption are strong functions of geosorbent type. It is commonly argued that when HOCs are sorbed on soils they are available to living organisms only after they have been transferred to coexisting aqueous phases. Therefore, the soil organic matter of a given geosorbent will play a major role in bioavailability because it will control the aqueous phase concentrations of HOCs. Laboratory studies have shown that bound residue formation is a primary fate mechanism of PAHs (Hurst et al., 1996). Humic materials are considered precursors to mature geosorbents and have been shown to have a higher sorptive capacity for PAHs than bulk sediment or soil materials (Garbarini and Lion, 1986). Humin fraction of the humic substances is usually the predominant organic material in most soils; humin organic–carbon typically represents more than 50% of the total organic carbon in soil (Kohl and Rice, 1998). Because the compounds can be recovered from the soil by extraction with strong organic solvents this indicates that they are non-covalently linked to soil constituents (Kelsey et al., 1997). Proposed interactions between non-ionic HOCs such as PAHs and soil organic matter (SOM) include absorption and adsorption (Weissenfels et al., 1992; Hatzinger and Alexander, 1995), and partitioning and entrapment of the molecules within soil micropores (Kan et al., 1994; Weber and Huang, 1996; Pignatello and Xing, 1996). Guthrie et al. (1999) demonstrated non-covalent binding of pyrene to humic materials in SOM. Several studies have suggested that the interaction between SOM and HOCs is probably due to the existence of van der Waals forces and hydrogen bonding (Gauthier et al, 1978; Pignatello and Xing, 1996), however, in our study hydrogen bonding is not involved in chrysene-soil interaction because the C-H bonds in chrysene are not

polarized. Although van der Waals forces are weaker relative to covalent and ionic exchange interactions, but they are apparently sufficient in strength to prevent the removal of HOCs by stringent solvent extraction methods (Lerch et al., 1997).

It has been suggested that the organic carbon content of soil is the single most important factor determining the sorption of HOCs such as PAHs because of their extremely high K_{ow} values and low vapour pressures (Weissenfels et al., 1992; Weston and Mayer, 1998). Johnson and Weber (2001) attributed desorptive behaviour of contaminants in large part to the different nature of natural organic matter that each soil or sediment type contains. Geosorbents containing primarily humic type organic matter will allow faster and more complete desorption than those containing primarily kerogen-like organic matter, which is chemically reduced, physically condensed and relatively non-extractable (Weber and Lanno, 2001). The soil used in our study had only ~15% organic matter and was not analysed for different types of organic matter present. We predict that this soil has probably more humic and fulvic acids than humin and does not have kerogen like organic matter. McCarthy and Jimenez (1985) suggested that there was a direct relationship between the hydrophobicity of PAH and the binding affinity to dissolved organic matter. Soil borne PAHs, if immobilized onto soil organic matter, are non-bioavailable hence non-biodegradable and are not released by rinsing soils with water (Weissenfels et al., 1992). When degradation of a single PAH or a mixture of seven PAHs by bacterial cultures enriched from contaminated soils was studied, 10% of the single PAHs and 20% of the PAHs in the mixture were found to be irreversibly incorporated into the dissolved and particulate organic matter (Ressler et al., 1998). Although both humic and humin fractions have hydrophobic sites, humin was found to have greater propensity to sequester pyrene (Guthrei et al., 1999) and was found to be a primary sink of bound ^{14}C-pyrene (Nieman et al., 1999), phenanthrene, naphthalene,

benzo(a)pyrene (Kohl and Rice, 1998). A significant fraction (29-73%) of PAH was found to bind rapidly and in many cases irreversibly to humin whereas 6.6-26% was found in humic/fulvic acid fraction of SOM (Kohl and Rice, 1998).

Effects of Ageing

Soil-PAH contact time (i.e., aging) is known to be one of the most critical bioavailability factors governing the fate and transport of PAHs in the soil. Several studies have reported that PAHs become increasingly less available with residence time in the soil (Hatzinger and Alexander, 1997; Kelsey et al., 1997; White et al., 1997). As organic compounds persist in soil, they become progressively less available for uptake by organisms, for exerting toxic effects, and for biodegradation and bioremediation by microorganisms (Alexander, 2000). It is believed that during aging, molecules slowly move into sites within the soil matrix that are not readily accessed by even the smallest of microorganisms (Alexander, 2000). The chief sorbent for hydrophobic molecules is the organic matter of soils, and this fraction is presumably where hydrophobic molecules become entrapped. If the molecules thus sequestered are inaccessible to organisms and if diffusion out of these remote sites is extremely slow, the bioavailability of those compounds will be governed by the very slow rate of release to an accessible site. In a reasonable short time period, therefore, little would be available to living organisms.

Minute pores or voids may also be important in soils. The organic fraction of soil contains an abundance of such nanopores and the sequestered compound may thus be localized in minute pores of the organic matter (Xing and Pignatello, 1997). However, for a molecule to become poorly available, it's desorption must be exceedingly slow. Test with beads having pore diameter of 2.5 –15 nm showed that phenanthrene was quickly desorbed and rapidly metabolised by microorganism if the pores did not have hydrophobic surfaces, but desorption was slow and biodegradability was enormously

reduced if the pore surface were hydrophobic (Nam and Alexander, 1998; Cornelissen et al., 1998).

The exposure of living organisms to a toxicant that is sequestered in soil is less than to the same concentration of the compound that is fully available, and the risk from the compound is consequently less (Chung and Alexander, 1998). Sequestration refers to a loss in availability of a compound. If the rates and extent of sequestration differ among soils with different properties, it is necessary either to determine the bioavailability of aged compound in each soil type or to find generalizations allowing a prediction of the effect of soil characteristics on the reduction in bioavailability as a function of time (Chung and Alexander, 1998). At present, assessments of the hazard of toxic chemicals in soil are made without taking into consideration decreases in bioavailability over time.

We conducted experiments with 6 and 12-month aged soils. In contrast to some studies, no reduction in ^{14}C-chrysene uptake by EVA film was observed in soils that were contaminated for 6 and 12 months compared to those aged for only 4 days. Although, there was a 38% reduction in the capacity of the aqueous compartment to hold chrysene in case of 6-month aged soil, no change in the aqueous compartment of 12-month aged soil was observed compared to non-aged experiments. We suspect that 12 month aging samples had collected moisture due to condensation caused by temperature fluctuations in the storage area. It has been suggested that in dry soils, sorption of non-polar organics is dominated by adsorption onto mineral surfaces, particularly clays and when mineral surfaces are saturated with water, the main interaction of compounds with soil is partitioning into SOM (Garbarini et al., 1986; Karimi-Loftabad et al., 1996). If this were true then longer aging and increased moisture should have caused less desorption compared to non-aged soil. HOC uptake by mineral

surfaces has been shown to be insignificant and non-specific (Schwarzenbach, 1981; Mader et al., 1997). We suggest that no sequestration of chrysene occurred because of the absence of NAPLs and this was also evident from the kinetics that showed presence of only one compartment. The presence of more humic/fulvic acids compared to humin in the organic matter and presence of more mineral domains in the soil used in our study is likely responsible for no difference observed between the aged and the non-aged soils.

Other researchers have found aging to have minimal impact on availability. Bordelon et al. (2000) fed soil spiked with coal tar (CT) in aged and unaged form to rats (Fischer 344 rats) and measured DNA damage through ^{32}P-post labelling. They found no significant differences in adduct levels between rats fed aged and non-aged CT-amended soils. Reeves et al. (2001) compared the availability of a complex mixture of PAHs from a freshly spiked soil with a soil aged 270 days using both in vivo and in vitro methods. Aging had no significant impact on 1-hydroxypyrene excretion by adult male Fischer 344 rats when comparing coal tar (CT) non-aged and CT aged groups. PAHs in the liver were not significantly different between the two groups indicating that aging did not limit PAH bioavailability from CT.

On the contrary, significant differences in the maximum extent of desorption was observed for aged soil (approximately 0.30%) and non-aged ^{14}C labelled compounds (2.5 – 12%) in a study done by Carmichael et al., 1997. Hwang and Cutright (2002) found that the aqueous PAH concentrations were greater in the aged soil systems than in the freshly spiked soil systems. They also found total biodegradation extent after 32 days was higher in the aged soil reactors than in the freshly spiked soil reactors which they attributed to production of rhamnolipids from *P. aeruginosa* upon PAH aging in the soil.

Laboratory studies have confirmed the unavailability of molecules that have been in soil for long periods to microorganisms. PAHs present in a soil at a manufactured gas site did not disappear over a 3-month period in the laboratory, but naphthalene and phenanthrene freshly added to the soil were rapidly metabolised (Erickson et al., 1993). Hatzinger and Alexander (1995) introduced phenanthrene, a hydrocarbon that is not readily lost by abiotic mechanisms, into sterile soil and added a phenanthrene-degrading bacterium after the hydrocarbon had aged for different periods of time. With a soil rich in organic matter, the extent of microbial conversion of phenanthrene to CO_2 and the rate of biodegradation declined with increasing time of contact of the contaminant with the soil. Similar observations were made with a soil with a lower level of organic matter although aging appeared to be slower. The recovery of phenanthrene that was freshly added to soil declined as the test compounds persisted in soil (Kelsey et al., 1997). Macleod and Semple (2000) found that the extractability of pyrene by methanol:water and 1-butanol decreased with increasing contact time. White et al. (1996) and Kelsey et al. (1997) also observed similar results. Increased soil-PAH contact time reduced the magnitude of the rapidly-desorbing phase and the extent of biodegradation (Alexander, 1995; Pignatello and Xing, 1996; Cornelissen et al., 1998) with the sorption of HOCs to sites in the condensed region controlling the slowly desorbing phase (Pignatello and Xing, 1996; Xing and Pignatello, 1997; Cornelissen et al., 1998)

Kinetics of Uptake by EVA Film/Desorption from Soil

Sorption to natural solids is an underlying process affecting the transport, degradation, and biological activity of organic compounds in the environment (Pignatello and Xing, 1996). The physicochemical interactions of PAHs with soils often result in strong binding and slow release rates that significantly influence their bioavailability (Luthy et al., 1997; Wilcke, 2000). Desorption is often considered the rate-limiting step

for soil systems (Hatzinger and Alexander, 1995). Sorption and especially desorption of HOCs like PAHs in soil aggregates can be exceedingly slow and a fraction of desorptoin resistant contaminant can be left in the soil.

We tried fitting our data to two-compartment model, but for most of the experiments, the rate constant describing slow desorption calculated by the model was essentially zero indicating that either a portion of PAH in the slow compartment would be unlikely to desorb or that there is only one compartment. Carmichael et al. (1997) observed desorption rates for the native PAH that had aged since 1950's in soil were markedly slower than the desorption rate for labelled phenanthrene and chrysene. However, in our study, desorption rate constants from aged soils were not significantly different from non-aged soil. This is consistent with a one compartment model because the soil used in this study was pristine. It contained natural organic matter only (probably mostly humic and fulvic acids) and no NAPLs such as oils, tars, etc; that are known to sorbing HOCs. Rate constants for complete experiments and 6-12 month aged soil experiments were in the range of 0.24 ± 0.17 h^{-1} to 0.49 ± 0.20 h^{-1}. In a study by Reeves et al. (2001), desorption experiments with tenax beads and coal tar contaminated soils resulted in rate constants ranging from 0.5 to 0.89 h^{-1} for 13 different PAHs. Chrysene had desorption rate constant of 0.5 ± 0.2 h^{-1} for non-aged and 0.7 ± 0.3 h^{-1} for aged (270 d) chrysene (Reeves et al., 2001). Desorption kinetics of 15 PAHs (possessing two to six aromatic rings) were determined using tenax solid phase extraction method by Cornelissen et al. (1998) and rate constants were in the order of 0.05-3 h^{-1}. Desorption rate constants for phenanthrene were in the range of 0.028 ± .021 h^{-1} to 0.598 ± 0.371 h^{-1} for three different soils that varied in organic matter content and type (Johnson and Weber, 2001).

Experiments comparing chrysene uptake by EVA and Caco-2 cells showed no significant difference in the rate constants. However, the fugacity capacity of EVA was calculated to be 23-fold higher than Caco-2 cells. The possible explanation for similar rate constants for both cells and EVA is that the resistance faced by the chrysene molecule at the EVA-aqueous interface is similar to the resistance faced by the molecules at cell-aqueous interface. Once in the EVA/cells, the movement of molecules may be slower in polymer than in cells because of the difference in the matrix structure. Another possible explanation could be that once chrysene enters the EVA, it has the ability to form a number of non-covalent bonds with EVA, which will stabilize the molecule and reduce the energy of chrysene. Thus, there is a higher energy barrier to chrysene leaving EVA for the aqueous phase. In contrast, in the Caco-2 cells, there is a lower ability to form these non-covalent bonds therefore a lower energy barrier exists for chrysene to leave Caco-2 cells to enter the aqueous phase.

Effect of Different Constituents of Gastro-Intestinal Fluids

To study the effect on the mobilization of a model PAH from contaminated soil, components of an in vitro digestion model were varied. To assess the health risk via ingestion of contaminated soil it is imperative that we know the fraction of the contaminant that is absorbed from the gastrointestinal tract. It is also necessary to know the effect of physiological factors and matrix type on mobilization of soil bound contaminants.

Comparison of Digestive Fluid and Water

We wanted to determine whether digestive fluid was a better solubilizer of soil-bound HOCs compared to water, therefore we compared mobilization of chrysene from soil using water and digestive fluids. It was not surprising to find that digestive fluids

resulted in higher bioaccessibility values (1.5-fold) compared to experiments with only water (no gastrointestinal components). The fraction of chrysene in the aqueous compartment had lowered dramatically. We studied this only at one time point (48 hours). A time-course study might have revealed longer equilibration times. Because the water phase had lower capacity to hold chrysene compared to digestive fluid, the rate of uptake into EVA would also be expected to be lower which would result in prolonged period to reach equilibrium. Our results agree with several other studies that indicated that extractability by digestive fluid might be more efficient and a far better measure of bioavailability than water (Mayer et al., 1996; Weston and Mayer, 1998). For example, partitioning coefficients derived from the sediment and the digestive fluid were consistently smaller than those derived between sediment and seawater, reflecting greater PAH solubility in digestive fluid (Weston and Mayer, 1998). Mayer et al. (1996) found that digestive fluids solubilized 0-10% of the total PAH loadings and seawater controls mobilized a maximum of 0.2% of the total PAH. Similar observations were made by Hack and Selenka (1996), who also found that neither water alone nor artificial saliva could mobilize considerable amounts of PAH from contaminated materials. Most of these studies attributed the enhanced mobilization of PAHs to the organic matter content of the digestive fluids indicated by the dark color of the fluid (Mayer et al., 1996), surfactant activity indicated by frothiness during pipetting and presence of amino acids (Mayer et al., 1996; Weston and Mayer, 1998). We attribute the enhanced mobilization of soil-bound chrysene observed in our study to neutralization after exposure to low pH of gastric fluids. In addition, buffering capability of digestive fluids may be a factor influencing the enhanced mobilization of chrysene.

Neutralization (pH Level)

When we investigated the effect of gastric fluid neutralization on the mobilization of chrysene from contaminated soil, we found that in the absence of $NaHCO_3$, the pH remained low (2.5), and ~ 3.5 fold less concentration of chrysene was mobilized into the aqueous phase and ~2 fold lower in the EVA film. We also observed that Tris could replace $NaHCO_3$ as both experiments resulted in a similar extent of mobilization of soil-bound chrysene indicating that it is the pH shift from 2.5 to 7 rather than $NaHCO_3$ per se that was responsible for causing enhanced mobilization. The effect of neutralization was not due to activation of intestinal enzymes or efficiency of bile salt mobilization because eliminating either of these components had no effect on chrysene mobilization. The increase in PAH desorption with increasing pH in our study may be explained by pH related changes in the humic structure as acidic groups become deprotonated and charged, thereby facilitating diffusion of PAH molecules through the organic matrix and to the water interface. Fulvic and humic acids are protonated at lower pH and therefore would likely bind to chrysene by non-covalent interactions (Engebretson and Wandruszka, 1994). At neutral pH, humic acids would be negatively charged and would repel chrysene due to the negative charge of the electron clouds of chrysene. We also observed visually that less colour developed in the digestive fluid when pH was low (2.5) compared to the darker colour at higher pH (7) which could be due to the relative insolubility of humic substances in their partially protonated forms in the acidic gastric environment.

The effect of pH on the mobilization of metals has been studied by many researchers but studies with HOCs like PAHs is limited. According to Hack and Selenka (1996), higher efficiency of pollutant mobilization by the intestinal juice compared with the gastric juice could be due to the presence of micelles, which are formed in aqueous

suspension by bile constituents. In their study, mobilization of pollutants under gastro-intestinal conditions was always more efficient than mobilization with the corresponding gastric juice, however, they did not test this hypothesis directly. In our study, the presence of bile salts did not enhance the mobilization of soil-bound chrysene. Engebretson et al. (1994) suggested that humic and fulvic materials dissolved in aqueous solution have a tendency to form micelle-like structures, with hydrophilic shells facing the aqueous phase and hydrophobic cores in the centre of the aggregates. This self-organized structure acts to enhance the solubility of HOCs in such solutions by allowing them to penetrate the hydrophilic shells. Yang et al. (2001) also found that desorption of soil-bound PAH compounds was pH dependent, with minimal release occurring at pH 2-3 and maximal release at pH 7-8 which is in agreement with the results from our study. Diffusion through the condensed SOM phase is more hindered than amorphous SOM because of its less polar nature and therefore contributes to the slow adsorption of HOCs, such as PAH compounds (Hatzinger and Alexander, 1995; Weber and Huang, 1996; Yeom et al, 1996; Luthy et al, 1997). Yang et al. (2001) indicated that soil-bound PAH desorption can be increased in the presence of chelating agents accompanied by considerable release of organic matter into solution. According to them, SOM is bound to inorganic matter by polyvalent metal ion bridges and these metal ions may serve as cross-linking agents within the organic phase by binding to multiple carboxy or phenolate groups from different strands of the humic macromolecues. Chelating agents like oxalate or citrate remove metal ions resulting in release of organic macromolecues and sorbed PAHs into the aqueous phase. Removal of the cross-liking agents in SOM can lead to change in SOM phase properties from a compact state to a more flexible or open state resulting in increased diffusivities of PAHs.

Effect of GI Tract Enzymes and Bile Salts

We investigated the effects of digestive enzymes and bile salts on mobilization of soil-bound chrysene. Pepsin is one of the most important of the digestive enzymes; it hydrolyses peptide bonds in proteins and polypeptides with a low degree of specificity, and it has been suggested that hydrolysed products perhaps aid in increasing solubilization (Rodriguez et al., 1999). In our study, when pepsin was eliminated from the in vitro digestion protocol, no significant change in chrysene mobilization was observed compared to the complete digestive fluids. A pepsin substitute, BSA, was substituted to maintain a similar protein content without the enzymatic activity of pepsin. BSA instead of pepsin increased the extent of chrysene uptake by EVA probably because of the higher percentage of chrysene that desorbed into the aqueous fraction. The higher solubilization potential of BSA compared to pepsin could be due to hydrophobic solubilization processes within the interior of the globular domain (Lawrence et al., 1996). BSA appears to have increased the fugacity capacity of the aqueous phase by providing binding sites for chrysene molecules. BSA itself may have some fugacity capacity for chrysene, which ultimately increases the total fugacity capacity of digestive fluids. The molar concentration of BSA added to the digestive fluids was half the molar concentration of pepsin, suggesting that the protein content may be an important factor responsible for increasing the aqueous phase concentration of chrysene. An increase in extraction of mono methyl mercury from sediment with increasing concentration of BSA was observed by Lawrence et al. (1996). Similar observation was also made by Chen and Mayer (1998) who suggested that dissolved total amino acids due to increase in BSA concentrations led to increased Cu release. Greater solubilization of PAHs due to the presence of higher amino acids has also been suggested by Mayer et al. (1996).

Absence of all intestinal constituents (bile salts, pancreatin, tris, calcium chloride) of digestive fluids did not change the extent of mobilization of chrysene from the contaminated soil, which agrees with the finding of Weston and Mayer (1998a) who observed lack of correlation between enzyme activities in the digestive fluid (lipase, protease, esterase, amylase, and cellulase) and the extent of PAH mobilization.

Bile salts are known to form mixed micelles with fatty acids, which act as transporters that are able to traverse the unstirred water layer adjacent to the intestinal wall (Mansbach et al., 2001). If hydrophobic compounds get incorporated into these mixed micelles they may be transported across the intestinal epithelium. Although a compound is thought to be absorbed in the freely dissolved form; it seems likely that lipophilic compounds sorbed to micelles and proteins may be available for absorption after digestive degradation and disintegration (Oomen et al., 2000). These authors suggested that bile salt micelles play a central role in mobilizing HOCs from a matrix and in making the HOCs bioaccessible. They found that 20-25% of total amount of HOCs (PCBs and Lindane) were sorbed to bile salt micelles; however the bile salt concentration used in their study was ~3-fold higher than the concentration used in our study. Weber and Lanno (2001) suggested that lipids might decrease intestinal uptake of lipophilic contaminants if they remain in unabsorbable excess in the intestinal lumen by retaining BaP in lipid/bile micelles whereas if triglycerides are hydrolysed into monglycerides/free fatty acid prior to absorption, lipophilic contaminant uptake may be facilitated by micelles. However, we did not observe any change in mobilization of soil-bound chrysene when bile salts were present or absent in the digestive fluids. Rather, changes in pH were the most important factor that mobilized PAH from soil.

Caco-2 Cells

The Caco-2 cell line represents an appropriate model for the study of intestinal transport mechanisms and for investigating diverse questions regarding nutrient and drug bioavailability and absorption. Several studies have been conducted primarily to study factors affecting uptake and metabolic functioning of different elements and a great deal of research has used Caco-2 cells to study drug bioavailability (Artursson and Karlsson, 1991; Artursson, 1991; Artursson and Borchardt, 1997; Ismail, 1999; Hilgers et al., 2003). Little if any effort has been devoted to development of Caco-2 cells system to estimate bioavailability of xenobiotics.

Wilcockson and Gobas (2001) suggested that there might be a useful relationship between lipid-normalized concentration in biological tissue and EVA film concentration. In our study, we therefore determined the lipid content of the Caco-2 cells and used the volume of lipid in these cells for fugacity calculations. However, differences in lipid solubility among various classes of lipids exist that can cause differences between lipid-normalized and thin film concentrations (Wilcockson and Gobas, 2001). Nevertheless, the entire lipid fraction which would contain phospholipids, triglycerides as well as sterols was extracted from Caco-2 cells for comparison with EVA.

In our experiments, Caco-2 cells were used to study partitioning of contaminants and to compare it with partitioning into EVA. Although both experiments comparing Caco-2 and EVA reached equilibrium within 6 hours, the extent of uptake by cells was significantly different in the two experiments. The technique of cell experiment needs to be improved and more comparative experiments are needed to make any firm conclusions. We should have exposed cells and EVA to the contaminant in the same experiment to measure the uptake from water phase in order to make better comparisons and to reduce the effect of confounding factors. The rates of uptake were

also significantly different between the two experiments. Similar kinetics of partitioning in both EVA and Caco-2 cells was observed, although EVA was found to have ~23-fold higher fugacity capacity than Caco-2 cells. This implies that EVA might be a good model for predicting relative oral bioavailability of HOCs in vivo.

Merits of the In Vitro Digestion Model

In vitro digestive fluid extraction is an effective and rapid technique for assessing the bioavailability of contaminants from ingested soils/sediments. The model employed in this study is unique as it combined digestive extraction technique along with an artificial absorptive membrane, EVA.

Human intestinal cell line, Caco-2 was used for comparison to EVA, however, using cell line for absorption/bioavailability studies has some limitations. Caco-2 cells are expensive to culture and maintain. Technical expertise i.e. person trained in tissue culture is required for model involving cells whereas EVA model does not require personnel with expertise. Caco-2 cells possess biotransformation enzymes and hence could cause metabolism of the compound being investigated. Cells are very sensitive and easily damaged, and may lift of the coverslips even when fixed whereas EVA films are tough and are not damaged easily. Because Caco-2 cells did not reach equilibrium when digestive fluid was used, PBS-Tween had to be used in order to shorten the time to reach equilibrium.

Caco-2 cell line has the advantage over EVA in being actual biota or tissue and not just a surrogate like EVA, so takes away certain assumptions associated with EVA model. EVA thickness is dependent on the individual coating the coverslips or vials and is not always consistent, however, it does not have the metabolic capabilities and has higher fugacity capacity.

An in vitro model facilitates investigating variables systematically and allows manipulations of soils/sediments or digestive fluid characteristics that would not be possible in vivo. For example, in our study we determined the effect of presence and absence of various gastrointestinal components. It is known that different soils are known to give different bioavailability or bioaccessibility values (Freeman et al., 1992; Ruby et al., 1996; Hamel et al, 1998); therefore, in vitro model is good for investigating the effects of a soil matrix on oral bioavailability. This model also facilitates site-specific investigations. Synthetic digestive fluids are necessary for routine and widespread use to avoid sacrificing lot of lab animals and for economical reasons. This approach may also provide a mechanism for avoiding both the physiological constraints and social barriers associated with in vivo experiments using lab animals.

The physiologically-based extraction technique combined with artificial intestinal membrane used in this study provides a potentially valuable approach for refining the input data used for formal risk assessment at sites subject to natural or anthropogenic soil contamination. Data obtained by in vitro gastrointestinal mobilization model may be helpful, both for local authorities and for experts, in assessing the health risk arising from polluted sites or from polluted materials. By estimating the fraction of soil contaminants available for human absorption, modelled risks will become more realistic and valid. At the present time, it is difficult to say whether a generic standard could be created because it is known that bioavailability of HOCs is dependent on type of compound and type of soil. In the long run, it would be ideal to have simple, bioavailability-predictive extraction tests for all contaminants of human health concern and for all matrices, so that risk for soil borne contaminants could be assessed based on the site–specific fraction of bioavailable contaminants. The gastrointestinal model presented herein is a suitable

technique to measure the quantities of contaminants that may be mobilized under physiological conditions in the gastrointestinal tract. Thus in vitro digestion model is a promising tool for studying the effect of the ingestion matrix on bioaccessibility.

Limitations of In Vitro Digestion Model

Hydrophobic compounds are absorbed by passive diffusion across the gut wall, and it is likely that a hydrophobic molecule dissolved within the digestive fluid would rapidly partition into the lipid membrane lining the gut lumen. Gastrointestinal absorption of HOCs in humans varies with the age, diet, and nutritional status of the subjects (Ruby et al., 1999). Our in vitro model used radiolabeled compounds to determine the absorption of the solubilized contaminant across the gut wall. It should be recognized that sorption and desorption behaviour of spiked PAHs are not equivalent to the identical compounds that may have had greater opportunity for aging or associated with different soil/sedimentary or contaminated (NAPL) phases. The radiolabeled compounds provide an exquisitely sensitive means to study partitioning but cannot be used as surrogate to predict environmental fate of in situ contaminants. The research described in this report was focussed on developing a sampling method that mimics intestinal mobilization, but of course actual gastrointestinal absorption is more complex than a chemical extraction tool such as in vitro digestion model with EVA film.

Conclusions

- We observed that ~50% of the added chrysene partitioned into the EVA indicating that the total extraction using chemicals may overestimate bioavailability.
- Digestive fluid is ~1.5-fold better solubilizer of HOCs like chrysene than water suggesting that using water for predicting bioavailability will underestimate the mobilized fraction of contaminant.

- Enzymatic hydrolysis by digestive enzymes did not affect the mobilization of soil-bound chrysene indicating that the partitioning of soil-bound contaminant to EVA through aqueous phase is driven primarily due to existence of a fugacity gradient.
- Neutralization of gastric content significantly increased the mobilization of soil-bound chrysene implying that it is the neutral or slightly alkaline pH that facilitates mobilization from soil organic matter into digestive fluids and further partitioning into EVA and/or intestinal epithelium.
- Ageing of chrysene onto soil did not enhance the binding affinity of chrysene to soil and the same proportion of chrysene was mobilized from aged soil as from non-aged soil indicating that soils with no NAPLs have only one compartment.
- Even though the fugacity capacity of EVA was ~23-fold higher than Caco-2 cell line, the kinetics of partitioning from aqueous to EVA or cells was similar suggesting that EVA may be a good model for predicting bioavailability.
- Difference in the rate constants describing movement of chrysene from soil to EVA and chrysene solution to EVA clearly shows that binding to soil limits the mobilization of soil-bound contaminants.
- An in vitro digestive model with artificial intestinal epithelium/EVA may be a useful tool to predict relative bioavailability in vivo.

Future Directions

Many questions remain unanswered regarding mobilization of soil bound contaminants. The following lists some issues that need to be addressed to be able to understand the underlying mechanism of mobilization from soil and absorption by EVA film.

- Work with different classes of compounds and a wide range of K_{ows} in order to investigate whether compounds with similar kows but different structures result in

similar bioavailabilities, and whether any correlation can be established between K_{ows} and % bioavailability.

- Test highly contaminated and aged soil and compare with spiked soil samples to determine the effect of NAPLs and ageing on desorption from soil to aqueous phase.
- Compare mobilization of soil-bound contaminants from soils with different organic matter content because several studies have shown that different types of organic matter (humin, humic and fulvic acids) have different affinities for HOCs.
- Investigate dietary effects on mobilization by varying the quantity of digestive fluids and by using different types of fats as studies have shown that food and fats can affect bioavailability.
- More comparative studies with Caco-2 cells and EVA are required to find correlation between the two regarding uptake of contaminants (rates and extents).
- Experimentally determine fugacity capacity for different compartments where possible rather than using literature values to reduce error and increase accuracy of estimates.
- Lastly, in vitro tests need to be validated by in vivo studies. Good correlations between the two methods may lead to the development of mathematical relationships from which predictions can be made to derive bioavailable concentrations in soils for risk estimates that have a lower degree of uncertainty and aid in the design and cost-effectiveness of remedial strategies at contaminated sites.

REFERENCES

Adam, A., Marzuki, A., Abdul-Rahman, H., Abdul-Aziz, M., 1997. The oral and intratracheal toxicities of roundup and its components to rats. Vet. Hum. Toxicol. 39: 147-151.

Alexander, R.R. and Alexander, M., 1999. Genotoxicity of two polycyclic aromatic hydrocarbons decline as they age in soil. Environ. Technol. Chem. 18: 1140-1143.

Alexander, M., 1995. How toxic are toxic chemicals in soil? Environ. Sci. Technol. 29: 2713-2717.

Alexander, M., 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. Environ. Sci. Technol. 34: 4259-4265.

Amellal, N., Portal, J.M., Berthelin, J., 2001. Effect of soil structure on the bioavailability of PAHs within aggregates of a contaminated soil. Appl. Geochem. 16: 1611-1619.

Aronstein, B.N., Calvillo, Y.M., Alexander, M., 1991. Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. Environ. Sci. Technol. 25: 1728-1731.

Artursson P., 1991. Cell cultures as models for drug absorption across the intestinal mucosa. Crit. Rev. Ther. Drug Carrier Syst. 8: 305-330.

Artursson, P. and Borchardt, R., 1997. Intestinal drug absorption and metabolism in cell cultures: Caco-2 and beyond. Pharm. Res. 14: 1655-1658.

Artursson, P., Karlsson, F.J., 1991. Correlation between oral drug absorption in humans and apparent drug permeability coefficients of human intestinal epithelia (Caco-2) cells. Biochem. Biophys. Res. Comm. 175: 880-885.

ATSDR, 1995. Toxicological Profile for polycyclic aromatic hydrocarbons (PAHs). Agency for toxic substances and disease registry, Atlanta, GA. Accessed online at http://www.atsdr.cdc.gov/toxprofiles/tp69.html

Belfroid, A.C., Sijm, D.T.H.M., van Gestel, C.A.M., 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. Environ. Rev. 4: 276-299.

Bodzek, D., Luks-Betlej, K., Jonoszka, B., 1998. Occurrence of PAHs in various elements of environment in Zabrze (Upper Silesia, Poland). Water, Air, Soil Pollut. 103: 86-91.

Bordelon, N.R., Donnelly, K.C., King, L.C., Wolf, D.C., Reeves, W.R., George, S.E., 2000. Bioavailability of the genotoxic components in coal tar contaminated soils in Fischer 344 rats. Toxicol Sci. 56: 37-48.

Brewster, D.W., Warren, J., Hopkins, W.E., 1991. Metabolism of glyphosate in Sprague Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived material following a single oral dose. Fundam. Appl. Tox. 17: 43-51.

Buckpitt, A.R., Frankin, R.B., 1989. Relationship of naphthalene and 2-methlylnaphthalene metabolism to pulmonary bronchiolar epithelial cell necrosis. Pharmacol. Ther. 41: 393-410.

Busche, U., Hirner, A.V., 1997. Mobilization potential of hydrophobic organic compounds in contaminated soils and waste materials. Part II: mobilization potential of PAHs, PCBs, and phenols by natural waters. Acta Hydro. Hydrobiol. 25: 248-252.

Calabrese, E., Barnes, R., Stanek III, E.J., Pastides, H., Gilbert, C.E., Veneman, P., Wang, X., Lasztity, A., Kostecki, P.T., 1989. How much soil do young children ingest: an epidemiologic study? Reg. Toxicol. Pharm.10: 123-137.

Calabrese, E.J., Stanek, E.J. and Barnes, R., 1996. Methodology to estimate the amount and particle size of soil ingested by children: implications for exposure assessment at waste sites. Reg. Toxicol. Pharm. 24: 264-268.

Calabrese, E.J., Stanek, E.J., James, R.C. and Roberts, S.M., 1997. Soil ingestion: a concern for acute toxicity in children. Environ. Health Perspect. 105: 1354-1358.

Cameotra, S.S., Bollag, J.M., 2003. Biosurfactant-enhanced bioremediation of PAHs. Crit. Rev. Environ. Sci. Technol. 30: 111-126.

Carmichael, L.M., Pfaender, F.K., 1997. Polynuclear aromatic hydrocarbon metabolism in soils: relationship to soil characteristics and preexposure. Environ. Tox. Chem. 16: 666-675.

Carmichael, L.M., Pfaender, F.K., 1997. Effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. Biodegradation, 8: 1-13.

Carmichael, L.M., Russell, R.C., Pfaender, F.K., 1997. Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. Environ. Sci. Technol. 31: 126-132.

Casteel, S.W., Cowart, R.P., Weis, C.P., Henningsen, M., Hoffman, E., Brattin, W.J., Guzman, R.E., Starost, M.F., Payne, J.T., Stockham, S.L., Becken, S.V., Drexler, J.W., Turk, J.R., 1997. Bioavailability of lead to juvenile swine dosed with soil from the smuggler mountain NPL site of Aspen, Colorado. Fundam. Appl. Toxicol. 36: 177-187.

Cerniglia, C.E., 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. Adv. Appl. Microbiol. 30, 31-71.

Chantal D.J., Rachel, R., Christian, M., and Chaplain, V., 2000. Use of surfactants to enhance the removal of PAHs from soil. Polycyclic Aromatic Compounds, 20: 143-154.

Charman, W.N., Porter, C.J.H., Mithani, S., Dressman, J.B., 1997. Physico-chemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH. J. Pharm. Sci. 86: 269-282.

Chen, Z., Mayer, L.M., 1998. Mechanisms of Cu solubilization during deposit feeding. Environ. Sci. Technol. 32: 770-775.

Chiou, C.T., McGroddy, S.E., Kille, D.E., 1998. Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments. Environ. Sci. Technol. 32: 264-269.

Chung, N., Alexander, M., 1998. Difference in sequestration and bioavailability of organic compounds aged in dissimilar soils. Environ. Sci. Technol. 32: 855-860.

Chung, N., Alexander, M., 1999. Effect of concentration on sequestration and bioavailability of two polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 33: 3605-3608.

Cornelissen, G., Rigterink, H., Ferdinandy, M.M., van Noort, P.C.M., 1998. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. Environ. Sci. Technol., 32: 966-970.

Creamer, B., 1974. Intestinal structure in relationship to absorption. Biomembranes, 4(A): 1-42.

Danielsson, A., 2000. Rate-limiting factors during bioremediation of soil containing HOCs. A Literature Study. Chemical Engineering, Lund Institute of Technology.

Darrell, R., Campen, V., Glahn, R.P., 1999. Micronutrient bioavailability techniques: Accuracy, problems and limitations. Field Crops Res. 60: 93-113.

Davis, A., Drexler, Ruby, M.V., Nicholson, A., 1993. Micromineralogy of mine wastes in relation to lead bioavailability, Butte, Montana. Environ. Sci. Technol. 27: 1415-1425.

Davis, A., Ruby, M.V., Bergstrom, P.D., 1994. Factors controlling lead bioavailability in the mining district, Montana, USA. Environ. Geochem. Health. 16: 147-157.

Davis, A., Ruby, M.V., Bergstron, P.D., 1992. Bioavailability of arsenic and lead in soils from the Butte, Montana, mining district. Environ. Sci. Technol. 26: 461-468.

Davis, S., Waller, P., 1990. Quantitative estimates of soil ingestion in normal children between the ages of 2 and 7 years: population-based estimates using aluminium, silicon, and titanium as soil tracer elements. Arch. Environ. Health. 45: 112-122.

De Sousa, C., 2001. Contaminated sites: The Canadian situation in an international context. J. Environ. Mgmt. 62: 131-154.

Degen, L.P.. Philips, S.F., 1996. Variability of gastrointestinal transit in healthy women and men. Gut. 39: 299-305.

Dennis, M.J., Massey, R.C., Grips, G., Venn, L., Howarth, N., Le, G., 1991. Factors affecting the polycyclic aromatic hydrocarbon content of cereals, fats and other food products. Food Addit. Contam. 8: 517-530.

Dieter, M.P., Matthews, H.B., Jeffcoat, R.A., Moseman, R.F., 1993. Comparison of lead bioavailability in F344 rats fed lead acetate, lead oxide, lead sulphide, or lead ore concentrate from Skagway, Alaska. J. Toxicol. Environ. Health. 39; 79-93.

DiToro, D.M., Zabra, C.S., Hansen, D.J., Berry, W.J., Swartz, R.C., Cowan, CE., Pavlou, S.P., Allen, H.E., Thomas, N.A., Paquin, P.R., 1998. Technical basis for establishing sediment quality criteria for non-ionic organic chemicals using equilibrium partitioning. Environ. Toxicol. Chem. 10: 1541-1583.

Doong, R., Lei, W., 2003. Solubilization and mineralization of polycyclic aromatic hydrocarbons by *Pseudomonas putida* in the presence of surfactant. J. Haz. Mat. B96: 15-27.

Duggan, C., Jennifer, G., Walker, W.A., 2002. Protective nutrients and functional foods for the gastrointestinal tract. Am. J. Clin. Nutr. 75: 789-808.

Ederbach, P.L., 1999. Influence of incubation temperature on the behaviour of triethylamine-extractable glyphosate (N-phosphonomethylglycine) in four soils. J. Agric. Food. Chem. 47: 2459-2467.

Ederbach, P.L., Douglas, L.A., 1991. Method for the determination of glyphosate and (aminomethyl)phosphonic acid in soil using electron capture gas chromatography. J. Agric. Food Chem. 39: 1776-1780.

Ellickson, K.M., Meeker, R.J., Gallo, M.A., Buckley, B., Lioy, P.J., 2001. Oral bioavailability of lead and arsenic from a NIST standard reference soil material. Arch. Environ. Contam. Toxicol. 40: 128-135.

Engebretson, R.R., Von Wandruszka, R., 1994. Microorganization in dissolved humic acids. Environ. Sci. Technol. 28: 1934-1941.

Erickson, C.C., Loehr, R.C., Neuhauser, E.F., 1993. PAH loss during bioremediation of manufactured gas plant site soils. Water Res. 27: 911-919.

Errampalli, D., Trevors, J.T., Lee, H., Leung, K., Cassidy, M., Knoke, K., Marwood, T., Shaw, K., Blears, M., Chung, E., 1997. Bioremediation: A perspective. J. Soil Contam., 6: 207-218.

Freeman, G.B., Dill, J.A., Johnson, J.D., Kurtz, P.J., Parham, F., Matthews, H.B., 1996. Comparative absorption of lead from contaminated soil and lead salts by weanling Fisher 344 rats. Fundam. Appl. Toxicol. 33: 109-119.

Freeman, G.B., Johnson J.D., Killinger, J.M., Siao, S.C., Davis, O., Ruby, M.V., Dhaney, R.L, Lovre, S.C., Bergstrom, P.D., et al., 1993. Bioavailability of arsenic in soil impacted by smelter activities following oral administration in rabbits. Fundam. Appl. Toxicol. 21: 83:88.

Freeman, G.B., Johnson, J.D., Killinger, J.M., Liao, S.C., Feder, P.I., Davis, A.O., Ruby, M.V., Chaney, R.L., Lovre, S.C., Bergstrom, P.D., 1992. Relative bioavailability of lead from mining waste soil in rats. Fundam. Appl. Toxicol. 19: 388-398.

Freeman, G.B., Johnson, J.D., Liao, S.C., Feder, PI., Davis, A.O., Ruby, M.V., Schoof, R.A., Chaney, R.L., Bergstrom, P.D., 1994. Absolute bioavailability of lead acetate and mining waste lead in rats. Toxicology 91: 151-163.

Freeman, G.B., Schoof, R.A., Ruby, M.V., Davis, A.O., Dill, J.A., Liao, S.C., Lapin, C.A., Bergstrom, P.D., 1995. Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. Fundam. Appl. Toxicol. 28: 215-555.

Fries, G.F., 1989. Influence of soil and matrix characteristics on the uptake of chemical contaminants. Comm. Toxicol. 3: 447-468.

Garbarini, D.R., Lion, L.W., 1986. Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. Environ. Sci. Technol. 20: 1263-1269.

Gasser, U.G., Walker,, W.J., Dahlgren, R.A., Borch, R.S., 1996. Lead release from smelter and mine waste impacted materials under simulated gastric conditions and relation to speciation. Environ. Sci. Technol. 30: 761-769.

Gauthier, T.C., Scitz, W.R., Grant, C.I., 1987. Effects of structural and compositional variations of dissolved humic materials on pyrene Koc values. Environ. Sci. Technol. 21: 243-248.

Ghassemi, M., Quinlivan, S., Dellarco, M., 1982. Environmental effects of new herbicides for vegetation control in forestry. Environ. Intl. 7: 389-402.

Gobas, F.A.P.C, Wilcockson, J.B., Russell, R.W., Haffner, G.D., 1999. Mechanism of biomagnification in fish under laboratory and field conditions. Environ. Sci. Technol. 33: 133-141.

Gobas, F.A.P.C., Mackay, D., 1987. Dynamics of hydrophobic organic chemical bioconcentration in fish. Environ. Tox. Chem. 6: 495-504.

Gobas, F.A.P.C., Zhang, X., Wells, R., 1993. Gastrointestinal magnification: The mechanism of biomagnification and food chain accumulation of organic chemicals. Environ. Sci. Technol. 27: 2855-2863.

Government of Canada. 1999. Canadian Environmental Protection Act, 1999. Canada Gazette Part III. Vol. 22, No. 3.

Groen, K., Vaessen, H.A.M.G., Liest, J.J.G., de Boer, J.L.M., van Ooik, T., Timmerman, A., Vlug, R.F., 1994. Bioavailability of inorganic arsenic from bog ore-containing soil in the dog. Environ. Health Perspect. 102: 182-184.

Gu, M.B. and Chang, S.T., 2001. Soil biosensor for the detection of PAH toxicity using an immobilized recombinant bacterium and a biosurfactant. Biosensors and Bioelectronics 16: 667-674.

Guha and Jaffe, 1996a. Biodegradation kinetics of phenanthrene partitioned into the micellar phase of non-ionic surfactants. Environ Sci. Technol. 30: 605-611.

Guha and Jaffe, 1996b. Bioavailability of hydrophobic compounds partitioned into the micellar phase of non-ionic surfactants. Environ. Sci. Technol. 30: 1382-1391.

Guthrie, E.A., Bortiatynski, J.M., Van Heemst, J.D.H., Richman, J.E., Hardy, K.S., Kovach, E.M., Hatcher, P.G., 1999. Determination of (^{13}C) Pyrene sequestration in sediment microcosms using flash pyrolysis-GC-MS and ^{13}C NMR. Environ. Sci. Technol. 33:119-122.

Guthrie, E.A., Pfaender, F.K., 1998. Reduced pyrene bioavailability in microbially active soils. Environ. Sci. Technol. 32: 501-508.

Hack, A., and Selenka, F. 1996. Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicol. Lett. 88, 199-210.

Hamel, S.C., Buckley, B. and Lioy, P.J., 1998. Bioaccessibility of metals in soils for different liquid to solid ratios in synthetic gastric fluid. Environ. Sci. Technol. 32: 358-362.

Hamel, S.C., Ellickson, K.M., Lioy, P.J., 1999. The estimation of the bioaccessibility of heavy metals in soil using artificial biofluids by two novel methods: mass-balance and soil recapture. Sci. Tot. Environ. 243/244: 273-283.

Harner, T., 1998. Octanol-air partition coefficient for describing particle/gas partitioning of aromatic compounds in urban air. Environ. Sci. Technol. 1998, 32: 1494-1502.

Harner, T., Farrar, N.J., Shoeib, M. Jones, K.C., Gobas, F.A.P.C., 2003. Characterization of polymer-coated glass as a passive air sampler for persistent organic pollutants. Environ. Sci. Technol. 37: 2486-2493.

Hatzinger, P.B., Alexander M., 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. Environ. Sci. Technol. 29:537-545.

Hatzinger, P.B., Alexander, M., 1997. Biodegradation of organic compounds sequestered in organic solids or in nanopores within silica particles, Environ. Sci. Technol. 16: 2215-2221.

Hillgers, A.R., Smith, D.P., Biermacher, J.J., Day, J.S., Jensen, J.L., Sims, S.M., Adams, W.J., Friis, J.M., Palandara, J., Hosley, J.D., Shobe, E.M., Burton, P.S., 2003. Predicting oral absorption of drugs: A case study with a novel class of antimicrobial agents. Pharm. Res. 20: 1149-1155.

Hillgren, K.M., Kato, A., and Borchardt, R.T., 1995. In vitro systems for studying intestinal drug absorption. Med. Res. Rev.15: 83-109.

Hippelein, M. and McLachlan, M.X., 1998. Soil/air partitioning of semivolatile organic compounds 1. Method development and influence of physical-chemical properties. Environ. Sci. Technol. 32: 310-316.

Huckins, J.N., Manuweera, G.K., Petty, J.D.M., Mackay, D., Lebo, A.A., 1993. Lipid-containing semipermeable membrane devices for monitoring organic contaminants in water. Environ. Sci. Technol. 27: 2469-2496.

Huesemann, M.H., Hausmann, T.S., Fortman, T.J., 2002. Microbial factors rather than bioavailability limit the rate and extent of PAH biodegradation in aged crude oil contaminated model soils. Biorem. J. 6: 321-336.

Hurst, C.J., Sims, R.C., Sims, J.L., Sorensen, D.L., McLean, J.E., Huling, S., 1996. PAH biodegradation as a function of oxygen tension in contaminated soil. J. Haz. Mat. 51: (1-3): 193-208.

Hwang, S. and Cutright, T.J., 2002. Biodegradability of aged pyrene and phenanthrene in a natural soil. Chemosphere. 47: 891-199.

IARC, 1983. Polynuclear Aromatic Compounds. Part 1. Chemical, environmental and experimental data. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol 32. International Agency for Research on Cancer.

Ismali. M., 1999. The use of Caco-2 cells as an in vitro method to study bioavailability of iron. Mal. J. Nutr. 5: 31-45.

Johnson, M.D., Weilin, H., Weber, W.J., 2001. A distributed reactivity model for sorption by soils and sediments. 13. Simulated diagenesis of natural sediment organic matter and its impact on sorption/desorption equlibria. Environ. Sci. Technol. 35: 1680-1687.

Johnson, M.D. and Weber, W.J., 2001. Rapid prediction of long-term rates of contaminant desorption from soils and sediments. Environ. Sci. Technol. 35: 427-433.

Juhasz, A.L. and Naidu, R., 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int. Biodet. and Biodeg. 45: 57-88.

Kadry. A.M., Turkall, R.M., Skowronski, G.A., Abdul-Rahman, M.S., 1991. Soil adsorption alters kinetics and bioavailability of trichloroethylene in orally exposed female rats. Tox. Lett. 58: 337-346.

Kalra,Y.P. and Maynard, D.G., 1991. Forestry Canada. Methods manuals for forest soil and plant analysis. Report No. R-X-319.

Kan, A.T., Gongmin, F., Tomson, M.B., 1994. Adsorption/desorption hysteresis in organic pollutant and soil/sediment interaction. Environ. Sci. Technol. 28: 859-867.

Karimi-Lotfabad, S., Pickard, M.A., Gray, M.R., 1996. Reactions of PAHs on soil. Environ. Sci. Technol. 30: 1145-1151.

Kelsey, J.W. and Alexander, M., 1997. Declining bioavailability and inappropriate estimation of risk of persistent compounds. Environ. Toxicol. Chem. 16: 582-585.

Kelsey, J.W., Kottler, B.D., Alexander, M., 1997. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. Environ. Sci. Technol. 31: 214-217.

Kluska, M., 2003. Soil contamination with polycyclic aromatic hydrocarbons in the vicinity of the ring road in Siedlce City. Polish J. of Environ. Studies. 12 : 309-313.

Koganti, A., Spina, D.A., Rozett, K., Ma, B., Weyand, R.H., 1998. Studies on the applicability of biomarkers in estimating the systemic bioavailability of PAHs from manufactured gas plant tar-contaminated soils. Environ. Sci. Technol. 32: 3104-3112.

Kohl, S.D., Rice, J.A., 1998. The binding of contaminants to humin: A mass balance. Chemosphere. 26: 251-261.

Kottler, B.D., Alexander, M., 2001. Relationship of properties of PAHs to sequestration in soil. Environ. Poll. 113: 293-298.

Kukkonen, J., Landrum, P.F., 1995. Measuring assimilation efficiencies for sediment-bound PAH and PCB congeners by benthic organisms. Aquat. Toxicol. 32: 75-92.

Laha, S. and Luthy, R., 1991. Inhibition of phenanthrene mineralization by non-ionic surfactants in soil-water systems. Environ. Sci. Technol. 25: 1920-1930

Landrum, P.F., 1989. Bioavailability and toxicokinetics of PAHs sorbed to sediments for the amphipod *Pontoporeia hoyi.* Environ. Sci. Technol. 23: 588-595.

Launen, L., Pinto, L., Weibe, C., Kiehlmann, E., Moore, M.M., 1995. The oxidation of pyrene and benzo(a)pyrene by nonbasidiomycete soil fungi. Can. J. Microbiol. 41: 477-488.

Lawrence, A., McAloon, K.M., Mason, R.P., Mayer, L.M., 1999. Intestinal solubilization of particle-associated organic and inorganic mercury as a measure of bioavailability to benthic invertebrates. Environ. Sci. Technol. 33: 1871-1878.

Leboeuf, E.J. and Weber, W.J., 1997. A distributed reactivity model for sorption by soils and sediments. Sorbent organic domains: Discovery of a humic acid glass transition and an argument for a polymer based model. Environ. Sci. Technol. 31: 1697-1702.

Leboeuf, E.J. and Weber, W.J., 2000. Macromolecular characteristics of natural organic matter. 2. Sorption and desorption behavior. Environ. Sci. Technol. 34: 3632-3640.

Legraverend, C., Guenthner, T.M., Nebert, D.W., 1984. Importance of the route of administration for genetic differences in benzo(a)pyrene-induced in utero toxicity and teratogenicity. Teratology. 29: 35-47.

Lee, H., 1991. A clam's eye view of the bioavailability of sediment associated pollutants. In Baker RA, Ed., Organic substances and sediments in water, Vol, 3. Lewis, Chelsea, MI, USA, pp 73-93.

Lee, H., Boese, B.L., Randall, R.C., Pelletier, J., 1990. A method for determining gut uptake efficiencies of hydrophobic pollutants in a deposit-feeding clam. Environ. Toxicol. Chem. 9: 215-219.

Leib, W.R., Stein, W.D., 1986. Transport and diffusion across cell membranes; Stein, W.D., Ed.; Academic Press Inc.: New York, p69.

Lennernas, H., 1998. Human intestinal permeability. J. Pharm. Sci. 87(4): 403-410.

Lerch, R.N., Thurman, E.M., Kruger, E.L., 1997. Mixed-mode sorption of hydroxylated atrazine degradation products to Soil: A mechanism for bound residue. Environ. Sci. Technol. 31: 1539-1546.

Leslie, H.A., Terlaalk, T.L., Busser, F.J.M., Drrak, M.H.S., Hermens, J.L.M., 2002. Bioconcentration of organic chemicals: Is a solid-phase microextraction fiber a good surrogate for biota? Environ. Sci. Technol. 36: 5399-5404.

Li, J. and Chen, B., 2002. Solubilization of model polycyclic aromatic hydrocarbons by non-ionic surfactants. Chem. Eng. Sci. 57: 2825-2835.

Liste, Hans-Holger, Alexander, M., 2003. Butanol extraction to predict bioavailability of PAHs in soil. Chemosphere. 46: 1011-1017.

Lucking, A.D., Huang, W., Soderstron-schwarz, S., Kim, M., Weber Jr., W.J., 2000. J. Environ. Qual. 29: 317-323.

Luster-Teasley, S.L., Yao, J.J., Herner, H.H., Trosko, J.E., Masten, S.J., 2002. Ozonation of chrysene; evaluation of byproduct mixtures and identification of toxic constituent. Environ. Sci. Technol. 36: 869-876.

Luthy, R.G., Aiken, G.R., Brussearu, M.L., Cunnigham, S.D., Gschwend, P.M., Pignatello, J., Reinhard, M., Traina, S.J., Weber Jr., W.J., Westall, J.C., 1997. Sequestration of hydrophobic organic contaminants by geosorbents. Environ. Sci. Technol. 31: 3341-3347.

Lydy, M.J., Landrum, P.F., 1993. Assimilation efficiency for sediment sorbed benz(a)pyrene by *Diporeia sp.* Aquatic. Toxicol. 26: 209-223.

Ma, W.C., van Kleunen, A., Imerzeel. J., de Maagd, P.G.-F., 1998. Bioaccumulation of PAHs by earthworms: assessment of equilibrium partitioning theory in situ studies and water experiments. Environ. Tox. Chem. 17: 1730-1737.

Mackay and Paterson, 1981. Calculating fugacity. Environ. Sci. Technol. 15: 1006-1014.

Mackay, 1979. Finding fugacity feasible. Environ. Sci. Technol. 13: 1218-1223

Mackay, D., 1991. Multimedia environmental models: the fugacity approach, Vol. 1, Lewis Publishers, Chelsea, Michigan.

Mackay, D., Ying Shiau, W., Ching, Ma.K., 1992. Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals; Vol. 1. Lewis Publishers: Chelsea, MI.

Macleod, C.J.A., Semple, K.T., 2000. Influence of contact time on extractability and degradation of pyrene in soils. Environ. Sci. Technol. 34: 4952-4957.

Maddaloni, M., Lolacono, N., Manton, W., Blum, C, Drexler, J., Graziano, J., 1998. Bioavailability of soil borne lead in adults, by stable isotope dilution. Environ. Health. Perspect. 106: 1489-1594.

Mader, B.T., Goss, K., Eisenreich, S.J., 1997. Sorption of non-ionic, hydrophobic organic chemicals to mineral surfaces. Environ. Sci. Technol. 31: 1079-1086.

Mansbach, C.M., Tso, P., Kuksis, A., 2001. Triacylglycerol movement in enterocytes. Intestinal Lipid Metabolism. Kluwer Academic/Plenum Publishers, New York. Pg: 215-229.

Mayer, L. M., Chen, Z., Findlay, R.H.M., Fang, J., Sampson, S., Self, R.F.L., Jumars, P.A., Quetel, Donard, O.F.X., 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. Environ. Sci. Technol. 30: 2641-2645.

Mayer, L.M., Schick, L.L., Self, R.F.L., Jumars, P.A., Findlay, R.H., Chen, Z., Sampson, S., 1997. Digestive environments of benthic macro-invertebrate guts: Enzymes, surfactants and dissolved organic matter. J. Mar. Res. 55: 785-812.

Mayer, P., Vaes, W.H.J., Winker, F., Karin, C.H.M., Kraaif, R., Tolls, J., Hermens, J.L.M., 2000. Sensing dissolved sediment porewater concentrations of persistent and bioaccumulative pollutants using disposable solid-phase microextraction fibers. Environ. Sci. Technol. 34: 5177-5183.

McCarthy, J.F. and Jinmenez, B.D., 1985. Interactions between PAHs and dissolved humic material: Binding and dissociation. Environ. Sci. Technol. 19: 1072-1077.

McKay, W.A., Memmott, S.D., 1991. An investigation of the availability of Cs and Pu for gut absorption in winkies following cooking and in vitro simulated gastro-intestinal digestion. Food Addit. Contam. 8: 781-786.

Meaney, C.M. and O'Driscoll, C.M. 2000. A comparison of the permeation enhancement potential of simple bile salt and mixed bile salt: fatty acid micellar systems using the Caco-2 cell culture model. Int. J. Pharm. 207: 21-30.

Miller, D.D., Schricker, B.R., 1981. In vitro estimation of food iron bioavailability. Am J. Clin. Nut. 34: 2248-2256.

Miller, M.M., Wasik, S.P., Huang, G-L., Shiau, W.Y., Mackay D., 1985. Relationships between octanol-water partitioning coefficient and aqueous solubility. Environ. Sci. Technol. 19: 522-529.

Minekus, M., Marteau, P., Havennar, R., Huis, V., 1995. A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. ALTA, 23: 197-200.

Morrison, D.E., Robertson, B.K., Alexander, M., 2000. Bioavailability to earthworms of aged DDT, DDE, DDD, and dieldrin in soil. Environ. Sci. Technol. 34: 709-713.

Mrozik, A., Piotrowaska-Seget, Z., Labuzek, S., 2003. Surfactant-enhanced remediation of contaminant soil: a review. Eng. Geology. 60: 371-380.

Nam, C., Alexander, M., 1998. Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. Environ. Sci. Technol. 32: 855-860.

Nam, C., Alexander, M., 1999. Effect of concentration on sequestration and bioavailability of two PAHs. Environ. Sci. Technol. 33: 3605-3608.

Nam, K., Alexander, M., 1998. Role of nonoporosity and hydrophobicity in sequestration and bioavailability: Tests with model solids. Environ. Sci. Technol. 32: 71-74.

Nebert, D.W., Jensen, N.M., Levitt, R.C., Felton, J.S., 1980. Toxic chemical depression of the bone marrow and possible aplastic anemia explainable on a genetic basis. Clin. Toxicol. 16: 99-122.

Nieman, J.K.C., Ronald, C.S.J., Sims, J.L., Sorensen, D.L., McLean, J.E., Rice, J.A., 1999. (^{14}C)Pyrene bound residue evaluation using MIBK fractionation method for creosote-contaminated soil. Environ. Sci. Technol. 33: 776-778.

Norris, D.A., Puri, N., Sinko, P.J., 1998. The effect of physical barriers and properties on the oral absorption of particulates. Advan. Drug Delivery Rev. 34: 135-154.

NTP, 1992. Technical report on toxicity studies of glyphosate (CAS N. 1071-83-6) administered in doses feed to F344/N rats and B6C3F1 mice. NIH publication 92-3135. Report of. U.S. department of health and human services, Research Triangle Park, NC, ST, USA.

Oomen, A.G, Hack, A., Minekus, M., Jeijdner, E., Cornelis, C., Schoeters, G., Verstraete, W., Wielde, T.V.C., Wragg, J., Romplelberg, C.J.M., Sips, A.J.A.M., Wijnen, J.H.V., 2002. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. Environ. Sci. Technol. 36: 3326-3334.

Oomen, A.G., Mayer, P., Tolls, J., 2000. Nonequilibrium solid phase microextraction (SPME) for determination of the freely dissolved concentration of hydrophobic organic compounds: matrix effects and limitations. Anal. Chem.,72: 2802-2808.

Oomen, A.G., Rompelberg, C.J.M., Bruil, M.A., Dobber, C.J.G., Pereboom, K.D.P.K.H., Sips, A.J.A.M., 2003. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. Arch. Environ. Contam. Toxicol. 44: 281-287.

Oomen, A.G., Sips, A.J.A.M., Groten, J.P., Sijm, D.T.H.M, Tolls, J., 2000. Mobilization of PCBs and Lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. Enivron. Sci. Technol. 34: 297-303.

Oomen, A.G., Tolls, J., Kruidenier M., Bosgra, S.S.D., Sips, A.J.A.M., Groten, J.P., 2001. Availability of PCBs and Lindane for uptake by intestinal Caco-2 cells. Environ. Health. Perspect. 109(7): 731-737.

Park, D.J.L. and Pritchard, P.H. 2002. Solubilization of polycyclic aromatic hydrocarbon mixtures in micellar nonionic surfactant solutions. Water Res. 36: 3463-3472.

Parkerton, T.F., Stone, M.A., Letinski, D.J., 2000. Assessing the aquatic toxicity of complex hydrocarbon mixtures using solid phase microextraction. Tox. Lett. 112-113: 271-282.

Paustenbach, D.J., Bruce, G.M., Chrostowski, P., 1997. Current views on the oral bioavailability of inorganic mercury in soil: Implications for health risk assessments. Risk. Anal. 17: 533-544.

Phillips, D.H., 1999. Polycyclic aromatic hydrocarbons in the diet. Mut. Res. 443: 139-147.

Pignatello, J.J., Xing, B., 1996. Mechanism of slow sorption of organic chemicals to natural particles. Environ. Sci. Technol. 30(1): 1-11.

Pinto, L., and Moore, M.M. 1999. Release of polycyclic aromatic hydrocarbons from contaminated soils by surfactant and remediation of this effluent by *Penicillium Spp.* Environ. Toxicol. Chem. 19, 1741-1748.

Potter, D.W. and Pawiiszyn, J., 1994. Rapid determination of PAHs and polychlorinated biphenyls in water using solid-phase microextraction and GC/MS. Environ. Sci. Technol. 28: 298-305.

Ramos, E.U., Meijer, S.N., Wouter, H.J.Vaes, H., Verhaar, J.M., Hermens, J.L.M., 1998. Using solid phase microextraction to determine partition coefficients to humic acids and bioavailable concentrations of hydrophobic chemicals. Environ. Sci. Technol. 32: 3430-3435.

Reeves, W.R., Mcdonald, T.J., Bordelon, N.R., George, S.E., Donnelly, K.C., 2001. Impacts of aging on in vivo and in vitro measurements of soil-bound PAH availability. Environ Sci. Technol.,35: 1637-1643.

Ressler, B.P, Kneifel, H., Winter, J., 1999. Bioavailability of PAHs and formation of humic acid-like residues during bacterial PAH degradation. Appl. Microbiol. Technol. 53: 85-91.

Robertson, B.K., Alexander, M., 1998. Sequestration of DDT and Dieldrin in soil: disappearance of acute toxicity but not the compounds. Environ. Tox. Chem. 17(6): 1034-1038.

Rodriguez, R.R., Basta, N.T., Casteel, S.W. and Pace, W.W. 1999. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ. Sci. Technol. 33: 642-649.

Ruby, M.V., Davis, A., Kempton, J.H., Drexler, J.W. and Bergstron, P.D., 1992. Lead bioavailability: dissolution kinetics under simulated gastric conditions. Environ. Sci. Technol. 26(6): 1242-1248.

Ruby, M.V., Davis, A., Nicholson, A., 1994. In situ formation of lead phosphates in soils as a method to immobilize lead. Environ. Sci. Technol. 28: 646-654.

Ruby, M.V., Davis, A., School, R., Eberie, S., Sellstone, G.M., 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ. Sci. Technol. 30: 422-430.

Ruby, M.V., Davis, A., Timothy, E.L., Schoof, R., Chaneu, R.F., Freeman, G.B., Bergstron, P., 1993. Development of an in vitro screening test to evaluate the in vivo bioaccessibility of ingested mine-waste lead. Environ. Sci. Technol. 27: 2870-2877.

Ruby, M.V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., Mosby, D.E., Casteel, S.W., Berti, W., Carpenter, M., Edwards, D. Cragin, D., Chappell, W., 1999. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. Enivron. Sci. Technol. 33: 3697-3704.

Safe, S., 1990. Polychlorinated biphenyls, (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). CRC Crit. Rev. Toxicol. 21: 51-88.

Samanta, S.K., Singh, O.V., Jain, R.K., 2002. Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends in Biotech. 20(6): 243-248.

Sambruy, Y., Ferruzza, S., Ranaldi, G., Angelis, D., 2001. Intestinal cell culture models. Cell Biol. Toxicol. 17: 301-317.

Schwarzenbach, R.P. and Westall, J., 1981. Transport of nonpolar organic compounds from surface water to groundwater, Laboratory sorption studies. Environ. Sci. Technol. 15: 1360-1367.

Sernka, T.J. and Jacobson, E.D., 1983. Gastrointestinal physiology – the essentials. Second Edition, Williams and Wilkins, Baltimore, USA.

Shea, D., 1988. Developing national sediment quality criteria. Environ. Sci. Technol., 22: 1256-1261.

Sheppard, S.C., 1998. Geophagy: who eats soil and where do possible contaminants go? Environ. Geology. 33 : 109-114.

Sheppard, S.C., Evenden, W.G., 1995. Systematic identification of analytical indicators to measure soil load on plants for safety assessment purposes. Int. J. Environ. Anal. Chem. 59: 239-252.

Sheppard, S.C., Evenden, W.G., Schwartz, W.J., 1995. Heavy metals in the environment. J. Environ. Qual. 24: 498-505.

Shimizu, Y., Takahashi, J., Matsubara, J., Ikeda, K., Matsui, S., 2002. Sorption of PAHs into liposomes (artificial cell membranes) and the effects of dissolved natural organic matter. Lakes and Reservoirs: Res. and Mgmt. 7(4): 295-299.

Shuttleworth, K.L., Cerniglia, C.E., 1995. Environmental aspect of PAH biodegradation. Appl. Biochem. Biotechnol. 54: 291-302.

Sijm, D., Kraij, R., Belfroid, A., 2000. Bioavailability in soil or sediment: exposure of different organisms and approaches to study it. Environ. Poll. 108: 113-119.

Sips, A.J.A.M., Bruil, M.A., Dobbe, C.J.G, van de Kamp, E., Oomen, A.G., Pereboom, D.P.K.H., Roimpelberg, C.J.M., Zeilmaker, M.J., 2001. Bioaccessibility of contaminants from ingested soil in humans. RIVM Report 711701012.

Sodergren, A., 1987. Solvent-filled dialysis membranes simulate uptake of pollutants by aquatic organisms. Environ. Sci. Technol. 21: 855-859.

Sprankle, P., Megit, W.F., Penner, D., 1975. Absorption action and translocation of glyphosate. Weed Sci. 23: 235-240.

Stanek III, E.J., Calabrese, E.J., 1995. Daily estimates of soil ingestion in children. Environ. Health. Persp. 103: 276-285.

Stephenson, R.G.A., Huff, J.L., Krebs, G., Howitt, C.J., 1992. Effect of molasses, sodium bentonite and zeolite on urea toxicity. Aust. J. Agric. Res. 43: 301-314.

Tang, J., Carroquini, M.J., Robertson, B.K., Alexander, M., 1998. Combined effect of sequestration and bioremediation in reducing the bioavailabilty of PAHs in soil. Environ. Sci. Technol. 32: 3586-3590.

Tang, J., Liste, H-H., Alexander, M., 2002. Chemical assays of availability to earthworms of PAHs in soil. Chemosphere. 48: 35-42.

Tang, J., Alexander, M., 1999. Mild extractability and bioavailability of polycyclic aromatic hydrocarbon in soil. Environ. Tox. Chem., 18(12): 2711-2714.

Tang, Y., Donnelly, K.C., Tiffany-Castiglioni, E., Mumtaz, M.M., 2003. Neurotoxicity of polycyclic aromatic hydrocarbons and simple chemical mixtures. J. Toxicol. Environ. Health. 66: 919-940.

Thomas D., Gauthier, T.D., Shane, E.C., Guerin, W.F., Seitz, W.R., Grant, C.L., 1986. Fluorescence quenching method for determining equilibrium constants for polycyclic aromatic hydrocarbons binding to dissolved humic materials. Environ. Sci. Technol. 20: 1162-1166.

Tremolada, P., Burnett, V., Calamari, D., Jones, K.C., 1996. Spatial distribution of PAHs in the UK atmosphere using pine needles. Environ. Sci. Technol. 30: 3570-3577.

Urso, P. and Gengozian, N., 1980. Depressed humoral immunity and increased tumor incidence in mice following in utero exposure to benzo(a)pyrene. J. Toxicol. Environ. Health. 6: 569-576.

Vasiluk, L., Pinto, L.J., Moore, M.M., 2004. Oral bioavailability of glyphosate: studies using two intestinal cell lines. (Unpublished data).

van Schooten, F.J., Moonen, E.J.C., van der Wal, L., Levels, P., Kleinjans, J.C.S., 1997. Determination of PAH and their metabolites in blood, faeces, and urine of rats orally exposed to PAH contaminated soils. Arch. Environ. Contam. Toxicol. 33: 317-322.

Van, V.P.A., 1990. Absorption and metabolism of dietary xenobiotic by the intestine of fish. Critical Rev. Aquat. Sci. 2: 185-203.

Verbruggen, E.M.J., Vaes, W.H.J., Parkerton, T.F., Hermens, J.L.M., 2000. Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concentrations of complex organic mixtures for estimation of baseline toxicity. Environ. Sci. Technol. 34: 324-331.

Verhaar, H.J.M., Busser, F.J.M., Hermens, J.L.M., 1994. A surrogate parameter for the baseline toxicity content of contaminated water: simulating bioconcentration and counting molecules. Environ. Sci. Technol. 29: 26-734.

Voparil, I., and Mayer, L.M., 2000. Dissolution of sedimentary polycyclic aromatic hydrocarbons (PAHs) into the lugworm's (*Arenicola marina*) digestive fluids. Environ. Sci. Tech. 34: 1221-1228.

Weber Jr., W.J., Huang, W., 1996. A distributed reactivity model for sorption by soils and sediments. Intraparticle heterogeneity and phase-distribution relationships under nonequilibrium conditions. Environ. Sci. Technol. 3: 881-888.

Weber, L.P. and Lanno, R.P., 2001. Effect of bile salts, lipid and humic acids on absorption of benzo[a]pyrene by isolated channel catfish (*Ictalurus punctatus*) intestine segments. Environ. Toxicol. Chem. 30: 1117-1124.

Weissenfels, W.D., Klewer, H-J., Langhoof, J., 1992. Adsorption of PAHS by soil particles influence on biodegradability and biotoxicity. Appl. Microbiol. Biotech. 36: 689-696.

Weston, D.P., Mayer, L.M., 1998a. In vitro digestive fluid extraction as a measure of the bioavailability of sediment associated polycyclic aromatic hydrocarbons; sources of variation and implications for partitioning models. Environ. Toxicol. Chem.,17: 820-829.

Weston, D.P., Mayer, L.M., 1998b. Comparison of in vitro digestive fluid extraction and traditional in vivo approaches as measures of polycyclic aromatic hydrocarbon bioavailability from sediments. Environ Toxicol. Chem. 17: 830-840.

White, J.C., Kelsey, J.W., Hatzinger, P.B., Alexander, M., 1997. Factors affecting sequestration and bioavailability of phenanthrene in soils. Environ. Tox. Chem. 18: 182-187.

White, K.L.J., Lysy, H.H. and Holsapple, M.P., 1985. Immunosuppression by PAHs: a structure-activity relationship in B6C3F1 and DBA/2 mice. Immunopharmacology. 9: 155-164.

WHO, 1983. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Polycyclic Aromatic Compounds, Part 1, chemical, Environ. Expt. Data, 32: 1-457.

WHO, 1994. Environmental Health Criteria, 159, Glyphosate. Env. Health. Criteria. 159: 177pp.

WHO, 2000. Polycyclic Aromatic Hydrocarbons. Pages 1-23.

Wilcke, W., 2000. Polycyclic aromatic hydrocarbons in soil – a review. J. Plant Nut. Soil. Sci. 163(3): 229-248.

Wilcockson, J.B., F.A.P.C. Gobas, 2001. Thin-film solid-phase extraction to measure fugacities of organic chemicals with low volatility in biological samples. Environ. Sci. Technol. 35(7): 1425-1431.

William, R. R., McDonald, T.J., Bordelon, N.R., George, S.E., Donnelly K.C., 2001. Impacts of aging on in vivo and in vitro measurements of soil-bound polycyclic aromatic hydrocarbon availability. Environ. Sci. Technol. 35. 1637-1643.

Williams, G.M., Kroes, R., Munro, I.C., 2000. Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. Reg. Toxicol. Pharm. 31: 117-196.

Williams, T.M., Rawlins, B.G., Smith, B., and Breward N., 1998. In-vitro determination of arsenic bioavailability in contaminated soil and mineral beneficiation waste from Ron Phibun, southern Thailand: A basis for improved human risk assessment. Environ. Geochem. Health. 20: 169-177.

Xing, B., Pignatello, J., 1997. Dual-model sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter. Environ. Sci. Technol. 31: 792-799.

Yang, Y., Ratte, D., Smets, B.F., Pignatello, J.J., Grasso, D., 2001. Mobilization on of soil organic matter by complexing agents and implications for polycyclic aromatic hydrocarbon desorption. Chemosphere. 43: 1013-1021.

Yeom, I-T., Ghosh, M.M., 1998. Mass transfer limitation in PAH-contaminated soil remediation. Environ. Sci. Technol. 37(8): 111-118.