A review of the current state of the art of physiologically-based tests for measuring human dermal *in vitro* bioavailability of polycyclic aromatic hydrocarbons (PAH) in soil

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Abstract

Polycyclic Aromatic Hydrocarbons are classed as Persistent Organic Pollutants, a large group of compounds that share similar characteristics. They are lipophilic, resistant to degradation in the environment and harmful to human and environmental health. Soil has been identified as the primary reservoir for Polycyclic Aromatic Hydrocarbons in the United Kingdom. This study reviews the literature associated with, or is relevant to, the measurement and modelling of dermal absorption of Polycyclic Aromatic Hydrocarbons from soils. The literature illustrates the use of *in vivo*, *in vitro* and in silico methods from a wide variety of scientific disciplines including occupational and environmental exposure, medical, pharmaceutical and cosmetic research and associated mathematical modelling. The review identifies a number of practical shortcomings which must be addressed if they are to be applied to high throughput laboratory analysis of contaminated soils for human health risk assessment.

Key words: PAH, soil, dermal absorption, bioavailability, contaminated land

1 Introduction

This is a review of recent literature associated with the human dermal bioavailability of Polycyclic Aromatic Hydrocarbons (PAH) in soil, in particular benzo[*a*]pyrene. PAH in soil can be naturally occurring (e.g. volcanic ash and its deposition and unburnt fossil fuels). However, the majority of PAH in soil are incidental bi-products of the combustion and incineration of carbonaceous material (Farrell-Jones, 2003). There are many hundreds of PAH but those that appear most commonly in the peer-reviewed literature are parent PAH that form the sixteen congeners defined by the United States Environmental Protection Agency (USEPA) as “Consent Decree” priority pollutants. PAHs are diverse in terms of their physico-chemical properties but generally an increase in molecular weight is

proportional to decreasing aqueous solubility ($2.2E^{-05}$ to 31 to mg/l), increasing log octanol-water partition coefficient (Log K_{ow} from 3.37 to 6.75) and increasing log organic carbon partition coefficient (Log K_{oc} 3.11 to 6.58) (Environment Agency, 2008). Overall, PAHs are hydrophobic and lipophilic meaning they tend to preferentially partition in fats and oils and in non-aqueous phases. PAH are widely recorded in soil, typically at mg kg^{-1} concentrations in urban soil and at lower concentrations in rural locations (Creaser *et al.*, 2007; Vane *et al.*, 2014). Some PAH are known and others are suspected to be carcinogenic and mutagenic to humans (Public Health England, 2008; U.S Environmental Protection Agency, 2013; International Agency for Research on Cancer, 2012). This assumption is mainly informed by a study on laboratory mice, which showed that BaP in a coal tar mixture fed to the animals in their food produced tumours (Culp *et al.*, 1998). Many other studies have also recorded malignant tumours following dosing with individual congeners such as benzo[*a*]pyrene (see International Agency for Research on Cancer, 2012).

The bioavailability of contaminants in soil refers to the proportion of a contaminant that is released from soil and crosses a biological membrane. Bioavailability is generally considered as a process that is measured in a laboratory. A recent paper on the use of bioavailability in risk assessment discusses the concept in detail (Ortega-Calvo, 2015). The bioavailability of contaminants in soil is dependent on the physico-chemical properties of the contaminant source (including the soil vehicle), the exposure pathway and the receptor.

A large proportion of bioavailability research completed to date relates to the ingestion pathway and inorganic compounds. In response to ethical and cost issues associated with *in vivo* bioavailability estimates, *in vitro* tests have been developed that estimate bioaccessibility as a surrogate for bioavailability (in vitro review paper) Collins *et al.*, 2015). Bioaccessibility is concerned with the release of contaminants from soil *in vitro* that are potentially available to cross a biological membrane (Wragg *et al.*, 2011). The relationship between *in vivo* and *in vitro* tests is important because it helps to confirm whether the use of *in vitro* estimates is a suitable replacement for *in vivo* methods. Research has shown acceptable relationships between ingestion *in vitro* and *in vivo* data for As, Ca and Pb (Denys *et al.*, 2012), leading to bioaccessibility tests being used in human health risk assessment of inorganic soil contaminants (Nathanail *et al.*, 2007).

Bioavailability methods are less well developed for organic compounds and as yet none of the current bioaccessibility methods have been able to reliably replicate *in vivo* ingestion results (Collins *et al.*, 2015; Ortega-Calvo *et al.*, 2015). This is because of difficulties in accurately quantifying compounds that are known to be at least partly metabolised *in vivo* (Ounnas *et al.*, 2009). Alternative approaches, such as analysing metabolites both *in vivo* and human biomonitoring studies, have been developed but there is still a lack evidence showing relationships between *in vitro* and *in vivo* data for the ingestion

(or dermal and inhalation pathways) for organic compounds in soil (Ortega-Calvo *et al.*, 2015). In silico studies are starting to appear in the literature that show that models are able to help explain some of the potential controls on bioaccessibility (Cave *et al.* 2015; Beriro, 2015). The physical structure of such models provide an opportunity to inform further research on the mechanisms that control the sorption of organic compounds to soil and their release once they are present in or on the receptor i.e. physico-chemical properties of the compounds and chemical properties of the soil (Cave *et al.* 2015).

The importance of the dermal absorption pathway is shown by Johnson and Kissel (1996) who found that 37 of 235 Superfund risk assessments conducted by the United States Environmental Protection Agency (USEPA) presented dermal pathway excess lifetime cancer risks higher than 1 in 10^{-4}. There is also evidence that PAH, especially benzo[*a*]pyrene (BaP), can cause skin cancers and adverse DNA interactions resulting in both localised and systemic effects (Public Health England, 2008; U.S Environmental Protection Agency, 2013; Nathanail *et al.*,, 2014).

The physiology of dermal uptake differs to that of the more extensively studied ingestion pathway. The latter involves the release and dissolution of chemicals from soil to digestive solutions prior to uptake across the lining of stomach or intestine (Cave *et al.*, 2011). The dermal absorption of chemicals from soil is essentially a one-step process, where the chemical is released from the soil and absorbed into the skin via a thin oily superficial mantle. The assumption that release and absorption of chemicals from soil to skin is effectively simultaneous in addition for the potential for localised effects calls for the following operational definition of dermal bioavailability. The dermal bioavailability of organic compounds in soil is therefore defined as:

> *The proportion of the total concentration of an organic compound or compounds in soil that, following exposure, is absorbed into any part of the skin, that then may remain in situ or be potentially available for uptake by the blood compartment or tissues for storage, release and distribution to one or more target organs.*

This operational definition is informed by explanations of dermal absorption provided in World Health Organisation Environmental Health Criteria 235, guidance by the USEPA and work edited by Roberts and Walters (Kielhorn *et al.*,, 2006; U.S Environmental Protection Agency, 2007). The definition has the benefit of being conservative since it assumes that any mass of chemical entering the skin has the potential to reach a site of action by residing in the skin and/or entering systemic circulation. It is acknowledged that the operational definition may not be universally accepted and may be restricted to the current work and any future applications. Unlike the ingestion pathway, the concept of bioaccessibility, i.e. the amount of a compound released in artificial human gastro-

intestinal fluids, is not relevant to dermal bioavailability because there is no intermediate solution to solubilise the compounds of interest prior to uptake. Estimates of human dermal bioavailability will therefore be referred to as *in vivo* and *in vitro* to differentiate between human or animal based laboratory methods respectively. For the purpose of this study the primary measure of dermal bioavailability is the dermally absorbed fraction (ABS_d) (Equation 1). ABS_d is commonly expressed as a percentage by percent of absorbed dose applied (PADA), after Roy *et al.* (1998). ABS_d and PADA are preferred because they are used in human health risk assessment for contaminants in soil (Environment Agency, 2009a).

$$ABS_d = \frac{Q_{soil}}{T_{soil}}$$

Where Q_{soil} is the amount of compound absorbed into any part of the skin and T_{soil} is the original total concentration in soil

Equation 1 – Percent absorbed of dose applied

The aim of this review is to synthesise current knowledge on the *in vitro* measurement and in silico modelling of the dermal absorption of PAH from soils. The objectives to be addressed to achieve this aim are:

i. Compile and review the literature associated with, or relevant to, the measurement and modelling of dermal absorption of PAH from soils with a greater focus on high molecular weight PAH i.e. ≥4 to 5 ring congeners;
ii. To identify limitations in current work and highlight new emerging methodologies which, going forward, will assist in the development of a suitable physiologically based dermal bioavailability method for PAH in soil.

2 Structure and function of the skin

2.1 Skin physiology

The skin is the largest human organ. In a typical adult the skin weighs approximately 5% of total body mass and covers an area of 1.8 m^2 (Pannatier *et al.*, 1978). Skin functions include provision of a physical and chemical protective barrier, sensory perception (i.e. pressure, temperature and pain) and temperature regulation. The skin is comprised of three principal layers: 1) epidermis; 2) dermis; and 3) hypodermis. The innermost and thickest layer of the skin is the hypodermis (also known as the subcutaneous or superficial fascia). The hypodermis cells are specialised in accumulating and storing fats and are grouped together in lobules separated by connective tissues. The hypodermis acts as an anchor to the overlying layers. The next innermost layer is a 0.2 - 0.3 cm thick layer of tissue called the dermis which comprises a fibrous protein matrix of collagen, elastin and reticulum that is embedded in an amorphous colloidal ground substance. The dermis carries blood vessels, sensory

nerves and lymphatic features and provides nutritional support for the outmost layer, the avascular epidermis. The *stratum corneum* is the outermost layer of the epidermis, consisting of dead cells.
The purpose of the stratum corneum is to form a barrier to protect underlying tissue from infection, dehydration, chemicals and mechanical stress.

2.2 Dermal absorption

The absorption of foreign substances into the skin takes place by dissolution and molecular diffusion through a composite, heterogeneous membrane, comprising of a two-phase protein-lipid interaction (Michaels *et al.*, 1975). The dermal absorption pathways include:

i. Intracellular bulk pathways;
ii. Intercellular bulk pathways; and
iii. Shunt pathways (Chilcott, 2008).

A simplified representation of dermal absorption can be made by comparing the system to a physical structure of bricks and motor used to construct housing (Elias, 1983) (Figure 1). The principal route of dermal absorption is the intercellular pathway, which is enhanced and facilitated by *stratum corneum* lipids (Michaels *et al.*, 1975; Elias, 1981; Grubauer *et al*,, 1987; Mao-Qiang *et al.*, 1993; Bouwstra *et al.*, 2001, 2003a; Ponec *et al.*, 2003).

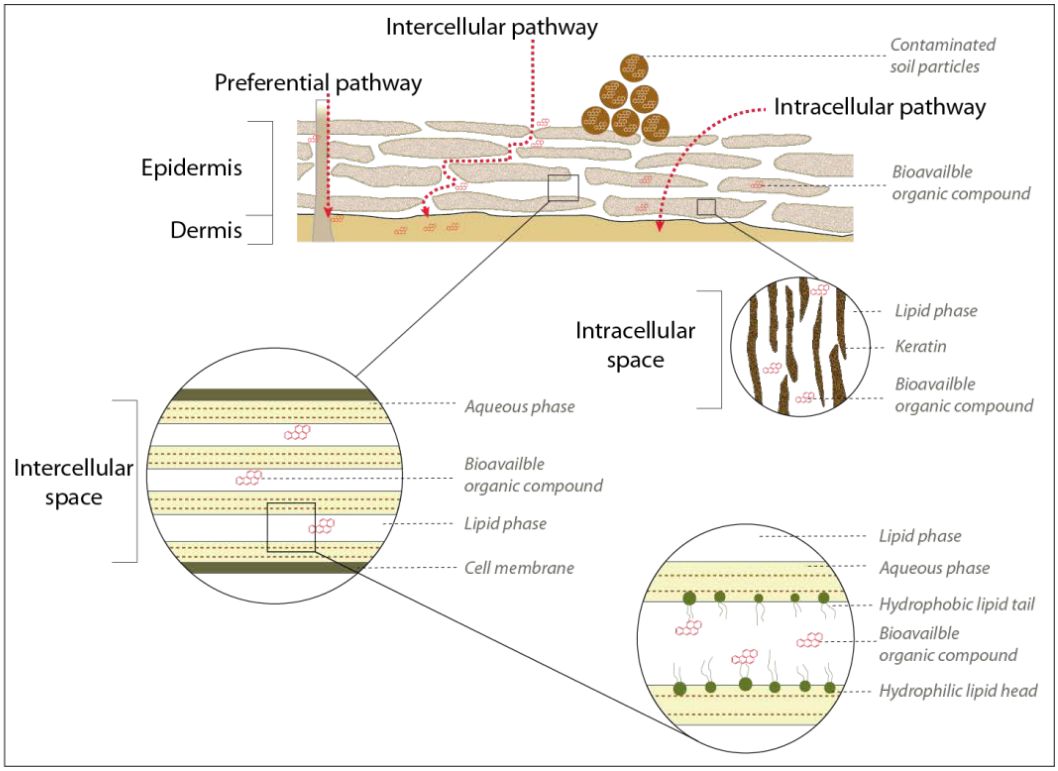


Figure 1 – Absorption pathways through the epidermis

The dermal bioavailability of a contaminant in soil depends of two factors: 1) the transfer of the compound from the vehicle i.e. permeation from the soil into the *stratum corneum*; and 2) penetration

i.e. diffusion of PAH through the *stratum corneum* to underlying or adjacent cells (Pugh and Chilcott, 2008). Both processes need to occur in order for the compound to be absorbed into the skin and be available for systemic circulation. It is important to recognise that the presence of foreign compounds in the skin can lead to localised effects e.g. Langerhan cells may increase the likelihood of carcinogenesis due to the metabolisation of dermally absorbed PAH (Modi *et al.*, 2012); similar observations of the genotoxicity of benzo[*a*]pyrene have also been made for metablolisation by kerotinocytes (e.g. Brinkmann *et al.* 2013). It is because there is a potential for localised effects caused by PAH that bioavailability is defined in this review as a substance "absorbed into any part of the epidermis" rather than only the fraction subject to systemic circulation.

Dermal exposure to foreign substances may be under equilibrium or non-equilibrium conditions depending on the dose applied and the physico-chemical properties of the substance. When a substance is absorbed into the skin there is a maximum concentration that can be achieved which, relative to time, is referred to as the penetration rate or flux (J) and is expressed by g cm^{-2} h^{-1} (Pugh and Chilcott, 2008). This exposure scenario is referred to as an infinite dose i.e. a theoretically unlimited supply of the substance. Should the penetration rate be limited by the amount of the substance available then the exposure scenario is referred to as a finite dose. The distinction between the infinite and finite dose is important for two reasons: 1) most bioavailability studies assume an infinite dose scenario in order to calculate kinetic parameters i.e. flux (J), permeability coefficient (K_p) and diffusion coefficient (D); and 2), chronic exposure to soil assumes a finite dose scenario (Environment Agency, 2009b).

Steady state flux (J_{ss}) is achieved after a lag phase (T_{lag}) has passed and is expressed graphically as the gradient of the penetration profile prior to any plateauing represented by the maximal concentration. The infinite dose across a membrane is expressed mathematically by Fick's First Law of diffusion (Equation 2 and Equation 3). The finite dose, referred to as diffusion within a membrane, is expressed mathematically by Fick's Second Law of diffusion (Equation 4) (Crank, 1975).

$$J = -D\frac{\delta C}{\delta x}$$

Where J is the flux (rate of transfer per unit area), δC is the concentration gradient i.e. the change in concentration, δx is the distance travelled and D is the diffusion coefficient.

Equation 2 – Fick's First Law of diffusion

$$J_{ss} = Kp.Co$$

Where Jss is the steady state flux per unit area, Kp is the permeability coefficient expressed in distance and time for a given organic compound in soil and Co is the concentration of that compound in soil. In soil studies Kp becomes Ks/soil known as the skin/soil partition coefficient) (Roy and Singh, 2001).

Equation 2 – Fick's First Law of diffusion (alternative expression)

$$\frac{\delta C}{\delta t} = D\frac{\delta^2 C}{\delta x^2}$$

Where the concentration of a compound within a membrane is derived by the differential mass balance and Fick's First Law of diffusion assuming homogenous conditions with no net loss of the compound.

Equation 3 – Fick's Second Law of diffusion

The amount of a compound in soil penetrating the skin can also be expressed mathematically (Roberts and Walters, 2008) (Equation 5).

$$Q = J_s A(T - lag)$$

Where Q is amount of compound absorbed, J_s is the flux through the epidermis, A is the area of application, *lag* is the effective lag time between application and absorption and T is the exposure time. The time taken to reach steady state flux is approximately twice the lag time.

Equation 4 – Amount of compound dermally absorbed

Physico-chemical properties of a penetrant compound affecting dermal bioavailability include molecular weight, solubility, charge and hydrogen bonding (Brain and Chilcott, 2008). The rule of thumb for the maximum size of molecule that can take advantage of these dermal absorption pathways is described by the 'rule of 500', which states that few molecules with a molecular weight above 500 Daltons are capable of diffusion through the skin (Bos and Meinardi, 2000). This rule may be broken by large long molecules such as heparin or DNA (Brain and Chilcott, 2008). The solubility of a chemical is principally determined by its partition coefficient. This is calculated as the proportion of the substance dissolved in an immiscible liquid mixture, typically octanol and water. The log of the ratio of the concentrations recorded in the two liquids is used to calculate the partition coefficient e.g. Log K_{ow}. Chemicals with a positive coefficient, between 1 and 3, are considered to be optimal for skin penetration resulting in entry to the systemic circulation, since higher or lower values will respectively: 1) remain in the lipid rich low moisture content stratum corneum; or 2) fail to access the stratum corneum (Brain and Chilcott, 2008). The stratum corneum carries a net negative charge as a result of proteins such as keratin with positively and negatively charged groups (Moody *et al.*, 1995). This situation is suited to most organic molecules and therefore does not pose any barrier to permeation.

3 Dermal bioavailability literature

A review of the current literature on estimating the human dermal bioavailability of PAH in soil was conducted by searching the Scopus and Web of Science bibliographic databases using a range of relevant search terms. Further searches were made using Google and Google Scholar. Secondary

references were examined in the articles found. The search continued until no new relevant references were identified. Studies were divided into four method groups: *in vivo* (including occupational exposure), *in vitro,* in silico and review. Each article identified was reviewed and the following information recorded: method group, year of publication, authors, lead institution, title, abstract, methods, source media, analytes, receptor, and species used in study and industry sector. Additional sources of supplementary information on human skin physiology, suppliers of laboratory bioavailability equipment and modelling software were also consulted and discussed where relevant.

A summary of the number of publications per year for each method group is given in (Figure 2). One hundred and thirty-four peer-reviewed journals articles and a small collection other material on or closely related to the dermal bioavailability of PAH from soil were reviewed. These articles covered the period between 1975 and 2015. The papers were divided into method group depending on the main function of the paper. The distribution of the peer-reviewed articles was broadly equal for *in vivo* (n=42) and *in vitro* (n=47) method groups over the period (Figure 2). In silico (n=27) studies accounted for approximately half of the *in vivo* articles (Figure 4). Dermal bioavailability review papers (n=18) appear in the literature from 2004 and cover the various aspects of dermal exposure assessment methods (Moody and Maibach, 2006; Van De Sandt *et al.*, 2007; Frasch *et al.*, 2014), compound group reviews (pesticides and organic flame retardants) (Tripp *et al.*, 2007; Abdallah *et al.*, 2015), metabolic pathways (Pannatier *et al.*, 1978), contaminants in soil (Spalt *et al*,, 2009; Andersen *et al.*, 2014), and in silico modelling (Moss *et al.*,, 2002a; Cronin and Schultz, 2003; Degim, 2006; Anissimov *et al.*,, 2013; Anissimov, 2014; Couto *et al.*,, 2014).

The articles reviewed were written by over 300 individual authors from various international academic, governmental and non-government organisations. Where the literature search results permitted, preference for detailed review was given to articles on the dermal bioavailability of PAH in soil. Where such studies were not available other relevant literature was reviewed. Relevant literature was defined as studies on the human and animal exposure to pharmaceuticals and other chemicals. For example, many European studies based in silico quantitative structure activity relationships (QSAR) research on individual chemical compounds, relates to regulatory requirements imposed by the European Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (European Commission Council Regulation, 2007).

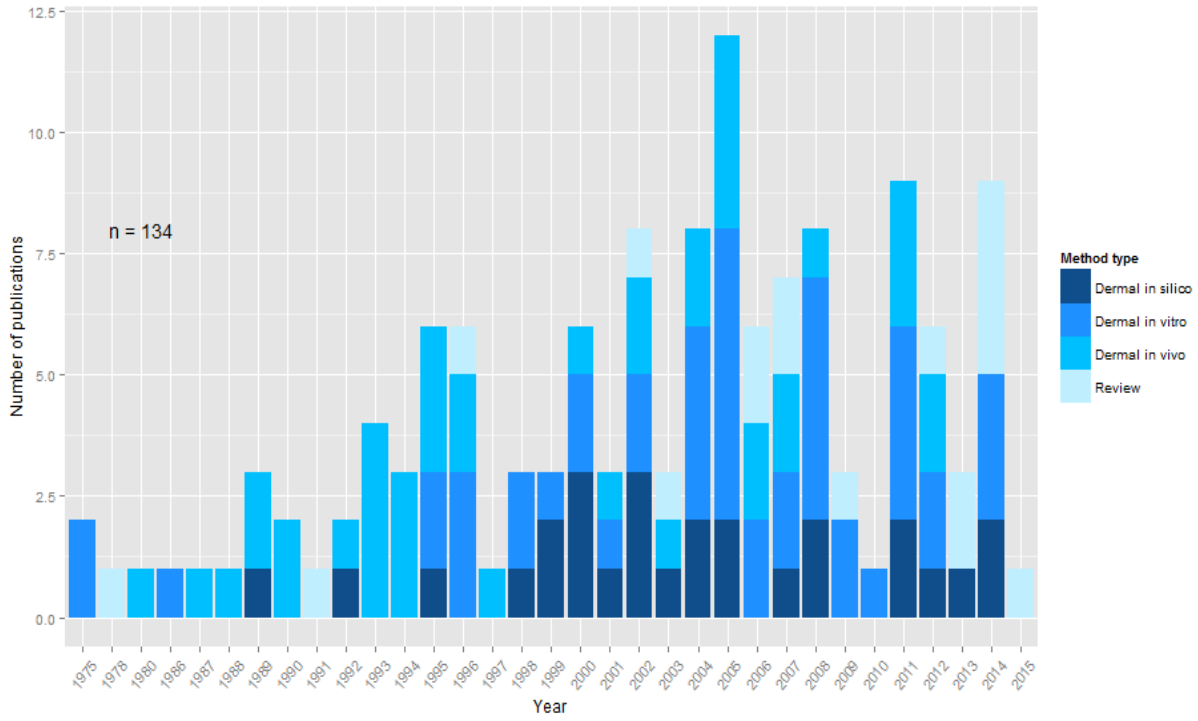


Figure 2 Summary of dermal bioavailability articles reviewed

Whilst this review is primarily aimed at *in vitro* testing methodologies for dermal bioavailability it is important to give an overview of the *in vivo* approaches as these supply data from physiologically and metabolically intact systems and are considered the "gold standard", from which base *in vitro* tests can be developed and compared. *In vivo* studies are, however, associated with strong ethical issues and there is an increasing movement towards the reduction of the use of animal testing e.g. (Keskin and Terzi, 2006) which will be emphasised in this review.

3.1 *In vivo* dermal bioavailability

3.1.1 Occupational human exposure

Occupational exposure to materials containing high concentrations of PAH has been described in a number of publications (Tsai, *et al.*, 2001; Vaananen *et al.*, 2005; Christopher *et al.*, 2011; Kriech *et al.*, 2011; Osborn *et al.*, 2011b; Smith *et al.*, 2011; McClean *et al.*, 2012; Serdar *et al.*, 2012; Fent *et al.*, 2014; Kamal *et al.*, 2014). Skin exposure is commonly measured using a wipe soaked in corn oil (Moody and Maibach, 2006) followed by extraction and quantification of the PAH content (Vaananen *et al.*, 2005; Christopher *et al.*, 2011; Osborn *et al.*, 2011b; Cavallari *et al.*, 2012; McClean *et al.*, 2012; Fent *et al.*, 2014). Moody and Maibach (2006) suggest that hand wiping is not necessarily best practice as it can increase the absorption of the contaminant by the "wash-in effect". Some studies have used passive samplers, which are attached to operatives that act as a surrogate for skin exposure measurements (Vaananen *et al.*, 2005; Olsen *et al.*, 2011a; Osborn *et al.*, 2011b; Cavallari *et al.*,

2012). The passive sampler described by Olsen (2011a) consists of a 5-layer passive organic dermal sampler. Vaananen *et al.* (2005) showed that the passive sampler method they used gave statistically indistinguishable results compared to skin wipes showing a strong correlation ($r = 0.757$, $P < 0.001$, $N = 23$ for total PAH).

In a number of these studies the internal PAH dose was quantified by analysing the PAH metabolites in urine samples. Metabolites of hydroxylated PAH (pyrene, phenanthrene, naphthalene and fluorene) have been analysed by Turkall *et al.* (1994); Elovaara *et al.*, (2006); McClean *et al.*,, (2012); Serdar *et al.*, (2012); and, Choosong *et al.*, 2014). Pliel *et al.* (2000) quantified exposure of marker compounds (e.g. naphthalene) in jet fuel using the exhaled air of military personal. More recently, studies have focused on using 3-OHBaP as a carcinogenic marker in urine of animals and humans (e.g. Heredia *et al.* 2013; Barbeau *et al.* 2015). Other researchers have measured PAH in urine using an Enzyme Linked Immunosorbent Assay (ELISA) kit which measures the total PAH content as phenanthrene equivalents (Smith *et al.*, 2011; Fent *et al.*, 2014). In addition to this, Serdara *et al.* (2012) used a different ELISA kit on urine samples to determine oxidative DNA damage by measuring urinary 8-oxo-2'-deoxyguanosine which showed a four-fold increase during work periods and correlated well with PAH urine concentrations. In those studies where urine analysis has been carried out, increased exposure to PAH in the workplace was clearly identified but they also showed exposure to PAH outside the workplace, which also includes that obtained from smoking and food. Two very comprehensive meta-analysis studies conducted by Bosetti *et al.*, (2007) and Rota *et al.*, (2014) bring together data on respiratory and urinary cancer health outcomes from epidemiological investigations of occupational exposure to PAH. The results show that increases in exposure in these industries leads to increased relative risk of cancer. Whilst the occupations studied (e.g. firefighters and road surfacing workers, military personnel) cover a variety of industries, there is no specific mention of workers exposed to PAH contaminated soil.

Although these occupational studies show that exposure to PAH results in increased PAH in the systemic circulation of workers and that covering vulnerable skin areas with protective clothing reduces exposure (Serdar *et al.*, 2012) they do not give a quantitative or even semi-quantitative breakdown of how much of the internal dose comes from dermal absorption. Whilst this is very important information which clearly identifies the need for measuring how much PAH from a soil sample ends up in the human body from the dermal absorption route, the actual scientific methods are not very helpful in designing a testing methodology which makes that measurement. The one piece of technology which will have relevance is the passive sampler (Vaananen *et al.*, 2005; Olsen *et al.*, 2011a; Osborn *et al.*, 2011b; Cavallari *et al.*, 2012) which is used to measure the skin exposure in the work environment. These devices have the potential for measuring the fraction of PAH in a soil which

is available to be absorbed by the skin and is therefore a conservative estimate of that which will actually absorbed.

3.1.2 Animal exposure

There are many *in vivo* studies on dermal bioavailability reported in the literature which are outside the scope of this review. The *in vivo* animal studies which relate specifically to PAH dermal bioavailability from soils have used monkeys, rats and guinea pigs (Yang *et al.*, 1989; Wester *et al.*, 1990; Turkall *et al.*, 1994; Kadry *et al.*, 1995; Moody *et al.*, 1995; Roy *et al.*, 1998a). These tests take the general form of applying the test chemical to a designated area of skin in an appropriate vehicle (for the purposes of this review this is soil). Body fluids, tissues, or excreta are collected at predefined intervals, and the quantity of chemical and /or metabolite is measured using a suitable analytical procedure. Finally at the end of the experiment the animal is sacrificed and the distribution of the test chemical between different body parts can be determined. The specific difficulty with soil is that it has to be held in place on the animal skin with a covering material which can lead to variability in skin contact area during the trial. For PAH, the additional difficulty is that the original compounds are metabolised as they go through the skin and enter systemic circulation and therefore both the original compound and the metabolites need to be analysed in the body fluids, tissues or excreta to get a true picture of its fate in the animal host. The *in vivo* studies for PAH in soil have overcome this problem by using soils spiked with radiolabelled PAH, following the distribution of the radioactive label in the host animal. The monkey study of Wester *et al.* (1990) used a less invasive approach, in which the ratio of radiolabelled BaP found in the urine of the monkey after application of the BAP spiked soil to the skin and the amount BaP found in the urine after intravenous injection of pure radiolabelled BaP in propylene glycol solution was calculated.

3.2 *In vitro* dermal bioavailability methods

Both the Organisation for Economic Co-operation and Development (OECD) (OECD, 2004) and United States Environmental Protection Agency (USEPA) (U.S Environmental Protection Agency, 2007) have set out guidelines for the *in vitro* determination of skin absorption which have also been or recognised by the World Health Organisation in Environmental Health Criteria 235 (Kielhorn *et al.*, 2006).

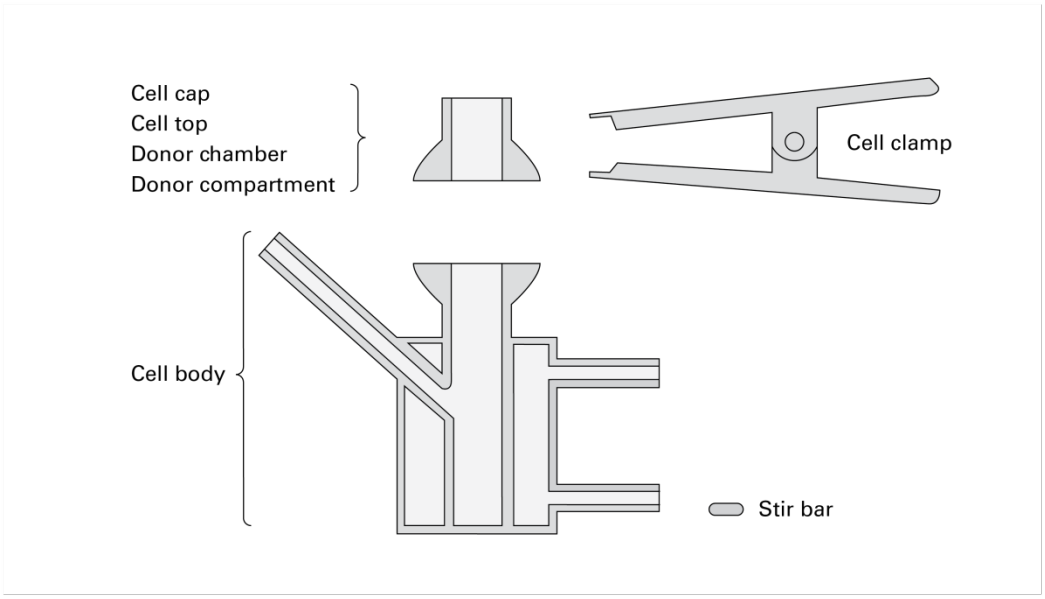
3.2.1 Diffusion Cell Design and operation

The OECD guidelines state “A diffusion cell consists of a donor chamber and a receptor chamber between which the skin is positioned. The cell should provide a good seal around the skin, enable easy sampling and good mixing of the receptor solution in contact with the underside of the skin, and good temperature control of the cell and its contents. Static and flow-through diffusion cells are both acceptable”. The two most popular designs are the Franz static diffusion cell (Franz, 1975) and the

Bronaugh flow through cell (Bronaugh and Stewart, 1984) (Figure 3). The OECD recommends maintaining a temperature of 37 ˚C whereas the USEPA states 32 ˚C. Both guidelines suggest that for lipophilic compounds that the receptor fluid should contain solvent mixtures (e.g. polyethylene glycol, olelyl ether, Bovine Serum Albumin (BSA)) to ensure the receptor is not rate limiting in the permeation step due to limited solubility. Both guidelines suggest the use of radiolabelled test materials to ensure the fate of the material through the cell can be traced and provide complete mass balance. Further information on sample loading, experiment sampling times are also given.

Schreiber et al. (2005) points out that European chemical policy in general, and the REACH initiative in particular, will increase the number of chemical substances for toxicological evaluation by several orders of magnitude. The need for high throughput measurements for dermal bioavailability has lead the redesign of the traditional Franz and Bronaugh diffusion cells as well as the development of new approaches to dermal bioavailability measurements.

A.



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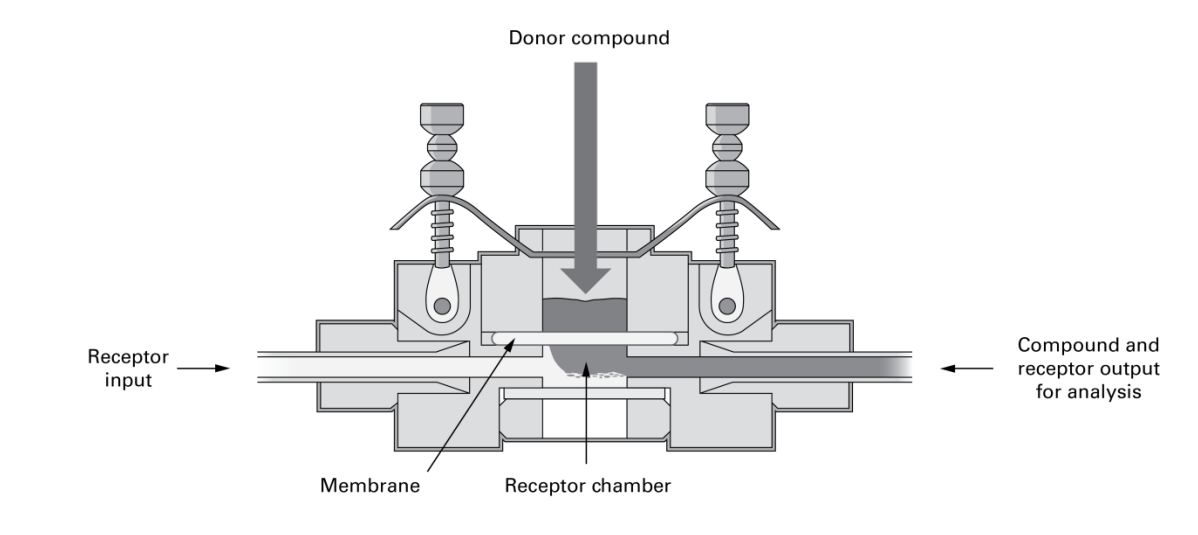

Figure 3 – Schematic diagrams of a typical Franz diffusion cell (a) and a Bronaugh diffusion cell (b)

3.2.2 Multi-plate systems

Jacques *et al.,* (2010) essentially miniaturised the Franz static cell design. They used 28 mm diameter discs of pig ear skin placed dermal side down in polycarbonate Transwell® inserts (28 mm inner diameter, with a 23 mm diameter and 8 µm pore size filter by Corning Life Sciences, Avon, France) placed in a 6 position well plate prefilled with 1.5 ml culture medium at 37 ˚C in a 5% CO_2 air incubator. Using this approach they were able to carry out 6 diffusion cell experiments in parallel. Sinko *et al.* (2012) have developed this further by modifying a 96 well Parallel Artificial Membrane Permeability Assay (PAMPA) (Figure 4) that had already been used for estimating passive gastro-intestinal absorption and blood-brain barrier permeability. In a follow up study, Karadzovska and Riviere (2013b) used the PAMPA methodology to compare the performance of synthetic skin membranes to porcine skin on a variety of organic compounds. The advantages of the cell array approach are that the plates are inexpensive and disposable and have the ability to carry out many tests simultaneously with the potential to automate dosing and sampling procedures. However, these cell arrays are made out of plastic which could provide PAH sorption sites. Whilst Jacques *et al.*(2010) reports that <3% of the radiolabelled BaP used in their study was absorbed to the plate walls, Karadzovska and Riviere (2013b) do not report any losses of the target compounds to the well plates. Miki *et al.*, (2015) have developed a high throughput PAMPA screening system for drug absorption into human skin with a copolymer containing poly(dimethylsiloxane) (PDMS) and poly(ethylene glycol) (PEG) supported on a membrane filter. This has been used successfully on both hydrophilic and lipophilic compounds (log Kow up to 3.86). For highly lipophilic PAHs such as BaP membrane testing may be required to ensure optimum performance.

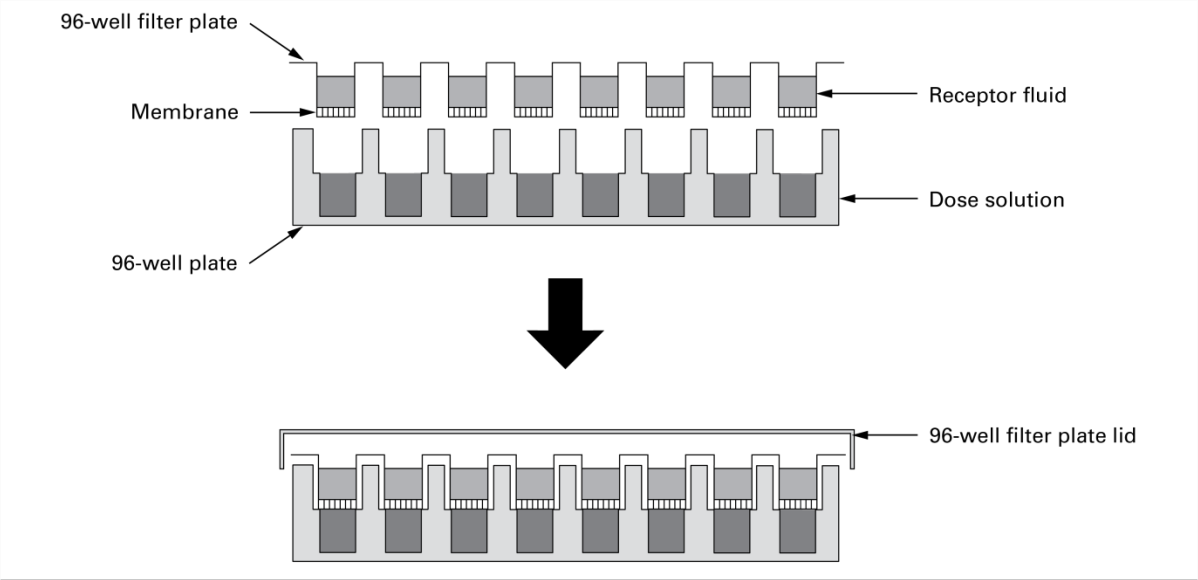

Figure 4 – Schematic of a multi-plate system for estimating *in vitro* dermal bioavailability

3.2.3 Fibre Arrays

A completely different approach to dermal bioavailability measurements has been reported in a number of studies (Riviere *et al.*, 2007; Baynes *et al.*, 2008; Karadzovska and Riviere, 2013a). The method uses a membrane-coated fibre (MCF) array to derive sorption data which is combined with regression modelling. A silica fibre is coated with absorbing media; these are used as a routine method of extracting and pre-concentrating target organic compounds from a variety of sample matrices prior to chemical analysis and are commercially available products.

The MCF array approach for skin permeability prediction is based on the similarity in the diffusion mechanisms of the MCF membrane and the *stratum corneum* of the skin. Several types of molecular interactions have been identified to be the primary factors in skin absorption: lipophilicity; hydrogen bonding and polarizable p*-electron interactions (Moss *et al.*, 2002b). These molecular interactions are simulated with number of MCFs that reflect the different physico-chemical properties of the compounds under test. A set of 'training' compounds is used to measure the relative dermal bioavailability for a particular vehicle (this could be soil, although soil has not been tested) and the *stratum corneum* using a conventional diffusion cell. At the same time, sorption data are derived for the same compound and vehicle using a MCF array. A predictive model is established via multiple linear regression modelling using the MCF partition coefficients as the predictor variables and the dermal bioavailability as measured by the diffusion cell as the dependant variable.

The dermal bioavailability of new compounds can be predicted by measuring the partition coefficients of these compounds in the vehicle using the MCF membranes. The main advantage of this approach is that the MCF measurements can be automated and would be a relatively straight forward high

throughput method to be implemented in a standard testing laboratory. As an example, a study used the MCF approach to evaluate the use of synthetic skins in a 96 well PAMPA assay compared to porcine skin in a convention diffusion cell (Karadzovska and Riviere 2013b). Three MCFs, polydimethylsiloxane (PDMS), polyacrylate (PA) and CarboWax (Wax), were used to determine the partition coefficients for a set of calibration compounds. Correlations between the MCF partition coefficients and the diffusion cell dermal bioavailability measurements were investigated and it was concluded that the Strat-M™ synthetic skin was a good analogue of *ex vivo* porcine skin.

3.2.4 Passive samplers

Passive samplers are primarily designed to simulate the amount of contaminant deposited on a skin surface, usually from occupational exposure. The samplers are placed on the skin at various locations around the human body and after exposure the target compounds are extracted from the sampler and analysed. Two studies of occupational exposure to PAH, one on road pavers from and one on operatives from a gasworks site, simply used proprietary polypropylene filters as passive samplers (Dor *et al.*, 2000; Vaananen *et al.*, 2005). Vaananen *et al.*(2005) compared the performance of passive samplers to skin washes and found equivalent performance. Olsen *et al.* (2011b) developed a more sophisticated 5 layer design. The 5-layers, from outside in, consisted of polypropylene, polyurethane foam, C-18 solid-phase extraction disk, ethylene tetrafluoroethylene, and an activated carbon cloth which they went on to use in further occupational studies on PAH exposure (Osborn *et al.*, 2011a). The layers were enclosed in aluminum foil and placed in a muslin envelope that had a 40.0 mm diameter opening. The passive samplers measures how much of the contaminant under study is deposited onto the skin surface and not how much is absorbed into the skin.

In a study of PAH contaminated soil, Hu and Aitken (2012) used a passive sampler to measure the amount of PAH released from a soil when placed on a C-18 extraction disk, at three different temperatures (20 ˚C, 30 ˚C and 40 ˚C), four soil moisture concentrations of (2%, 8%, 20% and 40%) and seven soil loadings (5-100 mg dry soil cm^{-2}) over periods of 6 days. Whilst this is not a measure of dermal bioavailability it measures the amount of PAH that is availble for absorption from the skin. This could be a useful measurement when working with soils since it provides measure of the availability of the PAH from the soil which in the first instance can be regarded as a conservative estimate of that which can be demally absorbed. The study showed that after bioremediation PAH desorption from the soil was completely eliminated.

3.2.5 In vitro membrane types used

The choice of skin to use as the diffusion membrane is of high importance. Both USEPA and OECD guidelines specify that human skin is the “gold standard” (Organisation for Economic Co-operation and Development, 2004; U.S Environmental Protection Agency, 2007). Human skin is, however, not

always easily available and a number of studies have used alternative animal skins. In studies where PAH dermal bioavailability, with and without soil have been studied (Wester *et al.*,, 1990; Moody *et al.*, 1995; Roy *et al*,, 1998b; Roy and Singh, 2001; Moody *et al.*, 2007; Moody *et al.*, 2009; Moody *et al.*, 2011), pig (Jacques *et al.*, 2010), rat (Yang *et al.*, 1989; Moody *et al.*, 1995; Roy *et al.*, 1998a) and guinea pig skin (Moody *et al.*, 1995) have been used.

An increasing trend is to move away from *ex vivo* animal skin to reconstructed human epidermis (RHE) skin substitutes (Netzlaff *et al.*, 2005; Schreiber *et al.*, 2005; Schaefer-Korting *et al.*, 2008). A number of synthetic skin products are commercially available and have been evaluated in the literature. EPISKIN®, EpiDerm™ and SkinEthic®, were tested against *ex vivo* human and pig skin to evaluate their suitability for *in vitro* dermal availability measurements. The substances used were tested under both infinite-dose and finite dose conditions in ten laboratories and under strictly controlled conditions. The data were subjected to independent statistical analyses. In general, permeation of the synthetic skins exceeded that of human and pig skin, yet the ranking of substance permeation through the three tested RHE models and the pig skin reflected the permeation through human epidermis. Whilst PAH were not tested, three compounds with log K_{oc} of 3.5 to 6.8 and a molecular weight (MW) of 288-875, similar to commonly found PAH, were studied.

Two recent studies have shown very promising comparative performance between artificial skin and human and pig skin. Karadzovska and Riviere (2013b) have studied three artificial membranes isopropyl myristate (IPM), certramides and a new synthetic skin (Strat-M™) using PAMPA for skin (Sinko *et al.*,, 2012). Although the compounds they used were not PAH, they used sodium diclofenac which has similar log K_{oc} and MW (4.5 and 318 respectively) to PAH found in soil samples. Resultant absorption data was compared to porcine skin diffusion cell data. A correlation between membrane retention and the amount remaining in skin had r^2 values of 0.73 (Strat-M™), 0.67 (certramides), and 0.67 (IPM). Uchida *et al.* (2015) confirmed the suitability of Strat-M™ as a replacement for animal skin. They used 13 chemical compounds with molecular MW of 152–289 and log Koc of 0.9 to 3.5. The performance of Strat-M™, human skin, or rat skin in a Franz-type diffusion cell was compared. The diffusion and partition parameters of chemicals in Strat-M™ were similar to those in the human and rat skins. They concluded that Strat-M™ could be used as an alternative to animal or human skin in dermal bioavailability studies.

3.2.6 Key in vitro dermal bioavailability studies

Most of the work on dermal bioavailability is directed at occupational exposure of potentially harmful substances including pharmaceuticals, jet fuel and cosmetics for pure substances. There has been recognition, however, that chemicals are rarely applied to the skin in their pure form and that the dosing vehicle as well as the presence of more than one contaminant, especially when in a mixture,

can have significant effects on the way in which the target chemical is absorbed (Riviere and Brooks, 2005; Karadzovska *et al.*, 2013b; Riviere *et al*, 1999). Spalt *et al.*, (2009) reviewed a large number of publications where the dosing vehicle is soil or sediment for a variety of contaminants. The Spalt study presents a comprehensive review of a subset of dermal bioavailability studies for PAH, where BaP is the most commonly reported congener, with a smaller number of studies focusing on naphthalene and phenanthrene. Key dermal bioavailability studies for PAH in soil are discussed with key points summarised in Table 1 (Yang *et al*,, 1989; Wester *et al.*, 1990; Moody *et al.*, 1995; Roy *et al.*, 1998a; Roy *et al.*, 1998b; Riviere *et al.*, 1999; Roy and Singh, 2001; Abdel-Rahman *et al.*, 2002; Moody *et al.*, 2007; Moody *et al.*, 2009; Jacques *et al.*, 2010; Moody *et al.*, 2011).

Yang *et al*, (1989)

Yang *et al.* (1989) used the same experimental *in vitro* set up used in Roy (1998) but instead used rat skin and compared their results to a parallel *in vivo* rat study. The study used a single air dried loam sample that was sieved to <150 µm and spiked with crude oil and tritiated BaP, although no ageing of the soil was carried out. The soil loadings were chosen to produce a monolayer of soil in both *in vivo* and *in vitro* studies. Within experimental error, the diffusion cell results agreed with the *in vivo* data and the dermal bioavailability of BaP from the crude oil alone was reduced by a factor of 4-5 when in the soil matrix. It appears that the *in vitro* data was derived from the concentration of BaP in the receptor solution only.

Wester *et al.* (1990)

The Wester *et al.*(1990) paper is important as the data reported here has been used in both the U.K and U.S as their generic values for BaP dermal absorption factors (CL:AIRE, 2013; Nathanail *et al.*, 2014). The study measures the dermal bioavailability of BaP using an *in vitro* test with human skin and an *in vivo* test with rhesus monkeys. The study uses only one soil (26% sand, 26% clay and 48% silt, no organic carbon content is given). The soil was spiked to 10 mg kg^{-1} with ^{14}C radiolabelled BaP. No ageing of the soil was carried out. The *in vitro* apparatus consisted of small flow through diffusion cells with a 1 cm^2 membrane surface area and a receptor solution of human plasma pumped at 3.0 ml h^{-1}. The *in vivo* study on rhesus monkeys used the same soil and measured the ratio of BaP found in urine after application of the soil to abdominal skin to the BaP in urine after an intravenous dose of BaP. The *in vitro* study found that, when applied in a soil matrix, 1.4 ±0.9% of the applied dose was absorbed into the skin with 0.01 ±0.004% in the plasma receptor fluid, whereas when radiolabelled BaP was applied directly to the skin in acetone solution 23.7 ±9.7% was absorbed into the skin and 0.09 ±0.06% in the receptor plasma. In the *in vivo* test, the percentage of applied dose absorbed for BaP in acetone was 51.0 ±22 and in soil was 13.2 ±3.4. Like the study of Roy *et al.* (1998), this shows that the skin itself is an important sink for absorbed PAH and should be included in the calculation of dermal bioavailability. The results also show that the soil matrix is important factor

in reducing PAH dermal bioavailability (a factor of 17 times lower for the *in vitro* test and factor of 4 for the *in vivo* study).

Riviere *et al.* (1999)

Riviere *et al.* measured the dermal absorption of marker compounds, including Naphthalene, in different Jet fuel mixtures using the *in vitro* isolated perfused porcine skin flap (IPPSF) method. This method is a self-contained system that mimics skin perfusion by maintaining vascular supply to the tissues using an artificial buffer solution. Further details of the method can be found in (Riviere *et al.*, 1986). The study shows that aliphatic marker compounds are absorbed slower than Naphthalene. In addition, the study shows that the penetration of Naphthalene is deeper the aliphatic markers, resulting in lower concentrations recorded in the stratum corneum in comparison to other markers. The study explains that measurements of systemic circulation, i.e. concentrations in the purfusate, and local accumulation in the stratum corneum may be different depending on the properties of the substance under test. Another key finding was the absorption profiles differed between the fuel types tested – it was suggested that the fuel additive may be the cause. Unfortunately, Naphthalene was the only PAH under test, but the results suggest that the individual hydrocarbons demonstrate different absorption and penetration profiles depending on the mixture, supporting the notion that physico-chemical properties are important in understanding compound bioavailability in localised and systemic contexts.

Roy *et al.* (1998) and Roy & Singh (2001)

The Roy *et al.* (1998) study was unique in that it used 9 naturally contaminated soils from manufactured gas plant sites. The soils were analysed for 18 target PAH but unfortunately the total concentration of the PAH in the soils was not reported and no other information on the mineral or organic carbon content of the soils was given. The soils were spiked with tritiated BaP and the dermal bioavailability was determined using Franz type diffusion cell with previously frozen human skin samples. The mass balance of the radiolabelled BaP in the diffusion cell receptor fluid, in the skin sample, on the skin surface and on the cell wall was determined. For all of the soils, less than 1% of the dose from tritiated BaP was found in the receptor fluid, ca. 1% in the skin and >80% on the surface of the skin (from a skin wipe) and 1-3% on the cell walls. A control experiment was carried out using PAH extracts from the soil without the soil matrix. When directly analysing the total target PAH in the receptor fluid the results showed a 2-3 order of magnitude reduction in PAH absorption compared soil extracts. The study had four main inconsistencies and problems: 1) the target PAH data for all nine soils was not presented; 2) the studies were carried out on freshly spiked soils; 3) a high concentration of PAH was used equivalent to an infinite dose rather than a finite dose encountered in PAH in soil exposure scenarios; and 4) the absorbed doses and contaminated flux data reported are for radiolabelled BaP not the naturally occurring PAH in the soils. Roy and Singh (2001)

addressed the last two points using the same experimental set up but they used a single “field soil” sieved to <150 µm with an organic carbon content of 0.43%. The soil was extracted with methylene chloride to remove unwanted organic matter prior to spiking with crude coke oven tar to give a BaP concentration of 65 mg kg^{-1} followed by aging at 20˚C in the dark for three time steps: 1, 45 and 100 days. The soils were also freshly spiked with tritiated BaP to check for the effect of ageing. The study looked at sample loading over the range of 1-10 mg cm^{-2} and showed that higher sample loading led to a reduction in percentage dose of BaP absorbed. This was assumed to be due to soil layering effects in which higher soil loadings lead to multiple layers of soil; where layers not in contact with the skin contribute less BaP. The experiment showed that the 110 days of aging reduced the dermal bioavailability by a factor of 2 compared to freshly spiked soil. The study also showed that the dermal flux of BaP from the crude coke oven tar was reduced by a factor of 10 when compared to the raw material to soil spiked with the tar. Again, it is unclear if the dermal bioavailability includes the amount of BaP in the skin as well as in the receptor fluid.

Table 1 – Summary of key *in vitro* dermal bioavailability studies

Experimental studies	PAH congeners	Animal / skin site	Study type	Soils	Reduction Factor in absorption due to soil matrix	Analytical method	Cell type	Receptor solution
Yang *et al.* (1989)	Benzo[*a*]pyrene	Rat Back	*In vivo & in vitro*	Yes	4-5	Radiometric analysis method not specified	Franz diffusion cell	6% Volpo-20 and 0.01% thimerosal antibacterial
Wester *et al.*(1990)	Benzo[*a*]pyrene	Rhesus monkey, human Abdomen	*In vivo, in vitro*	Yes	17 *in vitro* 4 *in vivo*	Liquid scintillation counting	Flow thru diffusion cell	Human plasma
Turkall *et al.* (1994)	Naphthalene	Rat, Abdomen	*In vivo*	Yes	NR	Liquid scintillation counting	na	na
Kadry *et al.*(1995)	Phenanthrene	Rat	*In vivo*	Yes	NS	Liquid scintillation counting	na	na
Moody (1995)	Benzo[*a*]pyrene	Guinea pig, rat, human	*In vivo & in vitro*	No	na	Liquid scintillation counting	Teflon Bronaugh diffusion cells	Hanks Balanced salt solution, 10% fetal bovine serum
Roy (1998)	60 PAH	Rat	*In vitro*	No	na	GC FID, GC MS	Franz diffusion cell	Aqueous 6% polyethylene glycol, 20% oleyl ether
Roy *et al.* (1998)	Benzo[*a*]pyrene	Human Abdomen	*In vitro*	Yes	100-1000	GC MS selected ion mode & Liquid scintillation counting	Franz diffusion cell	Aqueous 6% polyethylene glycol, 20% oleyl ether,0.01% antibacterial agent
Riveire *et al.* (1999)	Napthalene	Porcine	*In vitro*	No	na	Liquid scintillation counting	The isolated perfused porcine skin flap (IPPSF)	Perfusate
Roy and Singh (2001)	Benzo[*a*]pyrene	Human Abdomen	*In vitro*	Yes	10	Liquid scintillation counting	Franz diffusion cell	Aqueous 6% polyethylene glycol, 20% oleyl ether
Abdel-Rahman *et al.* (2002)	Benzo[*a*]pyrene	Pig, Abdomen	*In vitro*	Yes	Sandy soil 21 Clay soil 42	Radiometric analysis method not specified	Teflon flow through diffusion cells	Hanks Balanced salt solution, 10% fetal bovine serum
Moody (2007)	Benzo[*a*]pyrene	Human, breast	*In vitro*	Yes	3	Liquid scintillation counting	Teflon Bronaugh diffusion cells	Hanks Balanced salt solution, 10% fetal bovine serum
Moody (2011)	16 US EPA	Human, breast	*In vitro*	Yes	NR	HPLC with photodiode array detector	Teflon Bronaugh diffusion cells	Hanks Balanced salt solution, 10% fetal bovine serum
Jacques *et al.* (2010)	Benzo[*a*]pyrene	Pig, Ear	*In vitro*	No	na	Liquid scintillation counting, ion trap MS, NMR	Transwell system static diffusion	Dulbecco's Eagle Modified Medium (DMEM) , antibacterial

NOTES: na Not applicable; NR Not recorded; NS Not significant

Abdel-Rahman (2002)

Abdel-Rahman (2002) recognised the limitations of *in vivo* studies carried out by Turkall *et al.*, (1994) and Kadry (1995). The same soils (a sandy soil: 90% sand, 2% clay, 4.4% organic matter; a clay soil: 50% sand, 22% clay, 1.6% organic matter) were autoclaved and deionised water added to achieve a moisture content of 11%. The soils were spiked with tritium labelled BaP and aged by sealing in glass vials and storing in the dark for three months. The *in vitro* study used fresh pig skin mounted in a diffusion flow through cell. For pure BaP and spiked soils less than 1% of the total dose was found in the receptor fluid. For pure compound ca. 75% of the dose was found in the skin. With freshly spiked sandy and clay soil, 8.5% and 3.5% respectively, and aged soil, 3.7% and 1.8% respectively, were found in the skin. Clearly the aging reduces the dermal bioavailability of PAH. Unlike the Turkall *et al.*(1994) and Kadry (1995) studies, the sandy soil with higher organic matter had higher dermal bioavailability. This is probably due to the autoclaving step used in soil preparation which may have broken down the organic matter in the soil.

Moody *et al.* (2007)

Moody *et al.* (2007) from Health Canada carried out a very similar study to the *in vitro* testing of human skin described by Wester *et al.* (1990). In this case they used a single soil described as a "commercial gardening soil" (no information on mineral or organic carbon content was given), which was air dried, ground and sieved to <710 µm. Sub-samples (32 mg) of the soil were spiked with ^{14}C radiolabelled BaP with no ageing of the soil being carried out. The study used a Bronaugh Type flow through cell with a receptor solution consisting of Hanks buffer salt solution containing 4% BSA using female breast tissue as the skin membrane. Experiments were carried out over two time periods of 24 and 42 h. The percentage of the applied dose of BaP absorbed (including both in the skin and in the receptor solution) for the 24 h study was 14.8 ±6.17% (n=6) in the soil matrix and 56.4 ±10.49 (n=6) after direct application to the skin, and for the 42 h study they were 15.8 ±8.3 (n=4) and 49.7 ±9.35 (n=5) respectively. Whilst there was no significant difference between time periods the data show a clear reduction in dermal bioavailability when the BaP is applied in a soil matrix compared to direct application to the skin (a factor of ca. 3.5). The authors point out that the value for the absorbed dose from the spiked soil obtained in this study is in close agreement (i.e. 13.2 ±3.4) with the *in vivo* study of Wester *et al.* (1990). They note that the Wester study used an occluded (covered) application of soil in the *in vivo* but their study did not and that they may not be comparable. In addition to this, they were using a different soil sample and sample loading which may have very different BaP release characteristics from the Wester study. The agreement is therefore more likely to be down to chance. Moody discusses soil loading, particle size organic matter content of soils and their possible effects on dermal bioavailability but none of these were investigated in this study.

Moody *et al.*(2011)

The Moody *et al.*(2011) group from Health Canada address some important practical points in this study. All of the studies in Table 1 rely on the use of radiolabelled PAH to study the fate of the PAH from the soil, in and through the skin sample and finally into the receptor solution. In order to do this the soil sample has to be spiked with radiolabelled PAH. If the dermal bioavailability of a series of real-world contaminated soils is required, the PAH will not be radiolabelled, and the only way to quantify the dermal bioavailability will be through specific analysis of each PAH using standard analytical techniques for organics (e.g. GC-MS or HPLC).

Moody *et al.* (2011) used a "by difference" method to quantify the dermal bioavailability of real-world PAH that required the analysis of two media – the original contaminated soil and an analysis of the same soil after 24 h of exposure in a diffusion cell (the same apparatus and operating conditions as Moody *et al.* (2007)). The applied soil remaining on the membrane was removed using a soap-wash. The difference between the amounts of PAH in the original soil and that found in the soap wash expressed as a percentage of the mass in the original sample, providing a PADA value. Using this approach, it is neither necessary to analyse the amount of PAH in the skin membrane nor the amount of PAH in the receptor solution. The soil they used for this was from a coal-tar contaminated site in Canada and contained 255 mg kg^{-1} of the USEPA 16 PAH, where individual compounds ranged from 0.9 to 56 mg kg^{-1}. The problem encountered with this approach was that the amount of soil used in the test (32 mg) is low causing difficulties in detecting the low concentrations of PAH in the soap extracts with the HPLC and UV-vis detector system used for the study. Percentage dermal bioavailability data for only five of the 16 PAH originally analysed was reported: phenanthrene (60.8%); fluoranthene (49.4%); pyrene (48.7%); 1, 2-benzanthracene (26.4%); and chrysene (33.5%). These PAH showed an average reduction of 2 in their percentage dermal bioavailability compared to equivalent tests without a soil matrix and a linearly decreasing trend in dermal bioavailability with increasing octanol/water partition coefficients (Figure 5). It should be remembered that the study uses a single soil and that findings show K_{oc} has a larger effect on dermal bioavailability of PAH in soil compared to the pure compounds, but also that when Log K_{oc} <4.5, the effect of the soil matrix in suppressing dermal bioavailability is not significant.

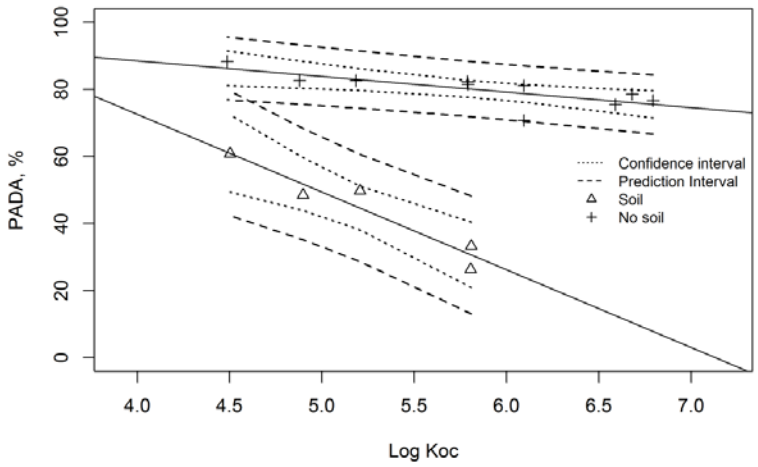

Figure 5 – Comparison of dermal bioavailability of PAH with and without a soil matrix (Moody 2011)

In addition to the studies listed in Table 1 which relate to dermal bioavailability of PAH contaminated soils, five other papers have been included which have studied direct dermal bioavailability of PAH compounds applied directly to skin in their pure form. These papers illustrate a number of important points that should be considered when developing a dermal bioavailability test for PAH contaminated soils. Three papers discuss the effect of skin type and skin preparation on dermal bioavailability. Moody *et al.* (2007) uses a similar *in vitro* flow through diffusion cell to Moody *et al.* (1995) measuring the dermal bioavailability of radiolabelled BaP combining together the BaP found in the receptor solution and absorbed in the skin. They compared rat, guinea pig and three types of human skin, all freshly prepared. They found percentage dermal bioavailability values ranging from 95% down to 23% under the same experimental conditions. In particular there was a significant difference from the skin of a 50 year old human (43 ±8.7%) and that of a 32 year old human (23 ±5.3%) clearly showing that skin age is an important parameter. In the same study they also carried out *in vivo* tests on the rat and guinea pig and found that for the rat the *in vitro* test (95 ±9.6%) over-estimated the *in vivo* value (70 ±7.6%) whereas for the guinea pig the *in vitro* test (51 ±3.0%) underestimated the *in vivo* value (68 ±9.3%).

Moody *et al.*, (2007) also investigated the effect of freezing skin samples on the dermal bioavailability of six environmental contaminants which included naphthalene and BaP. Using radiolabelled compounds they measured the dermal bioavailability combining together the BaP in the receptor solution and absorbed in the skin and found that for all compounds apart from BaP that freezing did not have a significant effect on measured values. In a more recent study, another research group (Jacques *et al.*, 2010) used a rather different experimental set up, working with pig ear skin in which skin punches were seeded dermal side down in polycarbonate Transwell® inserts (28 mm inner diameter, with a 23 mm diameter and 8 µm pore size filter) placed in a 6-well plate prefilled with 1.5

ml culture medium at 37 ˚C in a 5% CO_2 air incubator. The culture medium was Dulbecco's Eagle Modified Medium (DMEM) supplemented with l-glutamine (0.584 g l^{-1}), streptomycin/penicillin (100 g ml^{-1}), fungizone (2.5 g ml^{-1}), gentamycin (50 g ml^{-1}) and BSA (4%). This is equivalent to a miniaturised Franz diffusion cell. They used a 72 h experiment dosing both fresh and a frozen skin samples with radiolabelled BaP at 25, 50, 100, 200, 400, 600 and 800 nmol, corresponding to 1.51, 3.03, 6.06,12.12, 24.25, 36.39, 48.52 µg cm^{-2}, respectively), applied in 60 µl acetone to the skin surface. As well as measuring the distribution of the BaP left on the surface of the skin, absorbed in the skin and in the receptor solution they also quantified the metabolites of BaP in the receptor solution using Mass Spectrometry and Nuclear Magnetic Resonance analysis. A graphical summary of these results help illustrate the following three important observations (Figure 6):

i. In fresh skin, increasing amounts of the BaP are metabolised into in the skin and in the receptor solution to such an extent that the parent compound is only a minor fraction of the material collected in the receptor solution.
ii. BaP is not metabolised in frozen skin, and a larger proportion of BaP derived material remains in the skin compared to fresh skin. However, the sum of the amount of BaP in the skin and in the receptor solution was equivalent for both fresh and frozen skin (confirming the results of Moody *et al.*(2007))
iii. The fraction of the applied dose that is absorbed in the receptor solution decreases for both fresh and frozen skin with increasing applied dose.

Moody *et al.* (2011) suggest that the reason for very low recovery of BaP is that it is too insoluble to be partitioned into culture media even if 4% of BSA was added to the receptor fluid to facilitate the diffusion of lipophilic compounds (Bronaugh *et al.*, 1989; Ng *et al.*, 1992). This also accounts for the very low recoveries of BaP found in the receptor solutions of the relevant studies outlined (Table 1).

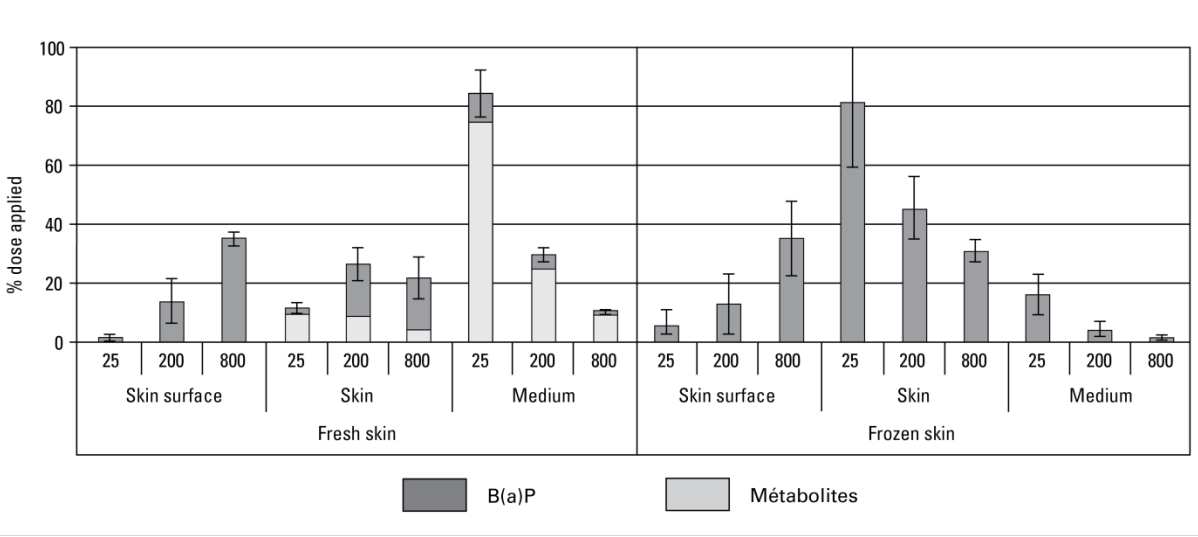

Figure 6 – Percentage of BaP and BaP metabolites present in culture media and skin after 72 h of incubation under different dosing levels using fresh vs frozen skin explants. Values are expressed as percent of the BaP applied dose and are mean±SD, n=3. (Jacques *et al.*, 2010).

3.2.7 Percentage absorbed of applied dose and experimental conditions

The majority of the work on the dermal bioavailability of PAH has concentrated on BaP, with a smaller amount of information on a few other PAH (Naphthalene: Turkall *et al.*,, (1994); Phenanthrene: Kadry *et al.*, (1995); USEPA 16: Moody *et al.*, (2011); 60 PAH: Roy *et al.*, (1998)). Roy *et al.* (1998) measured the dermal bioavailability of 60 PAH using a Franz diffusion cell with rat skin and a Volpo 20 ™ receptor solution with a 0.01% thimersol antibacterial agent over a 24 h period. The results were averaged over 4-5 experiments. The amount of PAH in the receptor solution measured using Gas Chromatography Flame Ionisation Detection (GC/FID) or Gas Chromatography Mass Spectrometry(GC/MS) was used to calculate PADA. This data clearly indicates that increasing K_{oc} (i.e. lipophilicity) is associated with a decrease in PADA and is in general agreement with the subsequent findings of Moody *et al.*(2011). PADA data were then used to develop three QSAR models using K_{oc} and other molecular descriptors. K_{oc} was found to be the most important predictor in all three models. Although these data have subsequently been used in follow up studies (Shatkin *et al.*, 2007 and Bouwman *et al.*, 2008) care needs to be taken in their use since they were derived only from the amount of PAH found in the receptor solution. It has been clearly shown, however, that significant amounts of PAH are absorbed into the skin (Abdel Rahman, 2002; Roy *et al.*,, 1998; Roy and Singh, 2001;Wester *et al.*, 1990; Moody *et al.*, 1995; Moody *et al.*, 2007; Moody *et al.*, 2009; Moody *et al.*, 2011; Jacques *et al.*, 2010).

A comparison between PADA and the Log K_{oc} for the Roy *et al.* (1998) study and Moody *et al.* (2011) data is presented in Figure 7. The Moody *et al.* (2011) data, which included absorption in the skin is, significantly higher than the Roy *et al*, (1998) data. Both datasets show the same trend of increasing PADA with decreasing log K_{oc} which can be fitted with linear regression models. Using PADA as the dependant variable and K_{oc} as the predictor variable, the Roy *et al.* (1998) data gives a model with a slope of 14.6 and an intercept of 99, explaining 58% of the variance in the model. The Moody *et al.*(2011) data gives a slope of -4.7 and intercept of 107, explaining 55% of the variance. Confidence intervals for the regression line and for predicting PADA are also presented (Figure 7). Both studies show that the PADA decreases as the hydrophobicity of the PAH increases but also that the inclusion of the PAH absorbed in the skin has significantly increased PADA.

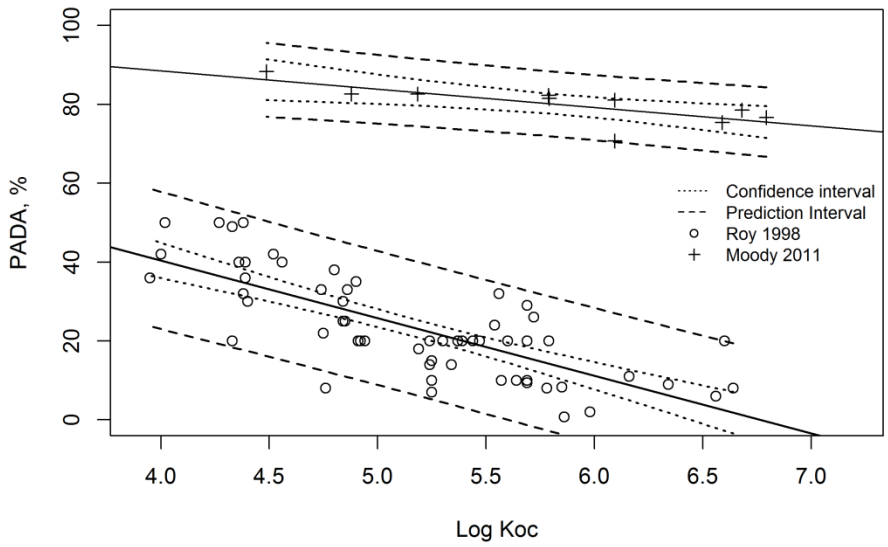

Figure 7 – Measured dermal bioavailability of 60 PAH (Roy *et al.*,1998b) plotted against log octanol water partition coefficients compared to the study of Moody et al 2011

Whilst there is a lack of consistency in the experimental methods and the way in which data are reported, there are a number of studies which give measured values for dermal bioavailability of PAH in soils. The majority of these data relate to BaP. The data from the summary of information of Spalt *et al.,* (2009) contains 44 measurements of percentage of dose absorbed and associated flux rates through the skin for BaP in a soils matrix. These data are derived from 7 references and represent 15 different soils. In some instances data for the same soil has been reported more than once under different experimental conditions (e.g. different sample contact times or different ageing times for soils). The data from Moody *et al.* (2007) has been added to this to give a further 2 measurements. All of the data comes from spiked soils and not from naturally aged contamination.

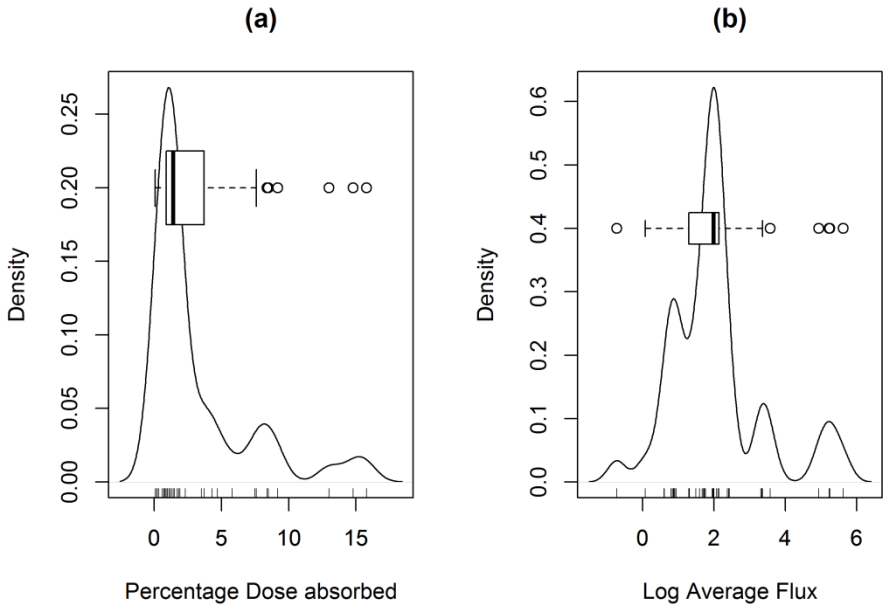

Figure 8 – Summary plots of literature reported BaP dermal bioavailability: (a) the percentage of the dose absorbed; and (b) the average flux of BaP absorbed into the skin.

Summaries of PADA as a density plot overlaid with a box and whisker plot and individual values shown as a tick marks on the x-axis are presented (Figure 8). This shows a range of values from ca. 0-15% with a median value of 1.4%. Spalt *et al.* (2009) suggests that PADA is not always the best way to report the data since it is dependent on the loading rate and time of the experiment and that the uptake flux of the contaminant is more useful, measured in mass of contaminant per unit area per unit time (e.g. pg cm^{-2} h^{-1}). The flux of BaP in soil is presented on a log10 scale for comparison (Figure 8). This shows that values cover 6 orders of magnitude with a median value of 100 pg cm^{-2} h^{-1}. The one study that measures the dermal bioavailability of PAH from a naturally aged soil (Moody *et al.*, 2011) does not report a value for BaP. Using a linear regression fit to their plot of PAH PADA vs Log K_{oc} (Figure 7) the predicted PADA for BaP in the soil used is 44% (assuming Log Koc =5.25 (Roy *et al.*,, 1998)), which is over double the highest value found in these other studies (Figure 8). The wide range of values reported clearly indicates that further work is required to understand which are the most important parameters controlling the dermal bioavailability process and, in particular, the effect of soil properties which has to a large extent been overlooked in current work. The range of experimental conditions that have been used are presented (Figure 9) in addition to BaP PADA (Figure 8).

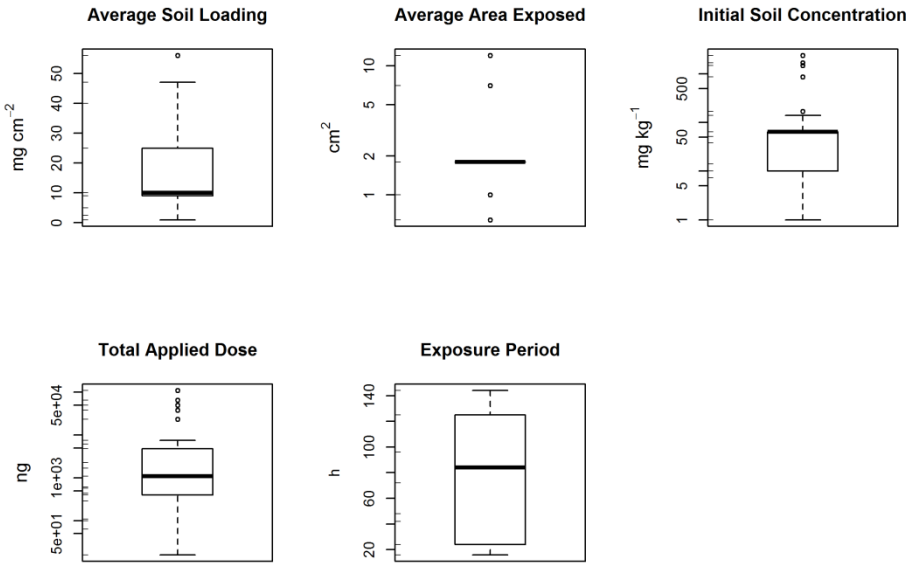

Figure 9 – Boxplot summaries of the experimental conditions used in the 46 literature studies reporting the dermal bioavailability of BaP from Soil (actual data points indicated by tick marks on the y axis)

3.3 In silico dermal bioavailability methods

In silico modelling of dermal bioavailability is the establishment of a computer simulation of the amount of chemical compound absorbed into the skin following exposure in any associated vehicle. The simulation can use a combination of input information including physical and chemical properties of the chemical compound and the vehicle being studied along with physical laws governing the diffusivity of a compound through a target media (e.g. Equations 1 to 5). The model can be theoretical where a series of linked mathematical equations are used to describe the underlying theory of all of the processes involved. Alternatively, an empirical model can be used in which the input parameters are the available data related to the chemical compounds, the vehicle and the skin and a regression of various different forms is used to set up a predictive relationship. Models of dermal bioavailability are always simplifications of a highly complex set of biological and physico-chemical interactions that drive passive diffusion of organic compounds from a heterogeneous matrix to a heterogeneous medium i.e. movement from soil to skin. The two approaches to modelling dermal bioavailability found in the literature are Quantitative Structure Activity Relationships (QSAR) and Physiologically-Based Pharmacokinetic (PBPK) models. Relevant examples of both type of study were identified and summarised (Table 2).

The two most common dependent dermal bioavailability variables are: 1) the concentration of compounds present in a given layer of the epidermis following application to the skin ($Bioav_{derm}$); 2) the ratio of $Bioav_{derm}$ and the total concentration present in the original soil sample (ABS_d or PADA) (Equation 1). Other dependent variables include flux, permeability coefficients (K_p) or the proportion

of the total applied concentration in a target organ or tissue (*in vivo*). Physico-chemical properties commonly include molecular properties (e.g. molecular weight and hydrogen bonding) or partition coefficients (e.g. K_{ow}) for the compound. These properties may be measured or predicted using computer software (e.g. NEMISIS (Oxford Molecular Ltd, 1992)). No studies were found that use the physico-chemical properties of soil, although some recent work on human ingestion bioaccessibility of PAH has started to investigate such relationships using infrared spectroscopy and organic carbon data (Beriro, 2015; Cave *et al.*, 2015).

Numerical predictive models of dermal bioavailability may be produced using stochastic (having a random probability distribution or pattern that may be analysed statistically but may not be predicted precisely) or deterministic (whose resulting behaviour is entirely determined by its initial state and inputs, and which is not random or stochastic) methods. Deterministic methods are either parametric or non-parametric and output the same results each time they are applied. Parametric methods make assumptions about the characteristics of the data or the format of the model. Non-parametric make fewer assumptions about the form input data and modelling process. Multiple linear regression (MLR) (Kirkwood and Sterne, 2003) is the most common parametric method used for estimating dermal bioavailability (Table 2).

Table 2 – Key in silico dermal bioavailability studies

Study	Method	Descriptive variables	Analytes and media	Predicted variable
QSAR				
Potts & Guy (1992)*	MLR	K_{ow} and molecular weight	177 pure organic compounds	Permeability coefficient (Log K_p)
Barrett *et al.,* (1995)**	MLR	Molecular volume, Log Kow and melting point	91 pure organic compounds	Permeability coefficient (K_p)
Roy *et al.,* (1998)	MLR	50 physico-chemical properties	60 pure PAH compounds	PADA (*in vitro* – rat skin)
Pugh *et al.,* (2000)***	Principal Components Analysis and MLR	Eigenvectors, molecular weight and Log K_p,	57 pure organic compounds	Flux as Log diffusion (D) coefficient / path length (h) (Log (D/h))
Devillers (2000)	Artificial neural networks	Used data from Flynn 1990 and Potts Guy 1992	Used pure compound data from Flynn 1990 and Potts Guy 1992	Used data from Flynn 1990 and Potts Guy 1992
Pannier (2003)	Adaptive neuro fuzzy inference system	MW and log octanol/water partition coefficients, hydrogen bond donor activity, hydrogen-bond acidity, hydrogen bond acceptor activity, dipolarity/polarizability and the molar refractivity.	Used pure compound data from Flynn 1990 and Potts Guy 1992	log skin permeability coefficient (log Kp)
Riviere & Brooks (2005)	Abraham's Linear Free Energy Relationship model	5 physico-chemical properties and 24 vehicle combinations	12 organic compounds in ethanol, propylene glycol Methyl nicotinate and sodium lauryl sulfate	Permeability constant (K_p) (*in vitro* – pig skin)
Wang *et al.,* (2008) ****	Gene Expression Programming	Range molecular descriptors (number not known)	60 pure PAH compounds	PADA (*in vitro*)
Moss *et al.,* (2009) *****	Gaussian process	6 physico-chemical properties	142 pure organic compounds	Log K_p
Buist *et al.,* (2010)	8 literature QSAR models	Molecular weight and Log K_{ow}	15 organic pharmaceutical / cosmetic compounds (finite dose)	K_p
PBPK				

Kissel & McAvoy (1989)[+ +]	Fugacity based model (Paterson and Mackay, 1986)	Physico-chemical properties of soil and skin	2,3,7,8-Tetrachlorodibenzodioxin (TCDD) in soil	PADA (*in vivo*)
Wester *et al.,* (2002)	PBPK (Ramsey and Andersen, 1984)	Partition coefficients and metabolism rates	Perchloroethene in soil	K_p
Shatkin *et al.,* (2002) [+]	Fugacity kinetics (McKone and Howd, 1992)	Physico-chemical properties of soil and skin	BaP in soil	PADA (*in vitro*)
Jongeneelen & Berge (2012)	PBPK IndusChemFate (Long-Range Research Institute, 2015)	Physico-chemical properties for inhalation and dermal exposure	Pyrene vapour	Urinary concentrations (human biomonitoring)
Ortiz *et al*. (2014)	human PBPK model extrapolated from a rat PBPK model	Physico-chemical properties for mainly dermal exposure	3-OHBaP	Numerous biological endpoints.

[*] Data from Flynn (1990) Scheuplein & Blank and Ackermann *et al*
[**] Data from Flynn (1990)
[***] Data from Wilschut *et al.,* (1995), Degim *et al.,* (1998), Pugh *et al.,* (1996), Abraham (1993) and Abraham *et al.,* (1995)
[****] Data from Roy *et al.,* (1998)
[*****] Data from Flynn (1990) and Moss *et al* (2006)
[+] Data from Roy *et al.,* 1998b and Reeves *et al.,* (2001)
[++] Data from Poiger and Schlatter (1980)

3.3.1 Quantitative structure activity relationships

QSAR (also referred to as quantitative structure permeability relationships (QSPR) and quantitative structure permeability relationships (QSPeR)) for dermal bioavailability statistically relate physico-chemical properties to experimentally determined percutaneous penetration of exogenous chemicals (Moss *et al.*, 2002a). These properties relate to the partitioning behaviour of a compound (e.g. K_{ow}) or linear-free energy relationships (LFER) (e.g. molecular size, hydrogen bonding) (Riviere and Brooks, 2005). The first MLR QSAR study applied was applied to pharmaceuticals and toxicology by Potts and Guy (1992) (Equation 6).

$$log K_p = 0.71 \log K_{ow} - 0.0061\, MW - 6.3$$

Equation 5 – Potts and Guy Log K_p regression model

Most QSAR studies use MLR to link PADA (e.g. Roy *et al.*,, 1998a) and K_p (Potts and Guy, 1992) with physico-chemical properties of multiple compounds (Table 2). A large amount of work on QSAR is driven by the need to help characterise the risk to human health under relevant state regulations (e.g. European Commission Council Regulation, 2007). Some QSAR studies have also incorporated principle component analysis (PCA) methods to assist with identifying key controls on dermal bioavailability (Pugh *et al.*, 2000).

Machine learning algorithms are also applied in QSAR, as shown in recent studies: gene expression programming (Wang *et al.*,, 2008); Gaussian profile (Moss *et al.*,, 2009) and similar stochastic techniques by others (Table 2).

Cronin and Schultz (2003) recognised that QSAR outputs should achieve the following criteria: 1) a well-defined and measurable endpoint; 2) use a chemically and biologically diverse dataset; 3) be based on chemical descriptors that are consistent with the endpoint; 4) use appropriate statistical methods; and 5) have a strong mechanistic basis. QSAR are usually completed for multiple compounds modelled in a pure form and infinite dose conditions. For example, Roy *et al.*, (1998a) modelled *in vivo* PADA data for 60 PAH individual compounds whilst Flynn (1990) modelled K_p for 97 organic chemicals. Studies have since been completed that have used Roy's and Flynn's data (e.g. Potts and Guy, 1992; Barratt, 1995). Remodelling such data is common because obtaining *in vivo* and *in vitro* estimates of dermal bioavailability is time consuming, expensive and can raise important ethical issues.

QSAR studies can be performed for complex vehicles containing the compound although this is much less common than modelling the permeability of pure compounds. Riviere and Brooks (2007) propose

the use of a hybrid LFER model that incorporates a mixture factor (MF) to account for the physico-chemical properties of the vehicle affecting the release of the compound prior to absorption. The hybrid model appears to appropriately account for the variance in the model, providing a useful approach to conducting QSAR for compounds contained in complex vehicles. Many studies now acknowledge the role of the vehicle and the susceptibility of compound to mixture interaction in dermal bioavailability. A review of such work is presented by Karadzovska *et al.,* (2013a). It should be noted that none of the studies mention the effect of soil or focus on PAH.

3.3.2 Physiologically-based pharmacokinetic models

PBPK modelling uses mathematical models to describe the absorption, distribution, metabolism and elimination kinetics of different chemical compounds (Dancik *et al.*, 2008). PBPK models of dermal bioavailability assume that the *stratum corneum* is the principal barrier to permeation and penetration (Dancik *et al.*, 2008). Such models are sometimes referred to as physiologically-based toxicokinetic (PBTK) models (Jongeneelen and Ten Berge, 2012). PBPK models are a unified model of many kinetic equations (e.g. Fick's Laws) for different animal compartments (e.g. epidermis, dermis, blood plasma) that are used to estimate the fate and transport of chemical compounds for a variety of biological endpoints (e.g. adipose tissue, urine, liver). Because of the complexity of such modelling, much of the work is completed using proprietary commercial software (e.g. ADMET Predictor™: Simulations Plus Inc. (2015)) or freeware (e.g. IndusTox: Berge and Jongeneelen (2015)). It is necessary to provide the software with physico-chemical properties of the chemicals being modelled. This information is often provided using an embedded QSAR model. As with QSAR this information may be passed to the software using MLR or stochastic techniques.

4 Estimating dermal bioavailability of PAH in soil

This review has presented a large amount of information to facilitate an understanding of the estimation of human dermal bioavailability. The aim of this review was to move closer toward developing an integrated *in vitro* and in silico method for the measurement and modelling of the human dermal bioavailability of PAH in soil. The limitations identified in the literature reviewed have been used to inform the key considerations discussed in the following sections. These are considerations that should be made when developing a suitable physiologically based method for estimating the bioavailability of PAH in soil.

4.1 Soil dosing

The importance of the amount of soil applied per unit area as well as considering whether the soil is saturated with the chemical under study have been highlighted (Kissel *et al.*, 2008; Spalt *et al.*, 2009). If the soil is fully saturated then the measurement is more closely aligned to measuring the dermal absorption of the free chemical in the form of an infinite dose and may fail to provide information on

the effect of soil. Kissel *et al.* (2008) and Spalt *et al.* (2009) point out that PADA was dependant on the amount of soil applied per unit area and that if multiple layers of soil are applied to the skin surface then the PADA decreases since the soil layers not touching the skin will supply less of the contaminant to the skin surface. This has been demonstrated or discussed in a number of studies (Kissel *et al.*, 2008; Spalt *et al.*, 2009; Frasch *et al.*, 2014) as well as specifically for BaP in soils (Roy and Singh, 2001). Spalt *et al.* (2009) provide an equation for loading a monolayer of soil (Equation 7).

$$SL_{monolayer} = \rho_{particle}\left(\frac{\pi d}{6}\right)$$

Where: $SL_{monolayer}$ is the soil load (mg cm^{-2}) representing a monolayer, $\rho_{particle}$ is the particle density of the soil (mg cm^{-3}), and d is the particle diameter (cm).

Equation 6 – Formula to calculate a soil monolayer

This equation makes the assumption of soil particles being spherical in a homogeneous close packed layer. This guidance was used in the Yang *et al.* (1989) study of *in vitro* BaP dermal bioavailability on rat skin with a loading rate of 9 mg cm^{-2}. Choate *et al.* (2006) report literature values for loading rate vary from 0.62 to 1.14 mg cm^{-1}. In practice the assumptions used in the literature studies are completely true to life because of the random nature of human activities and, although equations for corrections of multiple layer loading have been suggested (Kissel *et al.*, 2008; Spalt *et al.*, 2009), the best approach is to use Equation 7 as a guideline and to carry out the absorption experiments at a series of soil loadings to provide information on how this affects the specific contaminant and soil type.

The literature on loading rate relies on the selection of an appropriate soil particle size. This is an important aspect in the design of any dermal bioavailability study involving soil. A recent comprehensive review of literature to select an appropriate particle size for oral bioaccessibility studies is also relevant to quantifying dermal bioavailability (Ruby and Lowney 2012). Both science areas rely on understanding which size fraction is most likely to adhere to human hands. To this end, Ruby and Lowney recommend <150 µm. This value differs from other dermal exposure studies i.e. Choate *et al* (2006), who suggest using ≤3 µm. Both values are considerably lower than 250 µm, which is currently the most commonly used particle size for oral bioaccessibility.

It has been shown that the exposure times for the different studies vary considerably from <20 h to >140 h (Figure 8). In addition to the total exposure time, some studies take multiple intermediate measurements (Yang *et al.*, 1989; Turkall *et al.*, 1994; Roy *et al*,, 1998b; Roy and Singh, 2001; Abdel-Rahman *et al.*, 2002; Moody *et al.*, 2011), whilst others make a single measurement at the end time of the experiment (Wester *et al.*, 1990; Moody *et al.*, 2007). There does not appear to be any standardised approach to make the measurement at specific end-point i.e. when steady state is reached (in the case of infinite dose) or in the case of a flow through diffusion cell when the receptor solution

concentrations returns to baseline concentration of the chemical being studied (in the case of finite dose). Spalt *et al.* (2009) suggest that multiple exposure times should be measured to give information on the kinetics of the dermal absorption ranging from 1-2 h to >8 h to characterise the effect more clearly.

4.2 Soil maturity

Soil ageing has been shown to have a significant effect on the dermal bioavailability of PAH from soil (Roy and Singh, 2001; Abdel-Rahman *et al.*, 2002). Both studies found that soil ageing reduced the absorption by a factor of 2 compared to freshly spiked soil. This is very much in-line with wider studies on PAH sorption to soils, which also clearly shows how ageing of soils reduces the bioavailability of PAH e.g. Alexander (2000). Most of the studies reviewed do not use naturally aged soil, but rather spike natural soil or amended soil (Yang *et al.*, 1989; Wester *et al.*, 1990; Turkall *et al,*, 1994; Kadry *et al.*, 1995; Roy *et al,*, 1998b; Roy and Singh, 2001; Abdel-Rahman *et al.*, 2002; Moody *et al.*, 2007). Part of the reason for this is coupled with using radiolabelled PAH and associated scintillation methods for quantification (Table 1). In order to produce a method that is suitable for risk based land mangement, naturally aged soils covering a range of geological, industrial and contaminant conditions need to be examined. It is clear that soil properties, including aging, play an important part in the sorption and release of PAH from soil (Pignatello and Xing, 1996; Semple *et al.*, 2004; Juhasz *et al.*, 2008; Siciliano *et al.*, 2010; James *et al.*, 2011; Mayer *et al.*, 2011; Delannoy *et al.*, 2014).

4.3 Skin type

The effects of different skin types and skin preparation on dermal bioavailability of BaP have been investigated and have been shown to be important (Moody *et al.*, 1995; Moody *et al.*, 2007; Jacques *et al.*, 2010). Skin preparation techniques have also been suggested as source of uncertainty e.g.washing *ex vivo* skin may affect the role of the lipophilic acid mantle present on the outer epidermis (Chilcotte, 2008). High throughput methods are expected to benefit from use of a synthetic skin (Netzlaff *et al.*, 2005; Schreiber *et al.*, 2005; Schaefer-Korting *et al.*, 2008; Sinko *et al,*, 2012; Karadzovska and Riviere, 2013b; Uchida *et al.*, 2015) rather than *ex vivo* skins. However, no examples of their use for PAH in soils were found in the literature.

4.4 Experimental design

Another important point that comes from the reviewed diffusion cell studies, is that the skin membrane usually contains a higher proportion of BaP at the end of the experiment than the receptor fluid (Wester *et al.*,, 1990; Roy *et al.*, 1998b; Abdel-Rahman *et al.*, 2002; Moody *et al.*, 2007; Jacques *et al.*, 2010; Moody *et al.*, 2011). Whilst the BaP in the skin has not reached the systemic circulation it has been absorbed into the body and may be leached out slowly at a later date or cause localised health effects in the skin itself. It is clear, therefore, that only measuring the PAH in the receptor

solution could seriously underestimate the dermal absorption and that any testing protocol must make sure that both the PAH content of both the skin and the receptor solution should be taken into account when calculating the dermal absorption. This approach should also be complemented by an examination of the effect of different receptor solutions, which is not addressed in current literature. For lipophilic compounds such as PAH, the relative solubility of the compound in the receptor solution is likely to be an important driving force of the diffusion gradient through the skin (Jacques *et al.*, 2010). This raises the question of whether receptor solutions are actually necessary if it is simply the amount of PAH that is released from soil into the skin that the most important factor based on the definition dermal bioavailability presented in the introduction of the present study.

Yang *et al.* (1989) and Moody *et al.* (1995) provide two examples where the *in vitro* / *in vivo* comparisons have been carried out. Yang *et al.* (1989) used a rat model with a BaP spiked soil and found that, within the uncertainty of the measurements, the measured dermal absorption was the same. Moody *et al.*(1995) used a rat and a guinea pig model with BaP in the form of a pure compound and found that the rat *in vitro* test (PADA 95±9.6%) overestimated the *in vivo* value (PADA 70±7.6%) whereas for the guinea pig the *in vitro* test (PADA 51±3.0) underestimated the *in vivo* value (PADA 68 ±9.3). Outside of these specific studies on PAH, the general opinion, that is supported by a number of studies (Kielhorn *et al.*, 2006), is that *in vitro* testing using diffusion cells and *ex vivo* human and animal skins are acceptable alternatives to *in vivo* testing. Along with studies using synthetic skin (Karadzovska and Riviere, 2013b), developments in high throughput methods (Sinko *et al.*, 2012), new methodologies (Riviere *et al.*, 2007; Baynes *et al.*, 2008; Karadzovska and Riviere, 2013a) and research into *in vitro* methods is moving into a new era of development. In particular attention should be paid to the use of synthetic membranes (e.g. Netzlaff *et al.*, 2005; Schreiber *et al.*, 2005; Schaefer-Korting *et al.*, 2008) and new methods such as membrane coated fibres (e.g. Riviere *et al.*, 2007; Baynes *et al.*, 2008; Karadzovska and Riviere, 2013a), since they may offer suitably expedient accurate alternatives to more traditional methods. To date, no studies have been performed for soil borne PAH using these methods.

4.5 In silico modelling of dermal bioavailability

Rapid low cost prediction of the dermal bioavailability of organic compounds using in silico methods has received much attention over the past 20 years (Figure 2). The literature shows that physico-chemical properties, partition coefficients and diffusion kinetics can be used to produce predictive models to complement *in vivo* and *in vitro* estimates (Table 2). The two types of model that appear in the literature are QSAR and PBPK. In silico modelling studies tend to assume an infinite dose scenario, mainly because the permeability coefficient (K_p) assumes steady-state conditions and many studies used this as the predicted variable (Table 2). K_p is not directly related to the finite dose (Bouwman *et al.*, 2008), which is important because the use of the dermal bioavailability for PAH in

soil in human health risk assessment of contaminants in soil assumes a finite dose (Environment Agency, 2009b). Buist *et al.* (2010) used pharmaceutical and cosmetic compounds to produce QSAR models for non-volatile organic compounds for finite dose conditions. No in silico studies were found to examine environmental exposure to finite doses of exogenous organic compounds, either in their pure form or in soil. Riviere and Brooks (2005) propose a mixture factor approach to QSAR modelling of finite doses although their work is based on homogeneous solvent vehicles rather than soil. Despite the apparent absence of relevant work on the dermal bioavailability of PAH in soil, there are some recent studies in the human ingestion bioaccessibility literature which account for finite doses, complex heterogeneous vehicles (soil) and contaminants in mixtures (gasworks or coking works soils) (Beriro, 2015; Cave *et al.*, 2015). In each case these studies use machine learning methods to model the release of PAH from soil into simulated gastro-intestinal fluids.

5 Conclusions

This study has brought together literature *on in vivo, in vitro* and in silico methods for PAH dermal bioavailability methods from a wide variety of scientific disciplines including occupational and environmental exposure, medical, pharmaceutical and cosmetic research and associated mathematical modelling.

The current review has identified a variety of *in vitro* approaches which can and have been applied to measuring dermal bioavailability of PAH in soils, as well as identifying a number of practical shortcomings if they are to be applied to high throughput laboratory analysis of contaminated soils. These include: i) the need for better analytical protocols for measuring low concentrations of PAH in a variety of sample matrices; ii) a much better understanding of the soil properties and how these control dermal absorption; iii) account for advancements in diffusion cell designs; and iv) the effect of operating parameters such as receptor fluid composition; use of skin membranes and possible substitutes with new materials.

The current review has identified that mathematical modelling of dermal bioavailability is conducted by a combination of QSAR and PBPB methods. These approaches rely on both traditional statistical and kinetic models but also, and more recently, techniques such as machine learning. Future developments for modelling dermal bioavailability of PAH in soil is could follow a traditional statistical method approach or machine learning /alternative non-linear methods which account for the complexities associated with a finite dose, mixtures of contaminants and the heterogeneous nature of soil.

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