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Dermal absorption of high molecular weight parent and alkylated polycyclic aromatic hydrocarbons from manufactured gas plant soils using *in vitro* assessment

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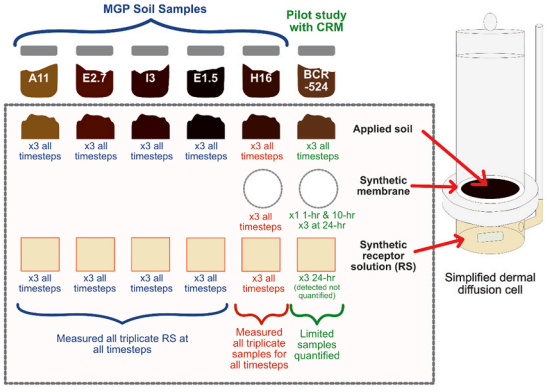
HIGHLIGHTS

- Conducted *in vitro* human dermal bioavailability experiments on 5 MGP soils.
- Measured 20 parent and 7 alkylated HMW PAHs dermal fluxes.
- Highest dermal fluxes measured for PAHs with fewer rings and lower alkylation.
- Membranes acted as PAH sink for all HMW PAHs.
- Alkylated C1-Fla/Pyr showed significant dermal flux at longer timesteps.

GRAPHICAL ABSTRACT



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ABSTRACT

An enhanced *in vitro* human dermal bioavailability method was developed to measure the release of twenty parent and seven alkylated high molecular weight (HMW) polycyclic aromatic hydrocarbons (PAHs) from contaminated soils collected from five former manufactured Gas Plants (MGP) in England. GC-MS/MS was used to quantify HMW PAHs in soil, Strat-M artificial membrane representing skin, and synthetic receptor solution (RS) representing systemic circulation at 1-h, 10-h, and 24-h timesteps. Fluoranthene and pyrene exhibited the highest fluxes from soils to membrane (ranging from 9.5 - 281 ng/cm^2/h) and soil to RS (<LOQ to 16.9 ng/cm^2/h). Chrysene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and the alkylated C1-fluoranthene/pyrene homologue series demonstrated fluxes higher than other HMW PAHs. The dermal fluxes were generally lower than those reported in previous investigations and suggests that dermal absorption varies between both HMW parent and alkylated PAHs and individual PAHs. The utilisation of real-world contaminated soils allowed for a more realistic

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representation; this is important because current risk assessment guidance is baseed on results from experiments that used artificially spiked soils. This research shows that the the ranges of dermal fluxes are PAH dependent and impact the mass of absorbed from soil after dermal exposure and therefore the potential risk contaminated soil poses to human health.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of thousands of individual organic compounds that are widespread in the environment, originating from both natural and anthropogenic sources [47]. Certain PAHs are known or suspected carcinogens and mutagens to humans, with benzo[*a*]pyrene (BaP) being a well-studied example of a confirmed carcinogen [22]. The 16 PAHs listed by the U.S. Environmental Protection Agency (EPA16) are commonly investigated during site investigation and risk assessment of contaminated post-industrial land [23]. However, the environmental presence of numerous other PAHs in complex mixtures with varying toxicities is notable, with non-EPA16 PAHs accounting for 69.3–95.1% of the overall toxic equivalents (TEQ) from 24 PAHs [39]. TEQ are calculated using individual PAH concentrations and their corresponding toxic potential relative to BaP, referred to as the toxic equivalent factor (TEF) [36,39]. Evaluation by the TEF method is one approach to estimate human health risks from chronic exposure to hazardous chemicals. Another is to use surrogate markers, which assumes that the cancer risk from a PAH mixture is proportional to the concentration of a surrogate marker PAH, typically BaP [36]. However, these methods become problematic as they assume either that all PAHs have similar dose-response relationships and share similar mechanisms. The approaches also assume that all PAHs exhibit similar concentrations and behaviours to BaP, which is not applicable for PAH profiles diverging from BaP [33].

Alkylated PAHs (alkyl-PAHs) are characterised structurally by the presence of aliphatic hydrocarbon chains of various lengths attached to the fused aromatic rings (*e.g.* C1-fluoranthene) and are widely present in mixtures [57]. Alkyl-PAHs have received limited attention in human health risk assessments (HHRAs) despite expectations that they are equally or more toxic than their parent compounds [29,39]. For instance, 5-methylchrysene possesses a TEF of 1 (BaP = 1), while its parent PAH chrysene (Chry) exhibits a TEF of 0.01 [39]. Additionally, 1-methylpyrene has demonstrated mutagenic and chromosome-damaging activities in mammalian cells, while alkylation has been shown to enhance the toxicity of Chry and benzo[*a*]anthracene (BaA) [26,39]. The limited research on alkyl-PAHs can be attributed to challenges associated with gas chromatography mass spectroscopy (GC/MS) and/or liquid chromatography mass spectrometry (LC/MS), including stronger fragmentation of the alkylated chain and overlapping peaks (Andersson and Achten, 2015). Understanding the behaviour of both parent and alkylated PAHs in soils is crucial for accurately assessing human exposure and managing associated risks with contaminated soils. Particularly because increasing the alkylation of PAHs increases the hydrophobicity and lowers the vapour pressure of the compound [2]. These properties mean that alkyl-PAHs are likely to be more persistent in soil than parent PAHs, potentially creating a higher contribution towards oral and dermal human expose pathways than parent PAHs over time [53].

Brownfield land forms an important contribution towards the sustainable growth of urban areas, as their reuse helps to avoid developing greenfield sites [38]. Brownfield land investigated in this research includes former manufactured gas plant (MGP) sites, which often exhibit extensive contamination by PAH mixtures due to the historical production, storage and distribution of coal tar and other hydrocarbon-rich solid and gaseous sources on these sites [50]. Williams-Clayson et al. [57] measured 30 parent PAHs and 21 alkylated PAHs in 48 MGP soils, reporting the PAH distributions and potential sources of PAHs and found that alkyl-PAHs accounted for 7–65% of the total concentration of the parent and alkylated PAHs measured. Over 80% of the 48 MGP soils in our previous study measured BaP concentrations above the current Generic Assessment Criteria (GAC) for residential use with plant uptake (>5 mg/kg) [8]. These findings suggest that many MGP soils are likely to pose risk to human health, requiring remediation prior to any change in land use. Prior to any soil remediation, risk assessments are conducted to determine whether soils pose unacceptable risks to human health and the environment. These assessments consider three main exposure pathways: oral, inhalation, and dermal. Limited research has been conducted to investigate the human dermal exposure risk from PAHs in soils [43,45,5].

High molecular weight (HMW) PAHs, containing $\geq$ 4 aromatic rings provide a higher dermal exposure contribution than low molecular weight (LMW) PAHs [33]. This feature is driven mainly by their lower vapour pressures. HMW PAHs are also generally more persistent in soils due to their low water solubility and high lipophilicity [5,33]. Current risk assessment software makes assumptions about the proportion of the total concentration of PAH in soil that following intake are available for uptake into body tissues and circulation [11,33]. This proportion is referred to as a bioavailable fraction (BAF) and assumes that the toxicity posed is primarily through its systemic (lymphatic and circulatory system) circulation rather than localised effects. For the dermal pathway localised effect such as the development of skin tumours are largely ignored [33]. To account for both potential systemic and localised effects, we define dermal bioavailability as the proportion of compound/pollutant absorbed into human skin (our artificial membrane), where it may remain or be further released to systemic circulation (our synthetic receptor solution) [5]. Dermal diffusion from a chemical containing matrix is typically quantified as flux [15], representing the mass of a chemical absorbed per unit area over a given time period (ng/cm2/h). The use of flux facilitates cross-study comparisons of dermal absorption results [10,34,5].

HHRAs assumption of the dermal bioavailability of PAHs from soil are largely based on one of the first studies investigating PAH dermal bioavailability by Wester et al. [54]. Wester et al. [54] used an *in vivo* method to quantify the absorption of BaP from soil into monkeys. Their study examines a single PAH (BaP) in one type of soil, neglecting the potential effects of soil properties and physicochemical characteristics of PAHs on dermal bioavailability and the variation in flux between PAHs. Subsequent research on the dermal bioavailability of PAHs from soils has been limited [43,45,5].

Several dermal studies reported the PAH dermal absorption as a fraction or percentage of the applied dose (*e.g.* percentage absorbed of the dose applied (PADA)) [30,54]. However, comparisons between studies using PADA are problematic, due to PADA being dependent on the loading rate and experimental time [32,45]. Dermal studies tend to primarily focus on a small number of parent PAHs (typically BaP) which are spiked into artificial soils [1,30–32,34,41,54,59]. Studies using spiked soil neglect to account for the influence of different PAH mixtures and the environmental impacts (such as weathering, biodegradation, and aging) on the desorption of PAHs from soils [19,4,55]. Whereas dermal studies using real-world contaminated soils exposed to the impacts of weathering, interactions with natural organic matter, and containing a diversity of PAH compounds can help establish dermal absorption results that are more representative of real-world dermal exposure scenarios [27].

To address these gaps in the dermal literature, our study presents an *in vitro* human dermal bioavailability method to measure the dermal absorption of 27 HMW PAHs from MGP soils. One of the novelties of our

research is measuring the dermal bioavailabilities of the alkyl-PAHs, as well as parent PAHs in historically contaminated MGP soils. Our study aims to provide a more comprehensive understanding of the dermal absorption in PAH mixtures in real-world soils. Our method builds on method development research in the same laboratory by Lort [27]. Our study enhances this method by measuring the *in vitro* dermal bioavailability of 20 parent PAHs and 7 alkyl-PAH groups using five MGP soils, in addition to a control reference soil study using the certified refence material (CRM) BCR-524. The research objectives of our study are as follows: 1) develop an *in vitro* method to measure the dermal bioavailability of both parent and alkylated PAHs in former MGP soils; and 2) compare and contrast the dermal PAH fluxes from soil to the membrane and receptor solution with similar studies. The findings of this research have practical implications for risk assessments and provide broader scientific understanding of dermal absorption rates for a range of PAHs and MGP soils.

2. Materials and methods

2.1. Sample selection

Detailed information regarding soil sampling, soil properties, sample preparation, and associated sample selection can be found in Williams-Clayson et al. [57] and in Section 2 of the Supplementary information file (SI). A total of 93 soil samples were collected from 10 UK former MGP sites and 2 soils from local parks (to act as background samples) using various collection methods. The five soils selected for this paper were collected from either boreholes (E2.7) or hand dug pits (A11, H16 & I3) or collected from spoil (E1.5). In summary, five representative freeze dried and sieved (<250 μm) soils were selected for this study from a larger sample set of MGP soils from England. K-means clustering (k = 5) was employed to define distinct clusters within the wider 94 sample set. Cluster parameters used in the K-means estimates were bulk organic matter (OM) properties obtained using Rock-Eval(6) Pyrolysis (RE). One soil sample was chosen from each cluster, and a filter applied to exclude soils with BaP concentration < 5 mg/kg (BaP GAC).

2.2. In vitro dermal absorption experiments

Fig. 1 provides an overview of the *in vitro* dermal absorption experiment design. A comprehensive description of the method can be found in the SI Section 3, based on Lort [27]. For each dermal experiment, 1.0 g of moistened soil (soil moisture content (SMC%) 25%) was applied at a thickness of approximately 2 mm to a synthetic membrane (Strat-M) using a brass stencil (surface area 8.55 cm^2). High soil loadings were deliberately used to create a supermonolayer to ensure complete coverage, an infinite dose, and maximise the potential to create steady-state conditions [17,34]. The membranes with applied soil were placed on top of the donor chamber of a Franz glass diffusion cell containing a magnetic stirrer bar and the receptor solution (RS) (~33 mL). The RS was formulated using Hank's Balanced Salt Solution (HBSS) (ThermoFisher Scientific), Bovine Serum Albumin (BSA) (Merck Life Science UK Ltd.) and a HEPES buffering agent (ThermoFisher Scientific). The glass diffusion cell was assembled, clamped, and placed in a water bath set to 32 °C for the specified timestep (1-hour (h), 10-h, or 24-h, timesteps were selected to simulate real-world exposure time scenarios and enabled comparisons with other studies). Upon completion of the experiments, soils were gently removed from the membrane with a spatula, which was then washed with deionised water. The collected soils were freeze-dried and stored in sealed 10 mL crimp top glass vials. The membranes were air-dried on a watch glass before being stored in sealed glass containers. The full volume of RS was pipetted into 50 mL Pyrex glass vials sealed with caps. Both soils and RS were stored in a refrigerator maintained at 4 °C until further sample preparation (max. 8 weeks). RS samples from all five MGP soils were measured in triplicate at each of the three-timesteps, while membrane samples were measured for H16 at all timesteps and BCR-524 at 24-h.

Modifications to Lort's method [27] were made to move our experiments closer to real-world MGP site conditions. Improvements included reducing the SMC% from 40% to 25% and applying a higher soil loading calculated based on the average particle size of dry soil. The lower SMC % was determined by calculating the mean SMC% from real-world data at eight monitoring sites closest to the MGP sites using the COSMOS UK daily hydrometeorological and soil data (2013–2019) [46].

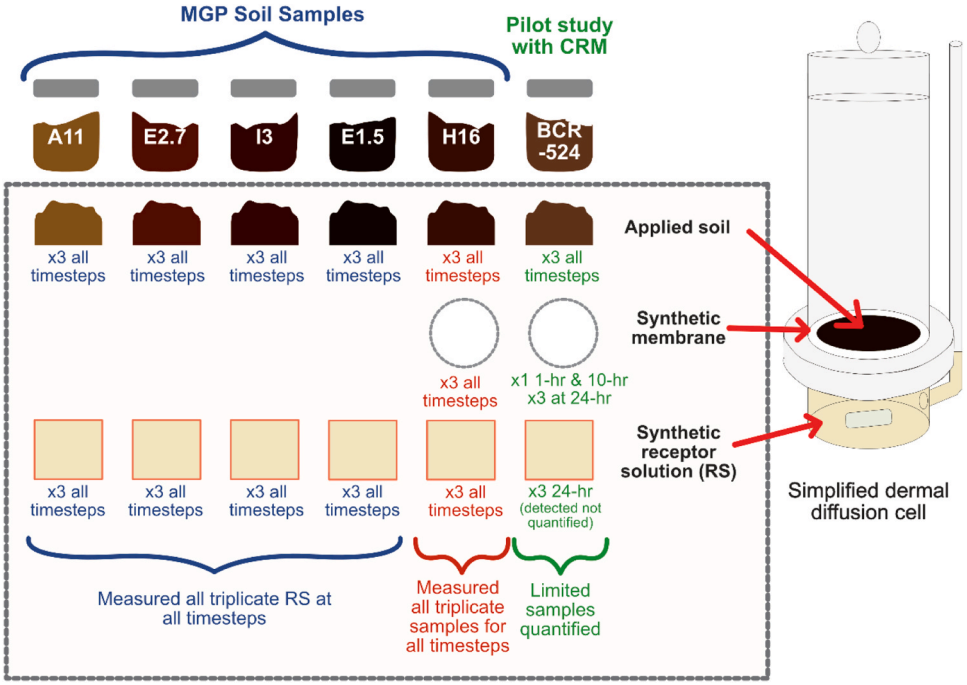

Fig. 1. Diagram illustrating the dermal experiments conducted in the control reference soil study and the five MGP samples, including which dermal matrices were measured for each sample at each timestep.

2.3. Sample analysis

HMW parent and alkylated PAHs in all three dermal matrices (soils, membranes, and RS) were measured using gas chromatography-tandem mass spectrometry (GC-MS/MS) (Thermo Scientific Trace 1300 GC coupled to a Thermo Scientific TSQ9000 triple quadrupole). The 20 parent PAHs measured included fluoranthene (Fla), pyrene (Pyr), benz[*a*]anthracene (BaA), cyclopenta[*c*,*d*]pyrene (CCP), triphenylene (TPh), chrysene (Chry), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*j*]fluoranthene (BjF), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), perylene (Per), indeno[1,2,3-*cd*]pyrene (IcdP), dibenz[*a*,*h*]anthracene (DahA), benzo[*g*,*h*,*i*]perylene (BghiP), dibenzo[*a*,*l*]pyrene (DalP), dibenzo[*a*,*e*]pyrene (DaeP), dibenzo[*a*,*i*]pyrene (DaiP), dibenzo[*a*,*h*]pyrene (DahP) and benzo[*c*]fluorene (BcFlu). The 7 alkyl-PAHs measured included C1-fluoranthene/pyrene (C1-Fla/Pyr), C2-Fla/Pyr, C3-Fla/Pyr, C1-benzo[*a*]anthracene/chrysene/triphenylene (C1-BaA/Chry/TPh), C2-BaA/Chry/TPh, C3-BaA/Chry/TPh and C4-BaA/Chry/TPh. Detailed information regarding the soil sample preparation and the instrument method can be found in Williams-Clayson et al. [57].

Membrane samples underwent identical sample preparation techniques as soils, using accelerated solvent extraction (ASE) and solid-phase extraction (SPE). However, the spiking concentrations, dilutions, and aliquots were adjusted to ensure appropriate detection levels of PAHs (see SI Section S4. B). RS samples were prepared using a different SPE procedure than membranes/soils, utilising Strata-PAH SPE cartridges (Phenomenex, UK). The RS samples were concentrated into 250 μL GC-MS vial inserts to enable sufficient concentration of PAH for detection (see SI Section S4, Table S5 and Section S7. C for details).

2.4. Dermal flux

The membrane flux represents the absorption rate of PAHs from the soil to the membrane per unit area, while the RS flux equated to the penetration rate of PAHs diffusing through the membrane into the RS. The total flux was calculated by summing the RS and membrane fluxes. Dermal fluxes (J) expressed as ng/cm^2/h were calculated using Equation 1, where Q is the measured PAH mass (ng) in the dermal matrix, A is the surface area of the membrane (8.55 cm^2) and T is the timestep of the exposure (h).

$$J = \frac{Q}{A \times T}$$

Equation 1. Calculation of dermal fluxes in dermal experiments for membrane and RS.

2.5. Data analysis

The R statistical software [37] was used to perform statistical analysis. The Kruskal-Wallis test and Dunn's tests were employed to assess differences between PAH fluxes within the same sample and timestep. T-tests and Wilcoxon signed-rank tests were performed to compare BCR-524 fluxes in this study with those reported by Lort [27].

2.6. Quality control

Comprehensive details of the quality control (QC) for the *in vitro* dermal absorption experiments and the analytical measurements are presented in SI Sections S6–7. Methods assessing the QC of the dermal experiments involved mass balance, conducting weighed matrix balance checks, and measuring PAH levels in dermal matrices from blank dermal experiments (without soil application). T-tests and Wilcoxon signed-rank tests were performed to compare BCR-524 fluxes in this study with those reported by Lort [27], and MGP sample fluxes were compared to other dermal studies. The performance of sample extraction and analysis was assessed through spiked solvent measurements, surrogate extraction efficiencies and by the measurement of two CRMs (BCR-524 and NIST-1944) whose measured PAH concentrations were compared to certified concentrations. Calculated detection limits (limit of quantification (LOQ) and limit of detection (LOD)) were compared to chromatogram peaks to ensure that membrane and RS peaks corresponded to analytes and not background noise.

3. Results

3.1. PAH concentrations in soils

The initial concentrations of PAHs in the real-world field soils are presented in Fig. 2 and can be found in the SI Table S15. The mean soil concentration for BaP was in a range of 6.53 – 160.5 mg/kg. All soil samples exceeded the GAC for BaP (5 mg/kg), indicating potential risks including fromdermal chronic absorption by humans for residential land use with plant uptake [8].

3.2. Membrane flux

Sample H16 membranes were measured for all timesteps in triplicate and the BCR-524 sample 24-h membranes were measured in triplicate, and one membrane from the 1-h and 10-h experiments (Fig. 1). Fig. 3 illustrates the initial rapid intake of PAHs (higher fluxes) into the membranes at shorter timesteps. The membrane fluxes were higher than the RS fluxes, for example, BaP flux means were 5.02 ng/cm^2/h for membranes and 0.0018 ng/cm^2/h for RS for the H16 sample at 24-h. Fig. 4 shows all HMW PAHs membrane fluxes at each timestep for each soil.

The number of HMW PAHs compounds detected in the membranes increased with longer timesteps, H16 detected 9, 15,18 HMW PAHs respectively for the timesteps 1-h 10-h and 24-h. In comparison H16 RS detected 2, 8, 10 HMW PAHs species with increasing timesteps, indicating a delayed diffusion of PAHs from membrane into RS. The membrane flux for each PAH was consistently highest at the 1-h timestep (when flux >LOQ) (Fig. 3). For example, BaP mean membrane flux for H16 decreased from 12.1 ng/cm^2/h to 5.54 ng/cm^2/h and then 5.02 ng/cm^2/h respectively.

Fla and Pyr showed the highest membrane fluxes for H16. C1-Fla/Pyr measured the third highest membrane fluxes at the longer timesteps 10-h and 24-h but was not detected at 1-h. No other alkyl-PAHs from either the alkylated Fla/Pyr or alkylated BaA/Chry/TPh homologue series were detected in the H16 membranes at the other timesteps except for C2-Fla/Pyr, which showed breakthrough into the membrane at 24-h. The BCR-524 24-h membranes detected the presence of C1-Fla/Pyr only, however the BCR-524 RS samples detected C2-Fla/Pyr, C3-Fla/Pyr, C1-BaA/Chry/TPh and C2-BaA/Chry/TPh compounds at the 24-h timestep suggesting that potentially alkyl-PAHs had passed through the membrane by 24-h into the RS.

The subsequent highest membrane fluxes for H16 after Fla and Pyr at 1-h were the 5-ring BeP > 4-ring Chry > 6-ring BghiP (mean membrane fluxes of 28.0 ng/cm^2/h > 21.3 ng/cm^2/h > 17.2 ng/cm^2/h). This suggests that ring size might not be a dominant factor for desorption rate of HMW PAHs from soils into membranes at shorter timesteps. Longer timesteps measured 4-ring PAHs with higher membrane fluxes compared to the 5-ring and 6-ring PAHs. The HMW parent PAHs that were not detected in any of the H16 membranes within 24-h included the 6-ring PAHs CCP, DaeP, DaiP, and DahP. The Kruskal-Wallis test was conducted on membrane fluxes, and it revealed statistically significant results ($p < 0.05$) for the H16 membranes at each time step. However, the Dunn's test did not identify any statistically significant differences in membrane fluxes among HMW PAHs.

3.3. Receptor solution flux

All five MGP samples RS at each timestep fluxes were quantified in triplicate, whereas the RS samples from the 24-h timestep for BCR-524

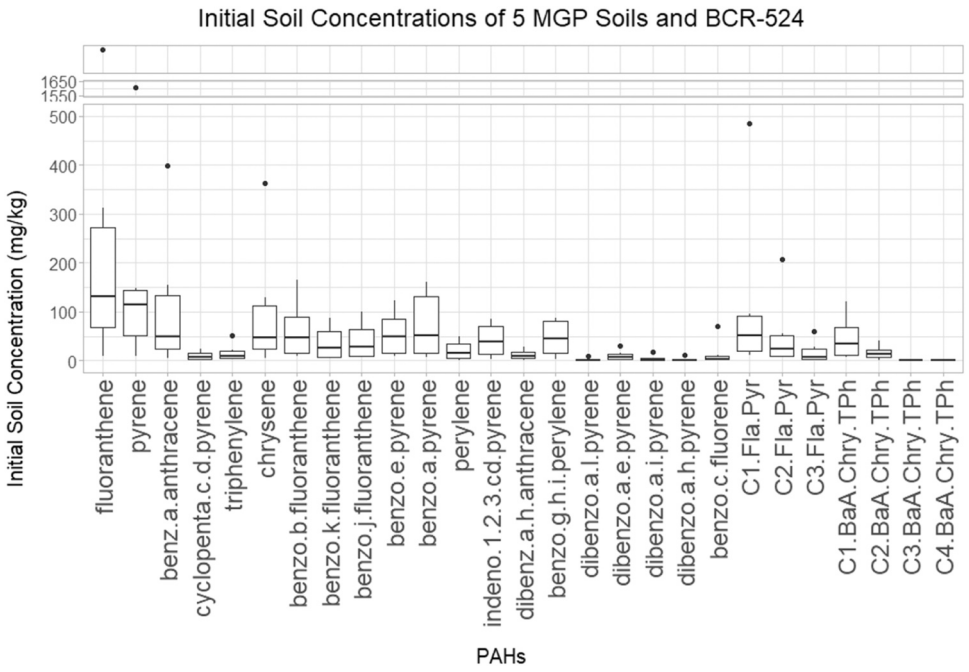

Fig. 2. Boxplot of the initial soil concentrations of the 27 HMW PAHs in the 5 MGP soils and BCR-524. Boxplot created using the mean soil concentration of three soil samples taken from the same soil. Lower and upper hinges correspond to 25th and 75th percentiles, median expressed as middle line. Outlier points reported when concentrations are 1.5 * interquartile range (IQR), y-axis breaks and change in scale used to show the extreme PAH concentrations for E1.5.

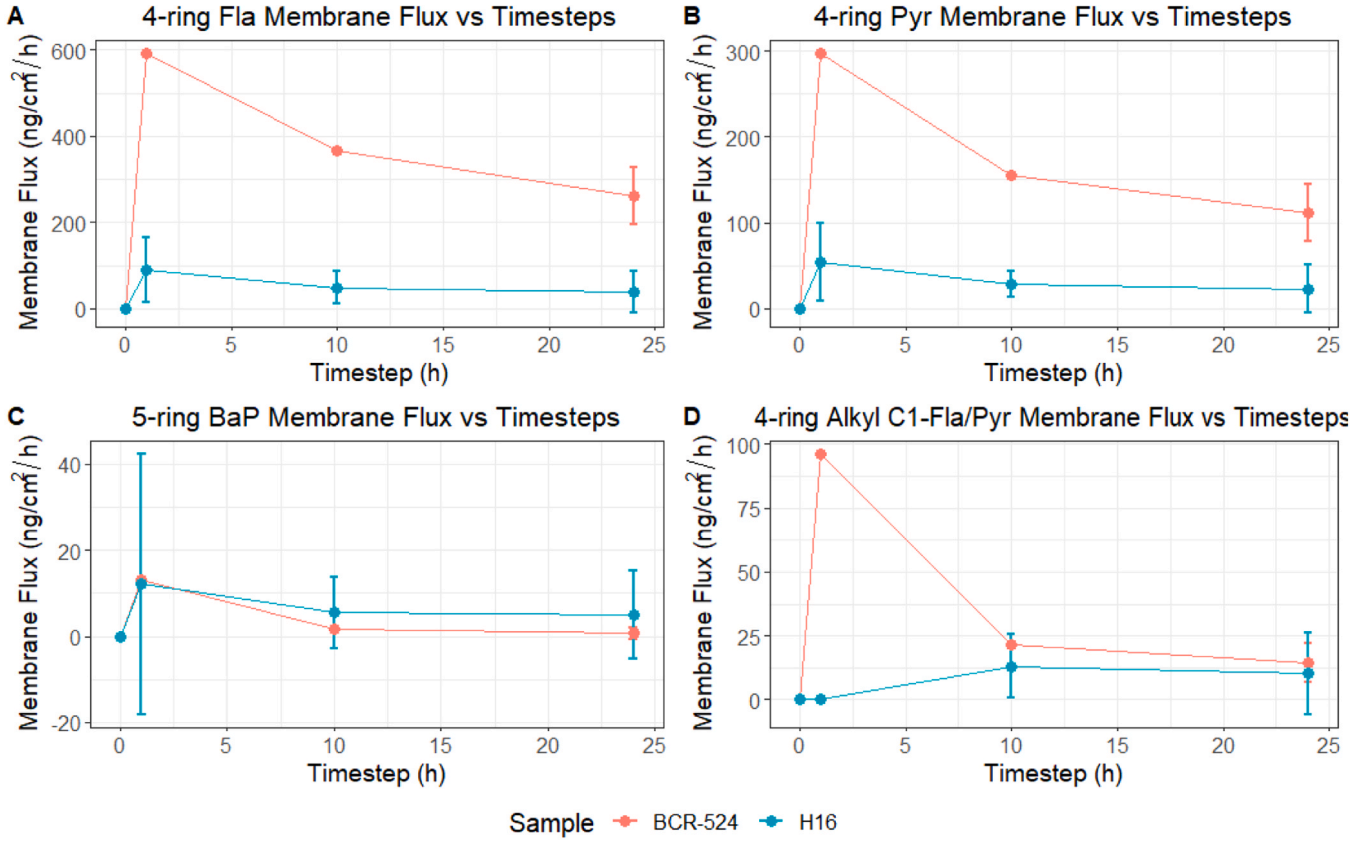

Fig. 3. Mean membrane fluxes for the four HMW PAHs (a) Fla, (b) Pyr, (c) BaP and (d) C1-Fla/Pyr over the timesteps 1-h, 10-h and 24-h. Data points represent mean membrane fluxes and error bars are the 95% CI. The H16 sample uses triplicate experiment results for each timestep, whereas the BCR-524 uses triplicate membranes for 24-h and one membrane measured at 1-h and 10-h. Note different y-axis scales in all plots and lines representing different samples.

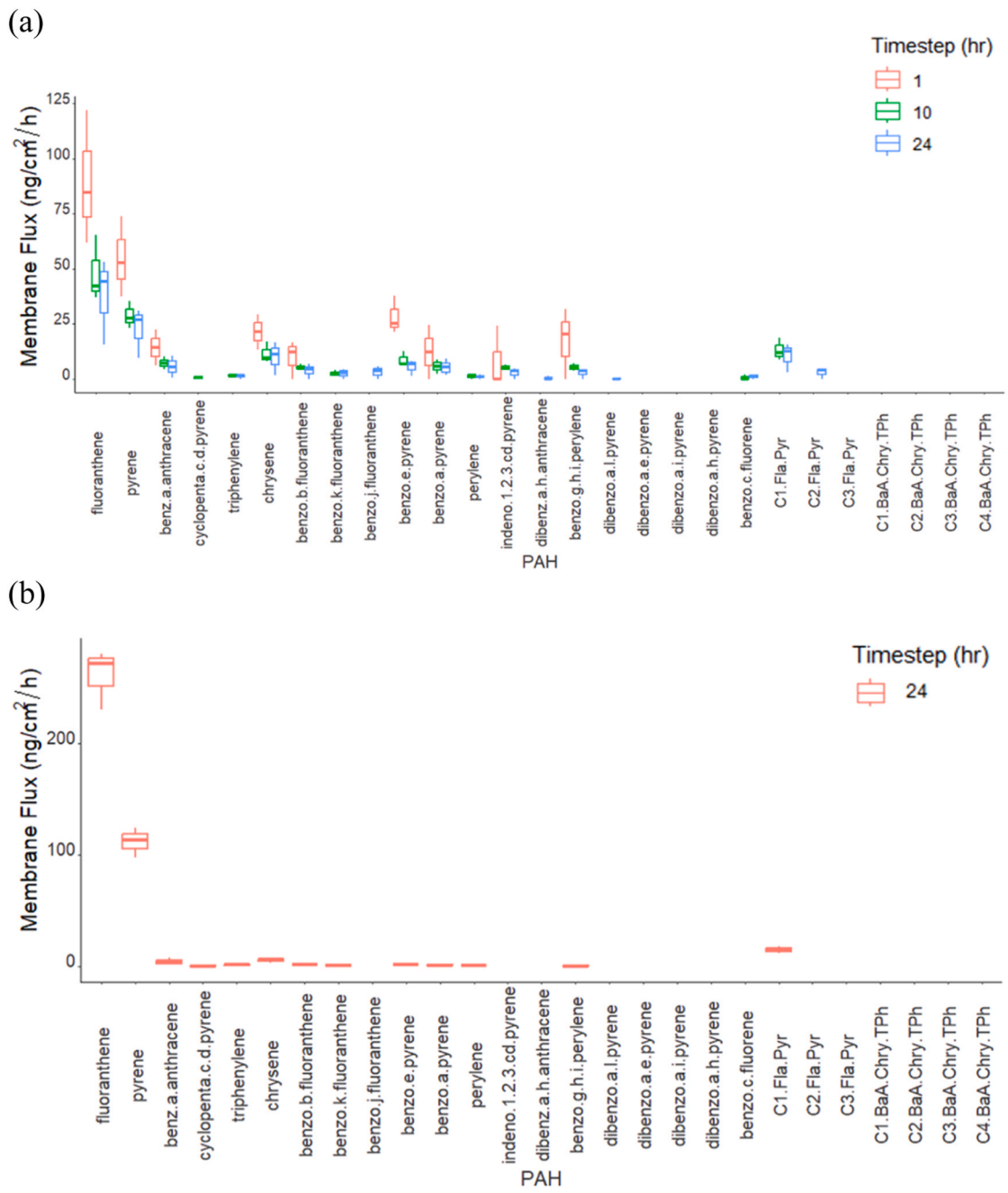

Fig. 4. Boxplots of the membrane fluxes for (a) H16 triplicate experiments at each timestep and (b) BCR-524 triplicates experiments for 24-h). Absence of boxplot indicates PAH concentrations are <LOQ in membrane.

were stated as detected or non-detected (not quantified) (Fig. 1). BCR-524 RS samples were not quantified due to issues with surrogate recoveries due to prolonged RS storage (>3 months) during method development and discussed in the SI Section S7. The fluxes from soil to RS (RS flux) varied between HMW PAH compounds, samples and timesteps. The dermal flux measurements are provided in the SI Table S15. Higher RS fluxes were generally measured for PAHs with lower ring sizes, longer timesteps and samples with higher initial soil PAH concentrations.

Fig. 5 shows that the mean RS fluxes of the 4-ring PAHs (Fla and Pyr) increased rapidly with longer timesteps, while 5-ring BaP showed increased RS flux with increasing timesteps but at lower flux rates. Samples with the highest initial soil concentrations (E1.5, H16 and I3 Fla & Pyr ranged between 100 – 2616 mg/kg) showed higher RS fluxes at longer timesteps. This is in contrast to samples with lower initial soil concentrations (A11 and E2.7 Fla & Pyr ranged between 8.1 – 54 mg/kg) which measured higher RS fluxes at shorter timesteps. Samples A11 and E2.7 showed higher variability for the RS flux for each PAH compared to the higher concentrated samples.

Boxplots presented in Fig. 6a-e illustrate the differences in RS fluxes between the 27 HMW parent and alkylated PAHs in each MGP sample. Fewer HMW PAHs were detected in RS at 1-h compared to longer timesteps, indicating lower soil to RS flux for the heavier PAHs with larger ring sizes. The differences in RS flux values between short and long timesteps and HMW PAH indicated changes in PAH availability for dermal absorption over time.

Comparing the fluxes between different HMW PAHs within each sample and time step revealed that Fla and Pyr consistently yielded the highest RS fluxes across all samples and timesteps, followed by C1-Fla/Pyr homologue series at longer time steps. Fla and Pyr were only

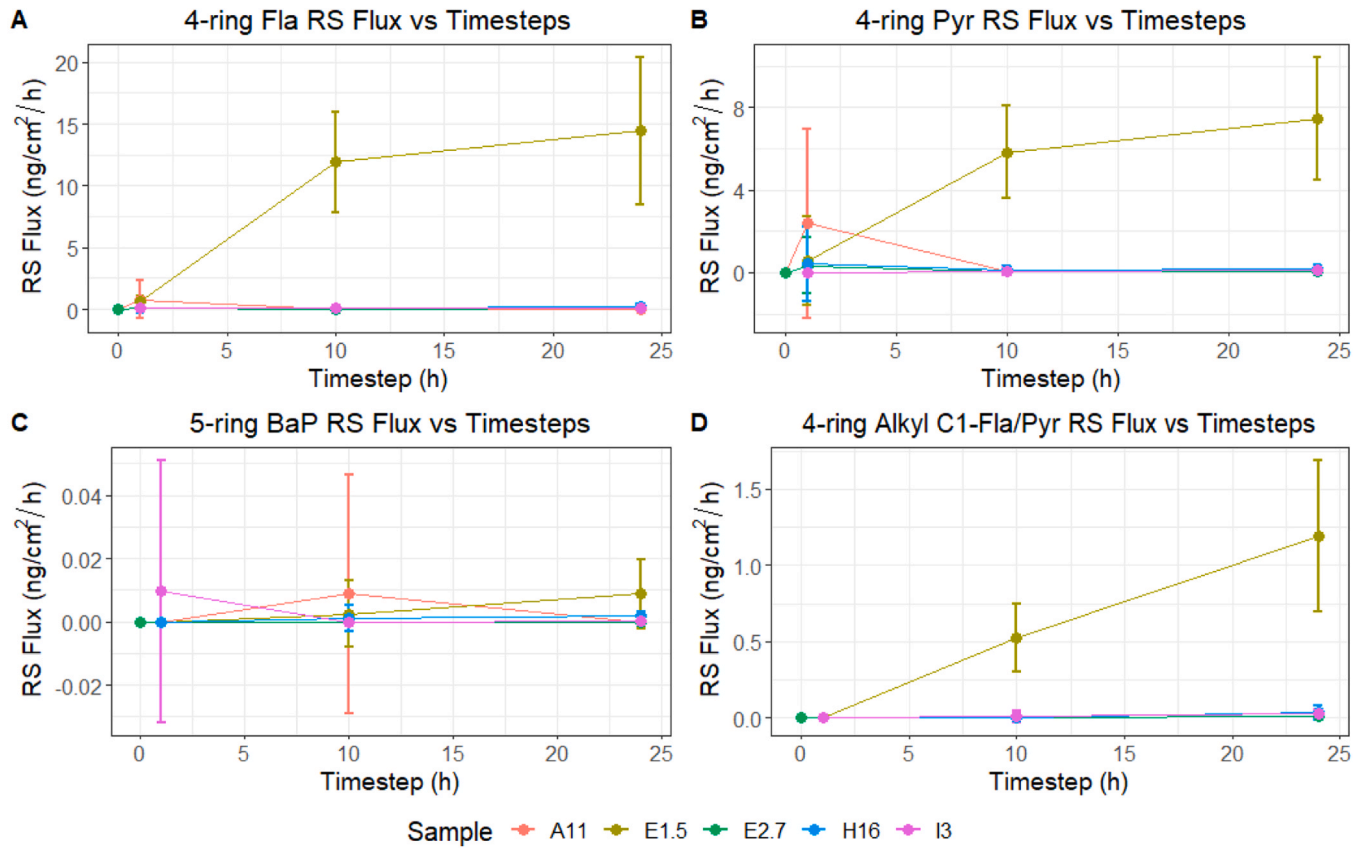

Fig. 5. Mean RS fluxes for the four HMW PAHs (a) Fla, (b) Pyr, (c) BaP and (d) C1-Fla/Pyr over the timesteps 1-h, 10-h and 24-h. Data points represent mean fluxes of the triplicate experiments performed for each timestep for each MGP sample, and error bars are the 95% CI. Note different y-axis scales in all plots and lines representing different samples.

statistically significant (p-value <0.05) from the other HMW PAHs fluxes for the samples I3 and E2.7 at 10-h when tested by the Dunn's test. Although the Kruskal-Wallis test indicated statistically significant results (p-value <0.05) for A11, I3 and E1.5 at the 1-h timestep, I3, E2.7, E1.5, and H16 at 10-h, and all samples at the 24-h.

C1-Fla/Pyr was detected in all RS samples at 24-h, with RS fluxes ranging between 0.007 to 1.19 ng/cm^2/h (mean: 0.25 ng/cm^2/h in comparison to BaP mean 0.002 ng/cm^2/h). However, the C1-Fla/Pyr was not detected in the RS for any sample at 1-h and only in two samples at 10-h, indicating a slower absorption than their parent derivatives. None of the other alkylated PAHs from either Fla/Pyr and BaA/Chry/TPh homologue series were detected in RS for the MGP samples at any timestep, except for C2-Fla/Pyr detected in E1.5 RS at 24-h (mean: 0.19 ng/cm^2/h). Although the control reference soil study with BCR-524 measured breakthrough of alkyl-PAHs with increased alkylated chain lengths (C2-Fla/Pyr, C3-Fla/Pyr, C1-BaA/Chry/TPh and C2-BaA/Chry/TPh) at longer timesteps. BCR-524 samples were collected from former wood treatment activities involving creosote oil.

In addition to Fla and Pyr, other HMW PAHs with elevated fluxes included BaA, Chry, BcFlu, and BbF. These PAHs measured mean RS fluxes of 0.035, 0.029, 0.043 and 0.003 ng/cm^2/h, respectively for the MGP samples. HMW PAHs not detected in the RS for either of the three-timesteps included one 5-ring PAH BjF, and the 6-ring PAHs DahA, DalP, DaiP, DahP, and DaeP.

3.4. Combined receptor solution and membrane flux

Combined membrane and RS fluxes (total flux) were calculated for samples H16 and BCR-524 (SI Fig. S7a-b) by adding the two flux values together. H16 total fluxes ranged from LOQ to 122.5 ng/cm^2/h (mean: 6.38 ng/cm^2/h) for all timesteps and HMW PAHs. Membrane flux consistently provided the highest contribution to total flux, therefore total flux trends within samples were similar to membrane flux. For example, the H16 sample membrane flux contributions to the total flux for each timestep was 100.00%, 99.98% and 99.91% for 1-h, 10-h and 24-h for BaP, in comparison Fla 99.92%, 99.79% and 99.18% for 1-h, 10-h and 24-h.

3.5. PAH physiochemical properties and initial soil concentration influences

The relationship between PAH physicochemical properties and RS fluxes was examined. Results for sample H16 are shown in Fig. 7, results for other samples are presented in SI Fig. S8. The plots show that RS flux rapidly decreased with increased Log K_{OW} and MW. Fig. 8 showed that several PAHs with high initial soil concentrations had higher dermal fluxes, however there were many PAHs with no correlation between initial soil concentrations and the dermal flux.

3.6. Quality control

The full details of the performance of the *in vitro* method, sample extraction and analysis are presented in SI Section S6 and S7. The QC regime results are presented in Table 1. In summary the results from the QC procedures provided confidence that the *in vitro* method was executed correctly and that the sample preparation and analysis were appropriate to quantify the range of HMW PAHs.

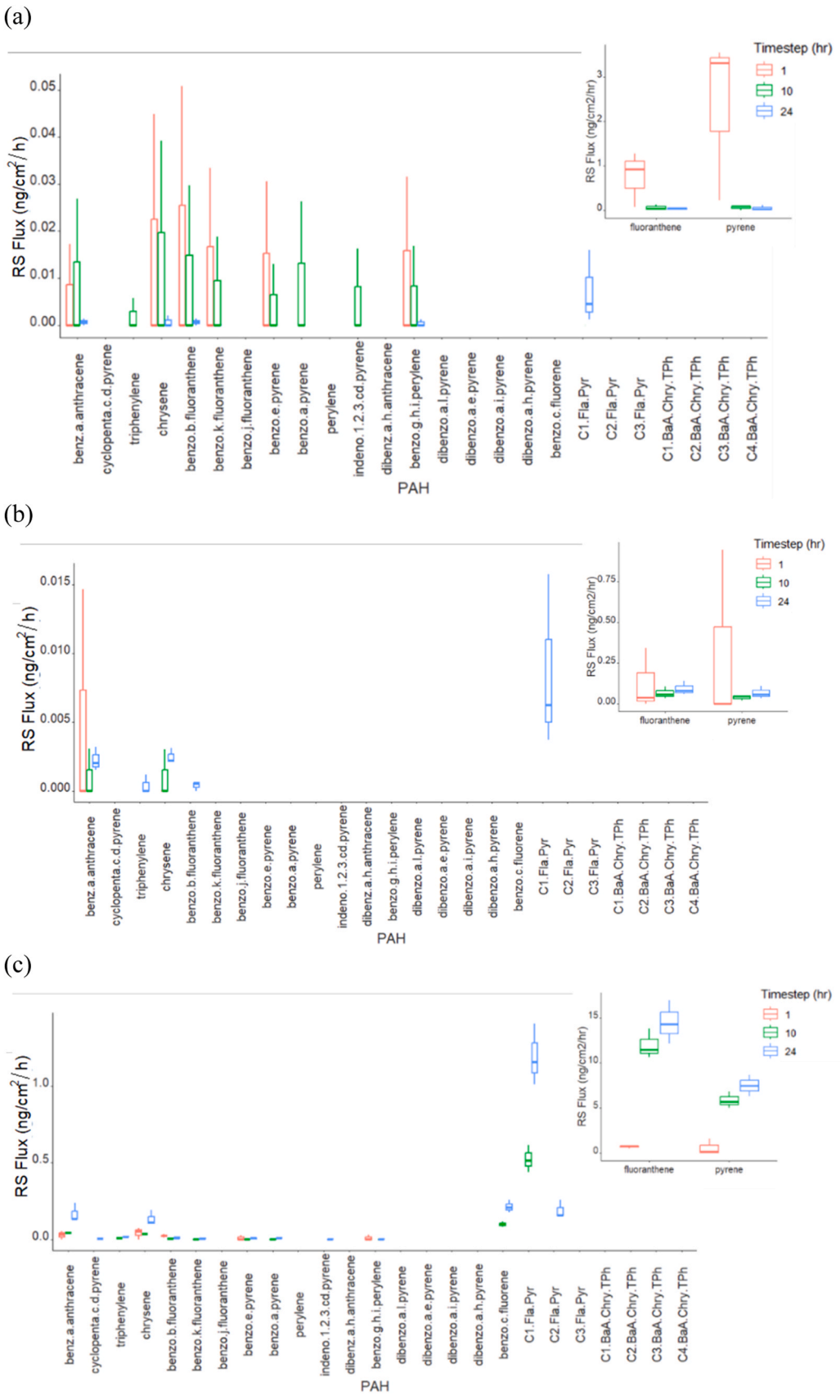

Fig. 6. RS flux for individual PAHs at each timestep, data based on three experiments per timestep, (a) A11, (b) E2.7 (c) I3, (d) H16 and (e) E1.5 reported as boxplots. Samples ordered by ascending soil PAH concentration, Fla and Pyr removed from larger boxplot to allow the scale of fluxes for other PAHs to be represented. Absence of boxplot indicates PAHs are <LOQ in RS.

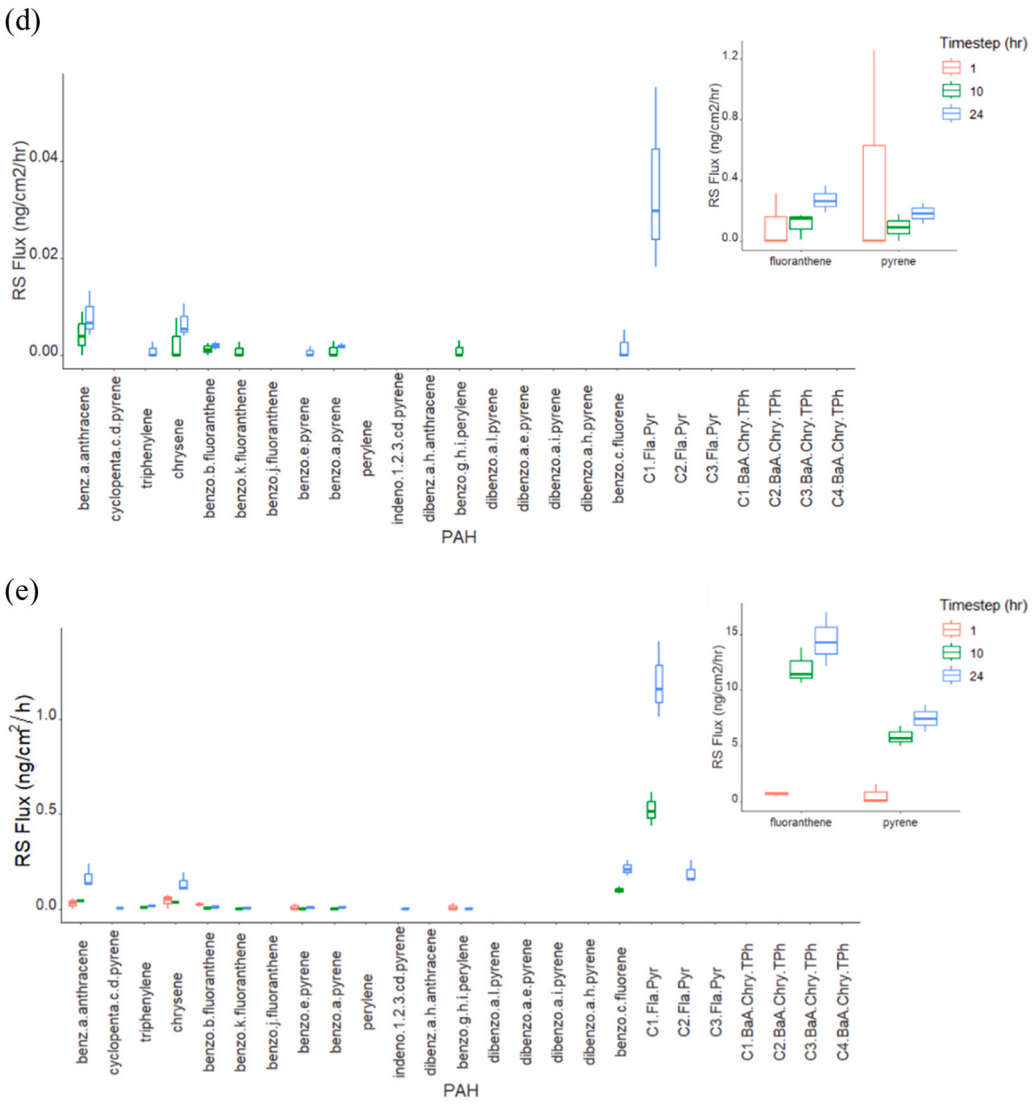

Fig. 6. (*continued*).

4. Discussion

4.1. HMW parent PAH dermal flux

This study shows that the majority of HMW PAHs enter the membrane within the 24-h timestep (19/27 PAHs were detected in the H16 membranes), but delays in diffusion through the membrane into the RS occurred for PAHs with higher ring sizes. The dermal flux values demonstrate significant variation between HMW PAHs across different soil samples and exposure times, with membrane fluxes consistently higher than RS fluxes. For example, Fla membrane fluxes were two orders of magnitude higher than the RS fluxes. Whereas higher ring size PAHs were not detected in the RS at shorter timesteps or had higher orders of magnitude differences between matrices (*e.g.* membrane flux for BaP at 24-h was three orders of magnitude higher than the RS flux). The membrane flux was higher than the RS flux, most likely due to HMW PAHs favouring partitioning into lipid medias such as skin/membranes [49].

The total flux was predominately contributed to by the membrane flux, this shows that the membrane is likely to be acting primarily as a sink but also a slow-release source for dermally absorbed HMW PAHs in soils. This suggests that to obtain a comprehensive assessment of the dermal risk from HMW PAHs in soils, the membrane and RS should be assessed to address for the potential future PAH release from the membrane when acting as a source for the RS. PAHs with the highest membrane fluxes at 1-h after Fla and Pyr did not follow the trend of increasing flux with increasing ring size. This suggests that ring size might not be a dominant factor for desorption rate of HMW PAHs from soils into membranes at shorter timesteps. However, the 5-ring and 6-ring PAHs penetration rates decrease with increased timesteps compared to the 4-ring PAHs.

4-ring Fla and Pyr consistently showed the highest fluxes in both the membrane and RS, regardless of the sample or exposure time or concentration. This result is similar to previous dermal studies which reported higher desorption of lower molecular weight (MW) PAHs in the EPA16 PAHs from soils [20]. Alalaiwe et al. [3] found Pyr to have the greatest flux across pig skin in an *in vitro* skin permeation experiment, further supporting its high dermal absorption potential. However, the relative fluxes of Fla and Pyr differed between our study and Alalaiwe et al. [3] who used an aqueous vehicle instead of soil. This difference is most likely due to the soil matrix effect, which decreases fluxes compared to aqueous media [54].

The higher fluxes for Fla and Pyr are concomitant with their physiochemical properties, including their 4-ring structure (smallest among HMW PAHs), low MW, lower boiling points, higher vapor pressures, higher water solubilities, higher Henry's Law constants, and lower Log

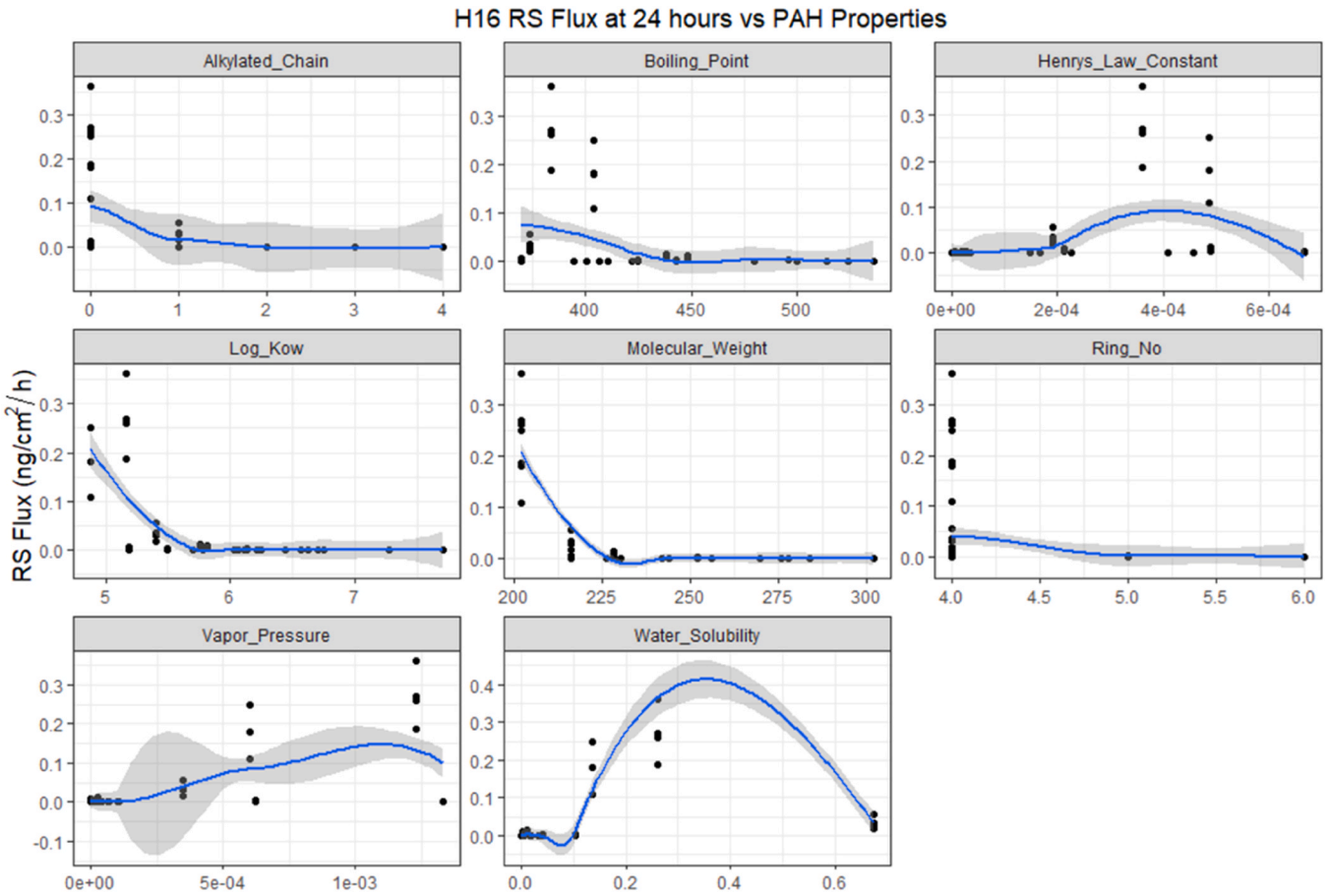

Fig. 7. Multiple plots of the sample H16 RS flux relationship with PAH physiochemical properties. The y-axis plots the H16 RS fluxes at 24-h for triplicate experiments and the x-axis plots the PAH physicochemical properties: alkylated chain length, boiling point (°C), Henry's Law Constant, Log Kow, Molecular weight (Da), number of rings in structure, vapour pressure (Pa) and water solubility (mg/L). Physicochemical property values taken from Achten and Andersson [2] whereby the mean physiochemical property values of individual alkyl-PAHs were used to represent the alkylated homologue series. Line of best fit calculated with locally estimated scatterplot smoothing (LOESS).

K_{ow} values. In summary, smaller, lighter more volatile HMW PAHs appear to promote rapid dermal absorption from soil into both the membrane and RS. Other 4-ring PAHs (BaA, Chry and BcFlu) also showed faster dermal absorption compared to higher ring number PAHs.

5-ring PAHs BbF, BaP, and BeP showed lower RS fluxes compared to the 4-ring PAHs, and their membrane fluxes decreased with increased exposure time. This suggests that the larger ring structure hinders their initial diffusion through the membrane into the RS but potentially results in increased flux to RS over time. BeP exhibited the highest membrane fluxes compared to BaP and BbF, which may be attributed to its lower vapor pressure, water solubility, and Henry's Law constant. On the other hand, HMW PAHs with opposing physiochemical properties to the 4-ring PAHs, such as CCP, DahA, DalP, DaiP, DahP, and DaeP, exhibited the lowest fluxes or were <LOQ in either the membrane or RS. These findings suggest a strong influence of PAH physio-chemical properties on dermal absorption.

4.2. HMW alkylated PAH dermal fluxes

The MGP samples showed similar alkyl-PAH behaviour where increases in the alkyl chain size decreases dermal absorption. A cut-off point was observed between one and two alkyl groups for Fla/Pyr compounds, where alkyl-PAHs with > 1 alkyl chain were not detected in RS or membranes. Notably, C1-Fla/Pyr exhibited some of the highest RS and membrane fluxes among all PAHs, surpassing most parent PAHs except for Fla and Pyr. Addition of an alkyl chain on BaA/Chry/TPh seemed to inhibit dermal absorption. This is shown by measuring flux for the 5-ring parent compounds (BaA, Chry, and TPh) while their corresponding alkylated derivatives were not detected in H16 membranes or any of the RS samples. This suggests that increasing the alkylated chain for alkyl-PAHs decreases the PAH ability to desorb from the soil into the membrane and will only reach the membrane at longer timesteps.

The control reference soil study utilising the CRM BCR-524 has previously been employed to investigate BaP desorption [35] and enabled comparison with Lort [27]. BCR-524 flux measurements show additional alkyl-PAHs compounds other than C1-Fla/Pyr were detected in the RS compared to the MGP samples. BCR-524 contains lower PAH concentrations than MGP soils despite the observation that concentration seems to relate to higher flux values in real-world soils. Possible reasons for this include a different contamination source than MGP soils and/or factors such as soil properties and the presence of other PAHs influencing the dermal absorption of alkyl-PAHs from soils [28,56]. Creosote is known to have a large number of alkylated compounds [18] which can effect solubility through co-solvency, which would support the increased presence of alkyl-PAHs within the dermal experiments with BCR-524. The differences in detecting other alkyl-PAHs in BCR-524 compared to the MGP soils suggests different contamination sources or matrix effects have different dermal absorption behaviours for alkyl-PAHs. These results suggest that soils contaminated by sources with numerous alkyl-PAH species might increase the dermal absorption of alkyl-PAHs.

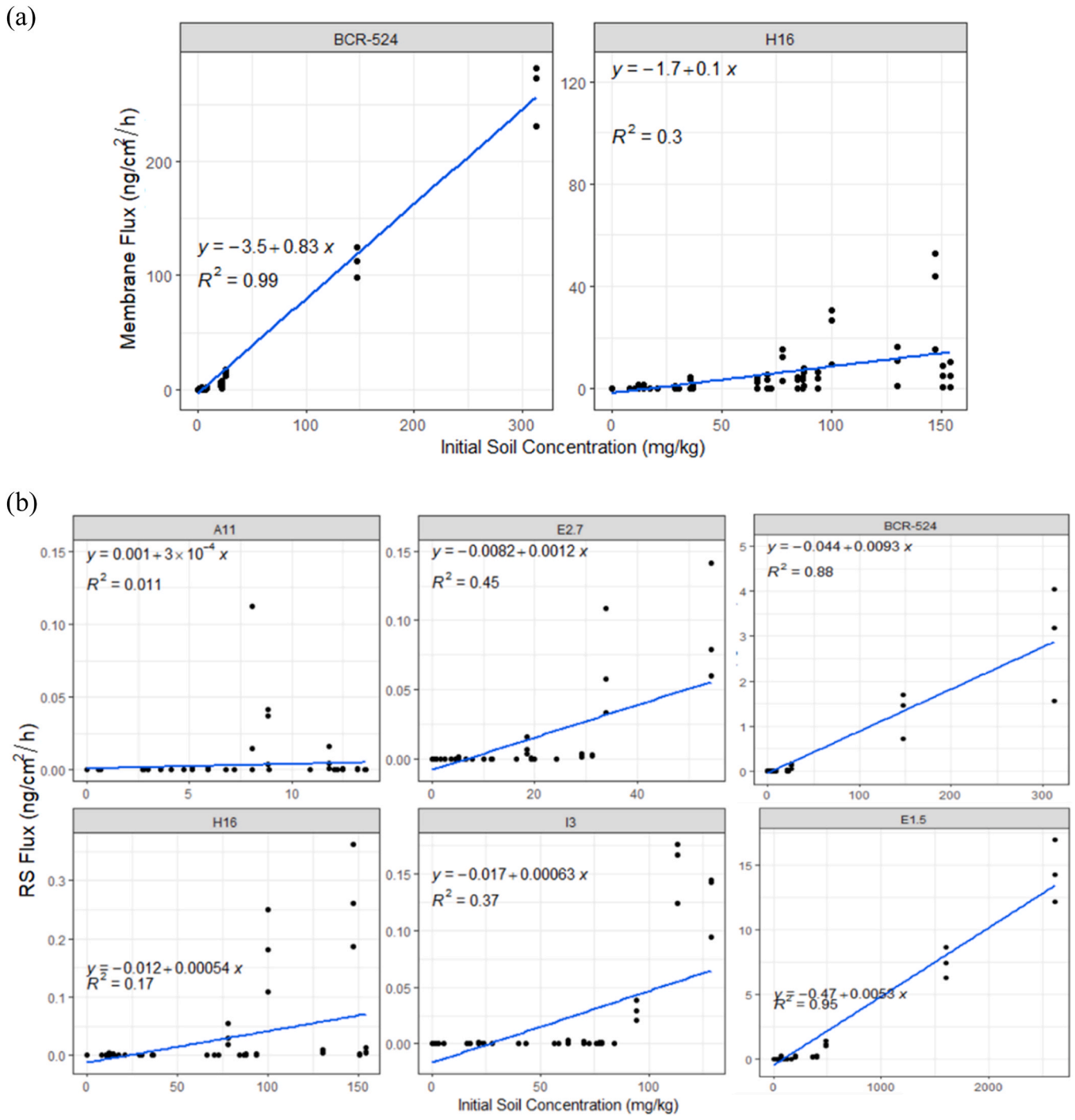

Fig. 8. The initial soil PAH concentration (mg/kg) of the 27 HMW PAHs plotted against (a) 24-h membrane fluxes for H16 and BCR-524 and (b) 24-h RS fluxes for all samples, line produced from linear regression.

4.3. PAH physiochemical properties and initial soil concentration influences

RS flux decreased with increasing Log K_{OW} and PAH MW, this direction of relationship between MW and flux is in agreement with other research [21,52]. PAHs with lower vapor pressures generally showed lower fluxes, however several PAHs with similar Log K_{OW} and PAH MW values exhibited varying RS fluxes, prompting an investigation into the initial soil concentration as a confounding variable affecting flux. Initial PAH concentrations in soils presented in Fig. 2 appear to play a role in PAH diffusion by creating a concentration gradient at the membrane surface for only a few PAHs (predominately Fla and Pyr), shown in Fig. 8. Considering Fick's Second Law of diffusion in Equation 2.

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

Equation 2. Fick's Second Law of diffusion.

Whereby δC is the concentration gradient, J is the flux, δx is the travelled distance and D is the diffusion coefficient [9]. The results in Fig. 8 suggest that higher soil concentrations provide a larger driving force for PAH diffusion, leading to increased fluxes for several PAHs [4, 60]. However, the majority of HMW PAHs did not show a trend, this suggests that other factors such as PAH physicochemical properties, PAH

Table 1
QC regime and results.

QC Procedure	QC Result
Spiked solvent measurements	%Bias[a] < 30% and relative standard deviation (%RSD)[b] < 15% as per Environment Agency [13].
Surrogate extraction efficiencies	Satisfactory mean recoveries: 81% for soils, 75% for membranes and 76% for RS. BCR-524 RS fluxes were not quantified due to unacceptable surrogate recoveries. These were caused by prolonged storage of RS samples during method development which impacted the RS extraction from SPE, instead of concentrations the detection of the presence of PAHs is reported for BCR-524 RS samples (see Section S7. B and Table S11 for details).
Calculated detection limits subjected to chromatogram verification	Verifications all aligned with their respective chromatograms for four PAHs, ensuring sample peaks were analytes and not background noise.
Measurement of two CRMs (NIST-1944 and BCR-524)	The majority of HMW PAHs exhibited %Bias < 30% and a %RSD < 15% (including C1-Fla/Pyr), hence further supporting the method's accuracy and precision in measuring a diverse range of HMW PAHs at varying concentrations.
Dermal experiments mass balance	Mean values of 98.3% and 92.1% for H16 and BCR-524.
Weighed matrix balance checks	Low membrane weight differences (mean of 1.72%) and collected dried soils close to targeted weight.
PAH concentration measurements from blank dermal experiments	RS and membranes indicated no additional HMW PAH contamination to samples, *e.g.* Fla in blank RS measured 0.085 μg/g compared the limit of quantification (LOQ) 0.083 μg/g.
Dermal study comparisons	The measured fluxes in the control reference soil study with BCR-524 did not significantly differ from the fluxes reported by Lort [27]. Furthermore, the fluxes obtained in this study fell within the range of fluxes reported in other studies, further reinforcing the standardisation of this study.

[a] Bias determines the accuracy of measurement evaluating the difference between the true CRM value and our measured value.
[b] RSD provides an indication of precision of method by interpreting the distribution of repeated measurements.

sources and soil properties are likely influences. 1-h RS fluxes were the highest among the other exposure times for the soil A11 with the lowest PAH concentrations. The causations of the different RS fluxes at different timesteps between soils with high and low initial PAH concentrations is difficult to interpret without further experimentation. One possible explanation is that soils with a lower concentration have lower absolute PAH to soil mass ratio. Previous studies have reported strong influences of PAH concentrations increasing the dermal absorption or flux [16,48]. In addition, supersaturated soils containing free PAHs are unable to help research determine the impact of soils properties on PAH release from soils, as these freely available PAHs as are not impacted by soil properties [40]. However, Xia et al. [58] reported that PAH dermal fluxes could not be explained by the PAH concentration differences in soils, but they did determine a strong positive correlation to freely dissolved PAH concentrations in soils. Our study shows that the relationship between RS fluxes and initial soil concentration varied among the MGP samples. This suggests that, like Xia et al. [58], other factors, such as PAH properties, PAH mixtures and soil properties, influence dermal absorption more strongly than concentration.

4.4. Comparison to Other Studies

We compared our BaP flux data with a range of other dermal studies including: Moody et al. [30], Peckham et al. [34], Roy and Singh [42], Lort [27], and Wester et al. [54]. Overall, our BaP fluxes fell within the lower reported ranges. Membrane fluxes showed no statistically significant differences (p-values >0.05) using the Wilcoxon signed rank test between our study and Lort [27] using a similar dermal *in vitro* method in the same laboratory. Lort [27] investigated three parent HMW PAHs (BaP, Pyr and DahA) with eight timesteps in two MGP soils and BCR-524. Lort [27] concluded that his method provided results in agreement with other published work, establishing its appropriate use for comparisons for PAH absorption in *in vitro* human dermal experiments and hence the method was improved and applied in this research. Differences in soil properties, soil moisture content (SMC%) [20,25], and analytical methodologies are likely to account for the variations in fluxes observed between studies. Our study demonstrated lower dermal fluxes for real-world MGP soils compared to studies using spiked soils [54], indicating the impact of soil properties including natural long-term weathering and sources of PAHs, such as bulk organic matter properties on fluxes.

Risk assessment guidance on the dermal exposure of PAHs from soils [12] is currently based on Wester et al. [54], which showed that 13% of the BaP dose applied is bioavailable using an *in vivo monkey* model. Spalt et al. [45] calculated the average uptake flux using Wester et al., data to be 2.2 ng/cm^2/h for 24-h. However, Wester et al. [54] collected urine for a total of 7 days, producing a lower flux (0.31 ng/cm^2/h) closer to the RS fluxes for BaP measured in our study (which mimic PAH uptake into the systemic circulation within 24-h). The higher membrane fluxes in our study suggest the membrane may be acting as a sink and then a source. Other studies have shown that BaP in the membrane have the potential to diffuse slowly into RS over time [4,48]. PAHs remaining in the skin after soil removal pose potential risks to human health, due to potentially absorption into the systemic circulation later or causing localised health problems to skin (skin tumours). Previous studies [24, 44,6] have identified PAHs as skin carcinogens, hence the presence of PAHs remaining in the membrane is a potential health risk. This is supported by Forsberg et al. [16], who showed continued absorption and diffusion of BaP from skin after soil removal suggesting the possible risks from PAHs remaining in skin.

Forsberg et al. [16] also showed influence of weathering, biodegradation, and aging on PAH desorption from soils. Roy et al. [41] determined that soil hinders the dermal flux rates by a factor of 160–900 compared to dermal experiments with PAHs from soil extracted solutions. This supports our findings of low fluxes with field-contaminated soil. Roy et al. [41] measured higher BaP fluxes in their *in vitro* study than our study. This is assumed to be because Roy et al. [41] calculated fluxes factoring the recovery of the spiked ^{3}H-BaP in each matrix rather than individual PAH released from real-world contaminated soils. The use of spiked PAHs in studies can overestimate dermal fluxes as compared to real-world soils, as PAHs undergo various transformations in natural environments [4,48,51]. Studies investigating the role of PAH sources [58] in dermal fluxes and soil properties in bioavailability studies [7] have indicated their significant impact on PAH release from soils. Soot, char, and other black carbon sources present in MGP soils can sequester PAHs and decrease their release, thereby affecting dermal absorption [43,51]. Our study shows lower 24-h RS fluxes than other dermal studies measured, with differences between samples, potentially highlighting the influence of soil properties and PAH sources on fluxes.

4.5. Future work and implications

There are several limitations in this study that could be addressed in future research.

The study measured dermal absorption of PAHs from a limited number of MGP samples (n = 5). It is recommended that future work uses a larger sample set including end member materials such as coal tar, Williams-Clayson et al. [57] highlighted the diversity of PAHs measured between different MGP sites with varying contamination histories and extend the different sources.

This study was able to fully analyse one MGP sample membrane fluxes at three timesteps, with results suggesting that the membrane accounts for the majority of PAH dermally absorbed from soil. A larger dataset on dermal flux from soil to membrane could be created as part of a wide range of *in vitro* experiments.

Three timesteps were investigated in this study to demonstrate the proof-of-concept of the enhanced *in vitro* dermal absorption experiment. Further research might increase the number of timesteps incorporated for both early stages and extension of the longest timestep.

BCR-524 was considered a useful proxy reference material in the absence of a formal dermal CRM. The use of BCR-524 showed that a larger number alkyl-PAHs were present in the RS than the MGP samples. This suggests that the contamination source may impact the release of PAHs, and therefore future work investigating soils contaminated by other anthropogenic sources would help identify the impact of contamination sources on dermal absorption.

For an *in vitro* method to be both regulatory and scientific accepted, the *in vitro* method should be correlated to a *in vivo* method [61,62]. Future work should validate the method against a *in vivo* study so that the findings using this method can be used in guidance.

Extending the research to account for the uncertainties in this study would help build a more comprehensive understanding of the PAH diffusion rates from soil to membrane and then RS for a range of parent and alkylated PAHs. The physicochemical properties of PAHs and the initial soil concentrations appear to influence the RS fluxes to some extent, but other factors, including PAH mixtures, source materials, and soil properties, also impact dermal absorption. Validating the *in vitro* dermal method with a *in vivo* method would allow the results in this study to be used to help shape HHRAs and impacting the future of contaminated land.

The findings presented here highlighted a variety of novel findings, including the high fluxes associated to C1-Fla/Pyr at longer timesteps. C1-Fla/Pyr is potentially a unidentified health risk, since alkyl-PAHs are expected to be as toxic or of greater toxicity than their respective parent compounds [14,29,39]. The specific individual alkylated compounds present in the calculated C1-Fla/Pyr flux remain unknown, as this study analysed the total concentrations of alkylated compounds in a homologue series. Additionally, more exotic parent PAHs dermal bioavailability were explored, including BcFlu, which has a TEF of 20. This indicates a carcinogenic potential about 20 times that of BaP [39] and therefore the higher fluxes experienced in E1.5 and H16 samples from BcFlu could be a significant risk, as BcFlu was dermally absorbed into both the membrane and RS.

5. Conclusion

This study utilised an *in vitro* method to examine the dermal fluxes of twenty HMW parent PAHs and, for the first time, seven HMW alkylated PAHs extracted from five real-world MGP soils. The results revealed that the PAHs with lower molecular weights exhibited higher dermal flux, particularly in the case of Fla and Pyr, including the alkylated C1-Fla/Pyr. Alkylated PAHs are suspected to be equally or more toxic than their parent compound counterparts which poses a potential risk to human health due to their high dermal fluxes compared to heavier PAHs. Membrane fluxes were found to be higher than the RS fluxes, indicating that the membrane acted as a sink and then a source for PAHs released from soils, driving delayed diffusion of HMW PAHs from the membrane into the RS. Consequently, HMW PAHs that diffused more slowly through the membrane had the potential to reach the RS (a proxy for human systemic circulation) at later timesteps, potentially greater than those measured in this study. A comprehensive assessment of dermal risk from PAHs in soils should consider the PAH content in both the membrane and the RS to assess the prolonged release from skin into systemic circulation.

Our study highlights the variations in PAH absorption between PAH mixtures in real-world contaminated soils, with the initial soil concentration and the physicochemical properties of PAHs potentially influencing HMW PAHs dermal fluxes. This research demonstrated that real-world contaminated soils exhibited lower fluxes than studies using spiked soils. These findings have important implications for decision-making processes in the fields of environmental remediation, land management, consultancy, and regulation, as they contribute to key uncertainties in the human health risk assessment associated with chronic exposure to carcinogenic chemicals in soils found in contaminated brownfield sites. We recommend further study of the membrane as a sink and source of PAHs, investigating larger soil subsets with a wider range of properties and their influences on both RS and membrane PAH fluxes.

CRediT authorship contribution statement

Matthew Jones: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Russell Thomas:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Christopher Taylor:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Darren J Beriro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Alison Williams-Clayson:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Christopher Vane:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization.

Environmental Implications

This study investigated the dermal bioavailability of 27 PAHs using *in vitro* human dermal bioavailability experiments and measured the fluxes of the 27 PAHs to determine differences between PAHs. The findings of this study can help with decision making in risk assessments (RAs) conducted by remediation companies, landowners, consultants, and regulators by estimating the PAHs of greatest concern to human health based on the MGP processes. Implications of more accurate RAs include safely remediating brownfield sites into beneficial use, therefore reducing the requirement for development on greenfield land.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.133858.

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