Time-Dependent Remobilisation of Non-Extractable Benzo[a]pyrene Residues in Contrasting Soils: Effects of Aging, Spiked Concentration, and Soil Properties

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ABSTRACT

The environmental and health risks associated with ‘non-extractable’ residues (NERs) of polycyclic aromatic hydrocarbons in soils and their potential for remobilisation remain largely unexplored. In this novel study, sequential solvent extractions were employed to interrogate time-dependent remobilisation of benzo[a]pyrene (B[a]P) NERs and associated kinetics after re-equilibration (REQ) periods lasting 30 d in four artificially-spiked soils aged for up to 200 days. Following sequential extractions of the re-equilibrated soils, remobilisation of B[a]P NERs was observed and further confirmed by decreases in the absolute amounts of B[a]P recovered following methanolic saponification after REQ. Remobilisation may occur through slow intercompartmental partitioning of more sequestered into less sequestered B[a]P fractions in soils. The amounts of B[a]P remobilised in soils decreased throughout aging following first-order kinetics and the rates of decrease were slow but 2 to 4 times faster than those of extractable B[a]P before re-equilibration. Sandy-clay-loam soils with large amounts of hard organic carbon exhibited less NER remobilisation compared to sandy soils. The amounts of remobilised B[a]P decreased significantly (p < 0.05) with aging. Specifically, butanol-remobilised B[a]P in soils spiked at 10 mg/kg and 50 mg/kg B[a]P ranged from 0.15 to 0.39 mg/kg and 0.67 to 2.30 mg/kg, respectively, after 200 d of aging.
INTRODUCTION

The putative remobilisation, impact, and significance of ‘non-extractable’ residues (NERs) of polycyclic aromatic hydrocarbons (PAHs) in soils remain largely unexplored. Polycyclic aromatic hydrocarbons are ubiquitous hydrophobic organic contaminants (HOCs) in the environment and are of global concern. Of the 16 PAHs listed by the United States Environmental Protection Agency (USEPA) as priority pollutants, Benzo[a]pyrene is often the risk-driver at most PAH-contaminated sites due to its persistence, toxicity, carcinogenicity, mutagenicity and potential for bioaccumulation. Common sources of PAHs, with soil serving as a sink, include coking and coal firing plants, vehicular emissions, bush fires, fossil fuel burning, and crude oil spillage. The amounts of PAHs in soils can be wide-ranging depending on land use and proximity to industrial activities. Average total concentration of the 16 USEPA PAHs in soils collected near gasworks sites can range from 300 to over 8000 mg/kg, and many people living close to these sites may be exposed.

In soil, PAHs may be lost through volatilisation, photolysis, leaching, microbial degradation, and biological uptake. Importantly, PAHs are readily sequestered in soil as they are highly hydrophobic. Sequestration of PAHs in soils occurs through partitioning into soft and hard organic carbon, clay, organo-mineral complexes, as well as diffusion into micropores, or strong sequestration to carbonaceous geosorbents such as black carbon. Detailed mechanisms of HOC sequestration have been described elsewhere. With aging, the amounts of readily available PAH fractions decrease while those of sequestered fractions increase. Even with successive exhaustive solvent extractions, the sequestered fractions in soils are not extracted substantially and referred to as ‘NERs’.

The NERs of HOCs in soils are sometimes regarded as unimportant in risk assessment. The focus of traditional risk assessments has been on total-extractable concentrations which
will be generally greater than bioavailable concentrations. It is assumed that NERs will always be strongly and irreversibly sequestered to soil particles. However, recent knowledge reveals that total NERs in soil could be of different types depending on binding mechanisms. Type I NERs are entrapped and adsorbed residues which are non-covalently bound in soil, with low to high stability but also with low to high potential for release as parent compounds or metabolites. Type II NERs are covalently bound, highly stable and with low potential for release. Type III or biogenic NERs are residues formed from microbial metabolic activities in soils and are ultimately incorporated into SOM; their release bears no risks to human and ecological health. Differentiation of NERs in soils ensures that potential risks associated with exposure are not overestimated. Methods such as silylation, methanolic saponification, and microbial turnover to biomass modelling have been proposed for the differentiation of the NER types; however, these methods are complex, laborious and still need to be widely validated. Investigating potential remobilisation of undifferentiated NERs can still provide relevant information on NER fate in soils, which is useful for the risk assessment and management of contaminated lands. Further, the concentrations of potentially remobilised NERs in soils must be considered for risk assessment purposes.

A number of earlier studies focused on the potential release of soil-bound pesticide residues to sentinel organisms, such as earthworms and plants. For PAHs, Eschenbach et al. reported that non-extractable ^14C-PAH residues in long-term contaminated soils were not remobilised by biological (fungal and microbial) and physical treatments. Enhanced release of non-extractable PAH residues in soil by the influence of low molecular weight organic acids in plant root exudates has been reported. Low molecular weight organic acids such as citric acid chelates metal cations within mineral-SOM complexes in soil, thereby facilitating release of sequestered PAHs within the SOM. Following removal of labile PAH fractions from sediments, non-labile PAHs repartitioned from solid into liquid phases during
an incubation period of 30 d as facilitated by solid-phase (Amberlite XAD-2 resin) extraction.

Contaminant repartitioning may have implications for the long-term fate of PAH NERs in soils since associated risks may be underestimated. Still, the nature and acceptability of potential risks will depend on the concentrations of the repartitioned contaminants in soil.

The cited studies have either only used a few soils or did not report concentrations of NER remobilised and its significance, especially regarding its potential to cause significant harm from a risk assessment perspective. Importantly, the potential remobilisation of NERs in soils, after a re-equilibration period, which would likely be time-dependent and may vary with soil properties and PAH concentrations has not been investigated. This is the first study to examine the time-dependent remobilisation of B[a]P NERs following sequential solvent extractions of four artificially-spiked soils.
EXPERIMENTAL METHODS

Chemicals. Analytical grade B[a]P (> 96% purity) was obtained as crystalline solid from Sigma-Aldrich Pty Ltd., Sydney, Australia. Also sourced from the same supplier were p-Terphenyl-d14, analytical grade acetone (Ace), acetonitrile (ACN), 1-BuOH (BuOH, density = 0.81 g/ml, ≥ 99.4%), dichloromethane (DCM), methanol (MeOH, HPLC Grade), toluene (Tol, 99.8%), potassium hydroxide (KOH), sodium persulphate, hydrochloric acid, and silica sand. Hexane (Hex, HPLC grade) was purchased from Fisher Scientific, Loughborough, UK.

Design. Twenty-five surface and 7 sub-surface soils previously collected from 25 sites located in Australia and characterised were used in this study (Table S1, Supplementary Information (SI)). The data for the physico-chemical properties (pH, EC, TOC, DOC, CEC, sand, silt, and clay contents) of the 32 soils were subjected to a principal component (PC) analysis to select soils with differing properties. Four soils (I, M, B, N) were selected based on average PC scores, relating to varying soil properties, and calculated following the Anderson-Rubin Method. Soils I (Kurosol), M (Ferrosol), B (Black Vertosol), and N (Tenosol) were classified according to the USDA textural classification as sandy-loam, sandy-clay-loam, sandy-clay-loam, and loamy-sand, respectively. Soil organic matter contents of soils I (13.3), M (21.4%), B (11.0%), and N (4.8%) were estimated by loss on ignition. The contents of hard organic carbon (Hard OC) in acid-hydrolysed soils were quantified (by combustion at 1350 °C using LECO CNS analyser) after removing soft carbon by wet oxidation with persulphate according to a previously described method. The difference between TOC and hard OC contents estimated Soft OC contents in soils. Thermogravimetric analysis (TGA) was also used to determine the contents of nonpyrogenic OC (weight losses at 200–470 °C) and black carbon (BC)-rich fractions (weight losses at 470–600 °C) in soils based on thermal stability of these fractions.
For the B[a]P studies, each air-dried soil was spiked at 10 mg/kg and 50 mg/kg B[a]P following methods described previously \(^\text{22}\). The soils were rehydrated with deionised water to their field moisture contents (25% to 40% on dry weight basis) and incubated in sealed amber glass in the dark at 22 ± 3 °C for 14, 33, 66, 120, and 200 d. Spiked silica sand was treated in a similar manner to test spike recovery. All treatments are presented in Figure S1. It was expected that substantial NER would be formed after 33 d of aging \(^\text{22, 23}\). After aging, approximately 1 g soil subsamples (\(n = 12\)) were dried at 37.5 °C and subjected to a sequential extraction to completely remove readily available B[a]P fractions first using BuOH and then DCM/Ace extraction as described previously \(^\text{22}\). Since pre-extracted soils were to be further re-equilibrated, anhydrous Na\(_2\)SO\(_4\) was not used (which would also include grinding) for drying soil prior to DCM/Ace extraction. This was to avoid any artefact, such as disintegration of micropores and drying-out of soils, which could impact on re-equilibration \(^\text{25}\). All percentage extractability and sequestration determinations were calculated based on initial amounts of B[a]P spiked into soil (10 mg/kg or 50 mg/kg), except where stated otherwise stated.

\[
\text{Extractability (\%) = } \left( \frac{\text{Amount of B[a]P Extracted by Solvent (mg)}}{\text{Amount of B[a]P Spiked into Soil (mg)}} \right) \times 100\% \quad (1)
\]

After exhaustive extraction, soils were randomised into three batches of 4 replicate samples per soil (Figure S1).

For each soil, no significant difference (\(p > 0.05\)) was found between mean total extractability (percent BuOH + DCM/Ace extractability) among the three batches using one-way ANOVA (Figure S2), as well as variances between batches using Levene’s Test \(^\text{21}\). Standard error of the mean for each batch (\(n = 4\)) was generally 5% or less, and the mean concentration of each batch was not statistically different (\(p < 0.05\)) from the mean of original samples (\(n = 12\)). Therefore, each batch (\(n = 4\)) was statistically indistinguishable from the
original, and independent of the other batches within the same soil group. The first batch of
pre-extracted soils (Batch A) containing NERs was hydrolysed using methanol/potassium
hydroxide (MeKOH) to extract the highly sequestered B[a]P residues\textsuperscript{26,27}. The second (B)
and third (C) batches (\(n = 4\) each) were re-wetted to field moisture contents in the same
centrifuge bottle used for sequential extraction. The sample bottles were then capped and
allowed to re-equilibrate in the dark at 25.5 ± 1.0 °C for 30 d and 60 d, respectively. After re-
equilibration (REQ), soils were dried at 37.5 °C and the sequential extraction was repeated.
The additional fractions of B[a]P that were extracted after REQ constituted the remobilised
fractions.

For each of BuOH and DCM/Ace extractions, the amount of B[a]P (mg/kg) extracted after
REQ was then added to that prior to REQ to obtain cumulative extractability (mg/kg or %). Benzo[a]pyrene extractability prior to and after REQ in the soils were then compared. If
cumulative B[a]P extractability (%) after REQ was significantly greater (\(p < 0.05\)) than that
prior to REQ, this suggested that previous NER had become remobilised after the REQ
period.

**HPLC Analysis of B[a]P.** The concentrations of B[a]P in extracts filtered with 0.45 µm
polytetrafluoroethylene (PTFE) syringe filters were determined with an Agilent 1100 Series
HPLC equipped with a fluorescence detector (excitation wavelength = 230 nm and emission
wavelength = 460 nm) as in our previous study\textsuperscript{22}.

**Modelling Extractability Kinetics of Aging and Estimation of Half-Life of B[a]P Loss**
in Soil. Benzo[a]pyrene Extractability versus aging data in this study generally showed first-
order exponential decreases\textsuperscript{28}. A 2 parameter first-order kinetics equation served to model
decrease in B[a]P with aging in BuOH, total and MeKOH extractability of B[a]P before and
after REQ as well as the decrease in the remobilised amounts\textsuperscript{23,29}. 
\[ \text{B[a]Pt} = \text{B[a]Po} \times e^{-kt} \quad (2) \]

Where \( \text{B[a]Po} \) and \( \text{B[a]Pt} \) are the percentage of \( \text{B[a]P} \) extracted at time 0 d and \( t \) d of aging, respectively, and \( k \) is the rate constant of the decrease in the extractable fraction (\( k, \text{d}^{-1} \)). The non-linear curve fitting package of Origin software (Microcal Software Inc., Northampton, MA, USA, version 6) was employed for modelling and obtaining \( \text{B[a]Po} \) and \( k \) values through 100 iterations. The half-lives of \( \text{B[a]P} \) loss in the soils were also estimated.

**Quality Assurance and Quality Control.** A standard calibration curve using 10 calibration points (0.1 ng/ml to 1 µg/ml) was used to determine extracted \( \text{B[a]P} \) concentrations and consistently gave \( R^2 \) greater than 0.999. Detection limit (LOD) using the linear regression method was calculated from the lowest detectable concentrations (0.1, 0.5, 1, 5, and 10 ng/ml) using 8 replicates for each concentration. The LOD and limit of quantitation were 0.07 ng/ml and 0.21 ng/ml, respectively. Background concentrations of \( \text{B[a]P} \) in the four soils and silica sand were below LOD. There were at least 4 replicate analyses per treatment and not more than 12 per soil sample. Solvent-rinsed clean glass bottles with PTFE-lined caps were utilised, and they were tightly-capped throughout the experiment. In a preliminary test, p-Terphenyl-d14 was used as a surrogate to monitor \( \text{B[a]P} \) losses in freshly spiked soils throughout experimental steps, including spiking, drying, extraction, vacuum concentration, and analysis. Recoveries ranging from 80.9 ± 9.3% to 101.9 ± 17.6% were obtained. Potential \( \text{B[a]P} \) losses through sample pre-treatment were not corrected for in this study.

**Operational Definitions and Data Evaluation.** All operational definitions are presented in Table S2. The statistical software used for data analysis and graphing were SPSS Statistics (IBM Corp, version 24) and Origin (Microcal Software Inc., Northampton, MA, USA, version 6). No data transformations were applied. A one-way ANOVA was used to test the effects of aging or soil properties on \( \text{B[a]P} \) extractability before and after REQ at \( p < 0.05 \). Tukey HSD and Games Howell post hoc tests were used to identify within group variances.
when the assumption of homogeneity of variance (Levene’s Test) was met or not. Where data were not normally distributed (as tested by the Shapiro-Wilk Normality Test), Kruskal Wallis test was applied. A 2-way ANOVA was used to assess the effects of REQ and soil properties on B[a]P extractability in the 4 soils when Levene’s Test of Equality of Error Variances was not significant ($p > 0.05$). A simple linear regression was used to evaluate the relationships between soil properties and potentially remobilised B[a]P, and the rate constants obtained from kinetics modelling.
RESULTS AND DISCUSSION

Interpretation of Solvent-Extractable B[a]P Fractions. A detailed interpretation of B[a]P fractions extracted by the solvents utilised is presented in Table S3. Briefly, BuOH-extractable B[a]P refers to fractions in soils that may be readily available to microbes, earthworms, and the human gut. Subsequent extraction with DCM/Ace relates to B[a]P fractions that are weakly and strongly adsorbed but not physically entrapped in soils. Total-extractable B[a]P is the sum of fractions extracted by BuOH and DCM/Ace.

Methanolic saponification of pre-extracted soils (after DCM/Ace extraction) results in partial cleavage of ester and ether bonds of soil humic matter. After cleavage, sequestered B[a]P molecules in soils may be partially released, resulting in greater recovery. Potential mechanisms for increases in the extractability of NERs after methanolic saponification are detailed elsewhere.

Spike Recovery. Spike recovery of B[a]P in silica sand was consistently above 95%. In the 4 soils, recovery ranged from 93.3 ± 1.5 to 118.8 ± 0.0% (n = 2) immediately after spiking with 1 mg/kg B[a]P; and from 60.6 ± 5.6 to 87.9 ± 5.1% (n = 2) and 84.2 ± 3.4 to 97.3 ± 8.0% (n = 2) at 10 and 50 mg/kg B[a]P, respectively, 2 d after spiking. The average standard deviation in recovery for each soil at both spiked concentrations was less than 10% throughout the experiment and comparable to other studies. Data variability was attributed to the widely different soil properties. Mass balances for all of the soils, before and after REQ, are presented in Figure S3. The incomplete mass balances of B[a]P in soils after 200 d of aging may be attributed to very strongly sequestered B[a]P residues in soils that were not extractable by MeKOH. The different soil properties in addition to the difficulty of artificially spiking HOCs, may result in spike heterogeneity in soils. Except for soil B (sandy-clay-loam) which showed a relatively low recovery, percentage recovery as well as the...
observed data variability were similar to those documented in other studies \(^{25,29}\), and thus validated the spiking and extraction procedures. Benzo[a]pyrene is intrinsically hydrophobic (Log octanol-water partition coefficient > 6.0) and has a very low aqueous solubility \(^{35}\), which may limit its transfer to the soil surface or aqueous phase, resulting in low bioavailability. Further, because of its strong partitioning to soil, low volatility, low water solubility and its molecular structure, B[a]P will be resistant to biodegradation \(^{8,36,37}\), and therefore persist in soil \(^{10}\). Low PAH extractabilities have also been reported in organic matter rich-clayey soils which were freshly spiked \(^{30}\), and incomplete mass balances of B[a]P in soils after 160 d and 200 d of aging \(^{25,29}\). Overall, decrease in B[a]P extractability after aging was attributed to sequestration processes \(^{29}\), although losses from biodegradation may not be completely disregarded.

**Differences in B[a]P Extractability in Soils with Aging before re-equilibration.**

Generally, B[a]P extractability in soils spiked at 50 mg/kg was consistently greater than in soils spiked at 10 mg/kg (Figure 1 and Table S4), which agrees with previous studies \(^{38}\). Total-extractable B[a]P was also greater than BuOH-extractable B[a]P. For BuOH, the amounts of B[a]P extracted from each soil significantly decreased \((p < 0.001)\) with aging, as shown by the first-order kinetic model \((R^2 = 0.80 - 0.94)\) (Figure 1 and Table S5). The modelled \(k\) values (Table S5) agreed with those \((10^{-3})\) reported for slowly-desorbing PAH fractions \(^{23,39}\), and did not differ \((p > 0.05)\) between the spiked B[a]P concentrations. In contrast, subsequent DCM/Ace-extractable B[a]P significantly increased \((p < 0.05)\) with aging, especially in the sandy-clay-loam organic matter-rich soil B and organic matter-rich soil M, suggesting that B[a]P became quickly and progressively sequestered in the soils. The amounts of total-extractable B[a]P (mg/kg) generally decreased rapidly \((p < 0.05)\), whereas NERs increased (Table S4). Although total B[a]P extractability tended to decrease
continuously or plateau beyond 33 and 66 d of aging, subsequent changes were not generally significant ($p > 0.05$).

![Exponential curve-fitting of observed B[a]P BuOH extractability in soil before and after re-equilibration.](image)

**Figure 1.** Exponential curve-fitting of observed B[a]P BuOH extractability in soil before and after re-equilibration. Open and shaded symbols represent B[a]P concentrations (%) before and after re-equilibration, respectively. Spiked B[a]P concentrations: 10 mg/kg (□) and 50 mg/kg (Δ). TOC is total organic carbon. Values are means of 4 replicates ± standard deviations.

Readily available PAHs in soils often tend to decrease or stabilise after approximately 30 d of aging. Soils with various sites for PAH sequestration, such as black carbon, organic materials in soils, and clay, are likely to show greater PAH sequestration than soils without or with less of these sequestration sites. The fractions of hard OC as a percentage of TOC, and the contents of clay in soils showed negative correlations ($r > -0.90$) with B[a]P extractability.
suggested decreased B[a]P extractability in soils with large amounts of hard OC.

![Graph A](image1.png)  ![Graph B](image2.png)

**Figure 2.** Correlations between hard OC fractions in soils and B[a]P extractability and sequestration. Cum. is cumulative extractability after re-equilibration (REQ).

Detectable amounts of highly sequestered B[a]P were found in all soils from 14 d of aging (Table S4) indicating rapid B[a]P sequestration. Benzo[a]pyrene NERs ranged from 5.8 – 8.0 mg/kg (10 mg/kg B[a]P) and from 25.8 – 36.9 mg/kg (50 mg/kg B[a]P) after 200 d of aging.

At 200 d of aging, B[a]P NERs recovered by MeKOH ranged from 8 – 22% (Figure 3), with the more sandy soils N and I showing larger recoveries than the sandy-clay-loam soil B or organic matter-rich soil M (Figure 3). This indicates that B[a]P residues in more sandy soils with less amounts of hard OC were less sequestered (Figure 2B), and may have greater potential to be remobilised than in the sandy-clay-loam organic matter-rich soils (Figure 2C).

The remaining B[a]P residues (78 to 92%) not extractable by MeKOH were regarded as very
strongly sequestered with very low potential for remobilisation.

Figure 3. Amount (%) of B[a]P NER recovered by MeKOH before and after re-equilibration. Only soils spiked at 10 mg/kg B[a]P. Open and shaded symbols represent B[a]P concentrations (%) before and after re-equilibration, respectively. TOC is total organic carbon. Values are means of 4 replicates ± standard deviations.

No obvious correlations were observed between soft OC fractions and B[a]P extractability and sequestration. Also, the strong positive relationships between hard OC and BC-rich fractions (Figure S5), as well as between soft OC and nonpyrogenic OC (Figure S5) indicate that increasing amounts of recalcitrant OC fractions in soils influenced B[a]P extractability and sequestration in soils, as with clay contents (Figure S4). In addition, a strong positive relationship (r > 0.8) was observed between the recalcitrant fractions and clay contents which may indicate that B[a]P sequestration sites in the soils may be a continuum comprising mostly hard OC fractions, clay, and clay-associated OC fractions (Figure S6). Therefore, the
extents of B[a]P extractability and sequestration in the soils may differ depending on its  
interactions with these sequestration sites. A wider range of soils may be needed to validate  
the observed relationships, hence interpretation of soil properties and B[a]P extractability and  
sequestration in this study should be treated with caution.

Over time, the decreasing trend in total B[a]P extractability or increasing trend in the NERs  
further indicates progressive B[a]P sequestration, particularly in soils with larger amounts of  
hard OC fractions, clay or clay-associated OC fractions. Aging effects on B[a]P extractability  
in soils before REQ have been well reported 11, 22, 25. The interested reader is directed to the  
SI for further discussions.

Differences in B[a]P Remobilisation in Soils with Aging and Associated Rates of Loss.

Effects of Re-equilibration Time. The re-equilibration time of 30 or 60 d significantly  
influenced (p < 0.05) the amounts of B[a]P extracted by each of the solvents, as cumulative  
extractability after REQ was consistently greater than prior to REQ in all 4 soils (Table S4).  
However, the effects of either a 30 d REQ or a 60 d REQ on B[a]P extractability at 14 and 33  
d of aging were statistically similar (p > 0.05) (Figures S7 and S8). Hence, pre-extracted soils  
after 33 d of aging were re-equilibrated for 30 d only. Another study also reported that the  
amounts of anthracene, benzo[k]fluoranthene and B[a]P repartitioned in an XAD-treated  
sediment did not differ after 30 or 90 d of incubation 19.

The increase in cumulative total-extractable B[a]P after REQ at each aging period in this  
current study also implied that B[a]P NERs remaining in soils after REQ were significantly  
more diminished (p < 0.001) when compared to the NERs before REQ. This is a further  
indication that B[a]P NERs became re-extractable after REQ. The amounts of total-  
remobilised B[a]P in soils were greater than BuOH-remobilised B[a]P (Figures 2C and 3),  
however, aging significantly decreased the amounts of B[a]P remobilised (Table S6).
The rates \((k, \text{d}^{-1})\) at which the remobilised B[a]P fractions decreased over time range from \(0.0040 \pm 0.0001 - 0.007 \pm 0.002\), and \(0.006 \pm 0.003 - 0.011 \pm 0.005\) in soils spiked with 10 mg/kg and 50 mg/kg B[a]P, respectively (Table S5). Although the \(k\) values of the remobilised B[a]P fractions aligned with \(k\) values before REQ \((10^{-3} \text{d}^{-1})\), the former were 2 to 4 times faster than the latter. This suggests that, over time, potentially remobilised B[a]P in soils may decrease very rapidly, particularly in long-term field-contaminated soils. Correlational analyses also suggest that the rates at which remobilisable B[a]P fractions decrease over time would be slower in soils with larger hard OC and clay contents (Figure 2D and Figure S9), further indicating that potentially remobilisable amounts are not changing substantially over time since B[a]P NERs are more strongly sequestered in these soils.

**Effects of Spiked Concentrations and Soil Properties.** Student’s t-test showed that the amounts (%) of B[a]P remobilised after REQ by BuOH from each soil spiked at 10 and 50 mg/kg differed particularly during earlier aging times (Figure 4). The amounts of B[a]P NERs remobilised by BuOH from 14 d to 200 d of aging ranged from 2 – 7% (10 mg/kg B[a]P) and 2 – 9% (50 mg/kg B[a]P) (Figure 4), and were largest in the more sandy soils. After 200 d of aging, the amounts of B[a]P remobilised by BuOH in the 4 soils were reduced and generally similar (Figure 4). For the subsequent DCM/Ace extraction, the amounts (mg/kg) of B[a]P remobilised increased significantly \((p < 0.05)\) especially until 66 d in soils I, M, and B after which remobilised B[a]P (mg/kg) decreased significantly \((p < 0.05)\) or remained unchanged. Amounts of B[a]P remobilised in soil N increased significantly \((p < 0.05)\) from 14 d (0.26 mg/kg) to 200 d (2.37 mg/kg), especially at 10 mg/kg B[a]P. Increases in the DCM/Ace-remobilisable B[a]P with aging further indicate the loss BuOH-remobilisable B[a]P in soils. The amounts (mg/kg) of total-remobilised (BuOH + DCM/Ace) generally decreased \((p < 0.05)\) from 14 d to 200 d of aging as with BuOH extractability (Table S6). The amounts of B[a]P totally remobilised differed significantly \((p < 0.05)\)
between all 4 soils at each aging time; these amounts ranged from 8% (0.5 mg/kg) to 46% 
(2.8 mg/kg) of B[a]P NERs (10 mg/kg B[a]P) and from 4% (1.1 mg/kg) to 15% (4.0 mg/kg) 
(50 mg/kg B[a]P) throughout the aging period. The amounts of B[a]P NERs totally 
remobilised were again generally greater in soils I and N compared to soils M and B (Figure 
5), further indicating the influence of soil properties.

![Figure 4](image.png)

**Figure 4.** Exponential curve-fitting of B[a]P remobilised (%) after re-equilibration through 
aging in soils. Spiked B[a]P concentrations: 10 mg/kg (■) and 50 mg/kg (▲). TOC is total 
organic carbon. Values are means of 4 replicates ± standard deviations.

Only soils M and N spiked at 10 mg/kg had greater amounts of remobilised B[a]P (mg/kg) 
after 200 d of aging (1.39 ± 0.05 and 2.76 ± 0.03, respectively) compared to that remobilised 
at 14 d (1.08 ± 0.06 and 1.06 ± 0.03), although remobilised amounts remained low. Overall, 
BuOH- and total-remobilisble B[a]P (mg/kg) were small in the four soils, especially after 
200 d of aging.
Further, there were negative relationships between the amounts of B[a]P remobilised from B[a]P NERs or from highly sequestered residues recovered by MeKOH and the fractions of hard OC in soils (Figure 5), and clay (Figure S9). Intercompartmental repartitioning of B[a]P NERs from strongly sequestered sites (BC-rich or hard OC-rich) into weakly sequestered sites where they are readily available may occur at very slow rates. Hence, the amounts of repartitioned and potentially remobilisable B[a]P were small, particularly in aged sandy-clay loam soils with BC-rich fractions.

**Figure 5.** Remobilised B[a]P fractions relative to the amounts of highly sequestered B[a]P NERs recovered by MeKOH and the amounts of NERs in soils.

After remobilisation in soils which had been re-equilibrated, the highly sequestered B[a]P residues (MeKOH-extractable) decreased significantly ($p < 0.05$) compared to those before REQ (Tables S4 and S6), as well as the percentage of the NERs recovered (Figure 3). This was an interesting finding because it indicated remobilisation of NERs. The decreasing trend
of the NERs after REQ further supports B[a]P remobilisation from NERs in pre-extracted soils. Also, increasing absolute amounts of the sequestered fractions (Table S2) indicate progressive transfer of B[a]P into more strongly sequestered fractions over time. In this study, absolute amounts of sequestered fractions were smaller after REQ than before REQ (Figure 6), indicating remobilisation of B[a]P NERs.

**Figure 6.** Absolute amounts of the highly sequestered B[a]P recovered before and after re-equilibration. Open and shaded symbols represent absolute amounts before and after re-equilibration, respectively. Spiked B[a]P concentrations: 10 mg/kg (□) and 50 mg/kg (Δ). TOC is total organic carbon. Values are means of 4 replicates ± standard deviations and were normalised (x 10²).

**Estimated Half-Life of B[a]P Loss in Soil.** The estimated half-lives of B[a]P loss in soils based on BuOH extractability, before and after REQ, ranged from 93 – 442 and 91– 221 d at 10 and 50 mg/kg spiked concentrations, respectively (Table S5). For total extractability
(Table S5), the half-lives of B[a]P loss in soils ranged from 125 – 866 d (10 mg/kg B[a]P)
and from 92 – 459 d (50 mg/kg). The half-lives of B[a]P loss were generally shorter for the
more sandy soils I and N compared to the sandy-clay-loam organic matter-rich soil B and
organic matter-rich soil M (Table S5) especially at 50 mg/kg, further confirming the
influence of soil properties and B[a]P concentration on B[a]P loss in soils. The half-life of
B[a]P loss in soils estimated in this study agrees with other studies’ findings: for example,
208 – 254 d in sterile sewage-sludge amended soil with radiolabelled B[a]P aged for 525 d \cite{25};
45 – 181 d in soils aged for 160 d \cite{29}; and 1155 d in more than a decade old aged
contaminated agricultural soil \cite{28}.

**Towards a Potential Mechanism of Remobilisation of B[a]P NERs in Soils.** This study
did not focus on differentiating NERs into their different types. The MeKOH-extractable B[a]P
fractions may be associated with Type I NERs in soils. The NERs described here may be linked
with hard OC fractions, clay, or clay-associated OC in soils. The influence of hard OC and
clay-associated OC fractions on PAH sequestration has been previously documented \cite{8, 23, 40, 41}.
Hard OC fractions contribute substantially to PAH desorption hysteresis \cite{8, 9}. Clay surfaces with
OC patches may also have better sequestration capacities for PAHs in soils than bare SOM or
clay \cite{42}. Due to the heterogeneous nature of sequestration sites in soils \cite{43}, non-covalently
sequestered PAHs may be potentially released at different rates and extents. This may depend
on the strength of associated interactions (Van der Waals, hydrophobic, hydrogen bonding, and
π – π interactions) \cite{12}. These interactions are influenced by the amounts and quality of OC (hard
or soft OC) and clay type (e.g. montmorillonite and kaolin) \cite{44-46}. Hence, Type I NERs (parent
compounds and metabolites) in this study may not be discreet fractions. Instead they may be a
continuum of NERs subjected to different degrees of sequestration in soils where Type 1A is
less sequestered than Type 1B (Figure 7).
Relative to the amounts of highly sequestered fractions (MeKOH-extractable) before REQ, the amounts of B[a]P remobilised ranged from 18% to 30% (10 mg/kg B[a]P) and from 21% to 41% (50 mg/kg B[a]P) (Figure 5). However, amounts of B[a]P totally-remobilised from the MeKOH-extractable fractions ranged from 86% to 214% (10 mg/kg) and 31% to 71% (50 mg/kg) (Figure 5). These observations suggest that small to large percentages of the highly sequestered fractions recovered before REQ by MeKOH were remobilised. The very high B[a]P remobilisation (up to 214%) in some soils indicated that the sequestered fractions in soils before REQ (Type 1A) may have been replenished by repartitioning of very strongly sequestered fractions (Type 1B) during the REQ period. This is explained in more detail below.

Following total remobilisation (BuOH + DCM/Ace) of B[a]P after REQ (Figure 7), the amounts of B[a]P NERs remaining in soils ranged from 4.4 mg/kg to 7.2 mg/kg (10 mg/kg B[a]P), and from 23.5 mg/kg to 35.8 mg/kg (50 mg/kg B[a]P). However, the amounts of B[a]P NERs in soils recovered by MeKOH after total remobilisation ranged from 5% (0.25 mg/kg) to 13% (0.5 mg/kg) at 10 mg/kg B[a]P, and from 3% (0.9 mg/kg) to 8% (1.9 mg/kg) at 50 mg/kg B[a]P (Figure 4E). These small amounts (0.25 to 0.5 mg/kg) of highly sequestered B[a]P fractions (MeKOH-extractable) that were still recoverable after total-remobilisation (10 mg/kg) (Figure 7), even though these fractions were completely depleted previously (i.e. 214%), may indicate that very small amounts of more strongly sequestered B[a]P NER fractions (Type 1B) repartitioned slowly into the less sequestered forms (Type 1A) with very low potential to be remobilised (Figure 4E). Slow repartitioning rates ($3.40 \times 10^{-3}$) of strongly sequestered PAHs into less sequestered PAH fractions in field-contaminated sediments have been reported. Overall, the amounts remobilised in soils after 200 d of aging were very small, and may be much smaller in long-term PAH-contaminated soils.
Figure 7. Proposed mechanism of remobilisation of B[a]P non-extractable residues (NERs) in soils after re-equilibration (REQ) through slow intercompartmental partitioning from strongly sequestered to less sequestered fractions. BuOH = butanol, DCM/Ace = dichloromethane/acetone, MeKOH = methanolic saponification. A = non-covalently sequestered B[a]P NER (parent compound or metabolites) comprising Types IA (less strongly sequestered) or Type IB (more strongly sequestered) before REQ; B = Soil humic matter disaggregated by MeKOH partially releasing B[a]P NERs (Likely Type IA); MeKOH-extractable B[a]P before REQ is an indication of the amounts of highly sequestered B[a]P that are potentially remobilisable following a REQ period. As a consequence of removing total-extractable fractions in soils, a concentration gradient results between readily available and sequestered compartments. During REQ of B[a]P NERs in soils, sequestered B[a]P (Type 1A) may repartition into readily available fractions at slow rates and low extents. C. Small amounts of BuOH-extractable B[a]P after the repartitioning process; D. Small amounts of total-extractable B[a]P after the repartitioning process. The remaining soil residue at this point is
similar to that after MeKOH extraction in B; E. MeKOH-extractable B[a]P relating to the previously repartitioned fractions that have not been completely depleted after DCM/Ace extraction (Type 1A) and/or newly repartitioned fractions (very low amounts and very slow rates) from strongly sequestered fractions (Type 1B) after complete depletion of previously repartitioned fractions. Note that the amounts of B[a]P NERs in this scheme may be overestimated, and that amounts repartitioned and potentially remobilised are likely to be very small and associated rates very slow, particularly in long-term contaminated soils. Also note that Type IA and Type 1B were depicted as discreet fractions for simplicity; in reality, they are a continuum.

**Does the very limited Remobilisation of B[a]P NERs in Soils have Implications for Contaminated Land Decision-Making?** Understanding the potential for remobilisation of NERs in soils may be important in risk assessment, particularly where NER concentrations exceed regulatory guideline values (5.8 to 36.9 mg/kg in this study); however, only a very small portion of the NERs may be remobilisable (Type I NERs). Therefore, it is important to consider the concentrations of NERs released or remobilised in soils for effective risk assessment and contaminated land decision-making, rather than consider the potential for release only. In Australia, for example, site-specific risk assessment and characterisation of contaminated soils considers bioaccessibility, particularly for metals and metalloids. The recently amended health investigation levels (HILs) for B[a]P in contaminated soils range from 3 to 40 mg/kg, based on B[a]P toxic equivalence factors, and are dependent on land use. Clearly, the concentrations of B[a]P NERs remobilised in soils by BuOH and exhaustive extractions were generally below 3 mg/kg in this study. From a potential remobilisation perspective, if only BuOH-extractable concentrations were considered, all soils would pass the HIL criteria and risks to human health may be acceptable. However, not all soils would pass the criteria if total-extractable concentrations were used. Based on this study, B[a]P
NERs in soils have the potential to be released at low concentrations over a long period of time further reducing associated risks to human and environmental health.

ASSOCIATED CONTENT

Supporting Information. The supporting information is available free of charge. Additions to Results and Discussion including: differences in B[a]P extractability in soils with aging; preliminary trends between soil properties and B[a]P extractability and sequestration; and supporting figures and tables; and references.

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Notes

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REFERENCES


14. Eschenbach, A. Characterization of non-extractable residues for their risk assessment in soil with special regard to pharmaceuticals. In *International Workshop - Pharmaceuticals in*


