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Rita M. Turkall Ph.D.
University of Medicine and Dentistry of New Jersey, turkalm@umdnj.edu

Gloria A. Skowronski Ph.D.
University of Medicine and Dentistry of New Jersey, skowroga@optonline.net

Mohamed S. Abdel-Rahman Ph.D., F.C.P., B.C.F.E.
University of Medicine and Dentistry of New Jersey, abdelrms@umdnj.edu

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EFFECTS OF SOIL MATRIX AND AGING ON THE DERMAL BIOAVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE SOIL

Rita M. Turkall1,2, Gloria A. Skowronski1, and Mohamed S. Abdel-Rahman1§

1University of Medicine and Dentistry of New Jersey, Pharmacology and Physiology Department, New Jersey Medical School and 2Clinical Laboratory Sciences Department, School of Health Related Professions, Newark, NJ, USA 07103-2714

ABSTRACT

The potential health risk from exposure to chemically contaminated soil can be assessed from bioavailability studies. The aims of this research were: (a) to determine the dermal bioavailability of contaminants in soil for representatives of the polycyclic aromatic hydrocarbon class of chemicals, namely, benzo(a)pyrene and naphthalene, and (b) to examine the relative contribution of soil matrix and chemical sequestration in soil with time (“aging”) on their bioavailability. In vitro flow-through diffusion cell studies were performed utilizing dermatomed male pig skin and radioactive chemicals to measure dermal penetration. Volatilization of naphthalene was predominant in reducing the amount of chemical available for dermal penetration. Immediate contact with either of two soils reduced volatilization, however, only the soil with higher clay content resulted in reduced penetration. Aging in higher sand content soil and higher clay content soil further reduced skin penetration by 23 and 70 fold, respectively, versus naphthalene in the absence of soil. Benzo(a)pyrene penetration was reduced >88% following immediate contact with either soil with further reductions occurring after aging. While aging in either soil reduced the dermal penetration of both naphthalene and benzo(a)pyrene, the effect on naphthalene was much greater. The results of this study suggest that the bioavailability from dermal exposure to the polycyclic aromatic chemicals examined can be significantly reduced by soil matrix and aging in soil, resulting in reduced potential health risk following dermal exposure.

Keywords: soil contaminants, dermal exposure, bioavailability effects

1. Introduction

Chemical contamination of soil is a widespread problem of concern to industry, employees, communities and regulatory agencies. Conservative assessment of human health risk following exposure to contaminated soil often is based on exposure to the total concentration of chemical
in the soil determined by rigorous extraction procedures (USEPA, 1986, 1992; Tang et al. 1999). This approach can result in overestimation of risk if only a fraction of the total concentration (the bioavailable fraction) is absorbed into the systemic circulation.

Soil is a complex matrix that can adsorb pollutants (Hamaker and Thompson, 1972). Organic carbon content, clay content, particle size, surface area and pH of soil can affect chemical sorption and desorption processes, and thus may have significant impacts on the bioavailability of chemicals from soils (NEPI, 2000; Pu et al., 2004). Moreover, the movement of chemicals from the surface of soil particles into less accessible sites with time (aging) (Linz and Nakles, 1997; Reid et al., 2000) can further impact chemical bioavailability from soil (Alexander, 2000). The mechanisms for chemical aging are not fully understood; however, it has been proposed that hydrophobic chemicals can partition into the solid phase of soil organic matter as well as become entrapped within soil nanopores where they may be retained and become less accessible (Steinberg et al., 1987; Brusseau et al., 1991; Pignatello and Xing, 1996). Adsorption, desorption and partitioning in soil are also affected by the chemical's size (Dragun, 1988; Winegardner, 1996), volatility and lipophilicity (Ibbotson et al. 1989).

The dermal route of exposure can contribute significantly to total exposure since adult human skin comprises more than 10% of total body mass and 1.8 m^2 of body surface (Roberts and Walters, 1998; USEPA, 2001). Adults are more likely to experience dermal exposures to contaminated soil during work related activities, waste disposal operations or accidental releases, while children do so during play activities. Adsorption through skin has the potential to deliver significant quantities of chemicals systemically to the body, while chemicals that cannot penetrate skin may be limited to local toxic effects on the skin. Therefore, it is necessary to know the capacity of a chemical for dermal absorption in order to assess its overall potential risk (Mattie et al., 1994).

The studies reported in this paper were conducted to assess the dermal bioavailability of contaminants in soil for representatives of the polycyclic aromatic hydrocarbon (PAH) class of chemicals, namely, benzo(a)pyrene (BaP) and naphthalene. PAHs are ubiquitous contaminants of soil derived chiefly from the incomplete combustion of organic materials as well as being introduced to soil through human activities such as gas manufacture from coal or oil resulting in deposits of coal tar residues (ATSDR, 1995; Loehr and Webster, 1997). New Jersey has the most sites with PAH contamination. Soil concentrations of BaP in National Priorities List (NPL) sites in the state range between 1.1 and 8,100 mg/kg (ATSDR, 1995; ATSDR, 1999). BaP has been classified as a probable carcinogen in humans. Dermal exposure to BaP can cause skin irritation with rash and/or burning sensations. Repeated exposure can produce skin changes such as thickening and darkening (NJDHSS, 1998). Naphthalene has been identified as one of the most abundant soil contaminants at hazardous waste sites (NRC, 1991). The mean concentration of naphthalene detected in soil/sediments from 106 of the 862 hazardous waste sites analyzed by the Contract Laboratory Program was 1,300 μg/kg (CLPSD, 1988). The primary health concern for individuals dermally exposed to naphthalene is hemolytic anemia (Schafer, 1951; Dawson et al., 1958) due to the potent hemolytic properties of the metabolite alpha-naphthol (Mackell et al., 1951).

The relative contribution of the soil matrix and chemical aging in soil on the dermal bioavailability of the representative chemicals were determined so that the impact of the results
on health risk could be evaluated. Bioavailability was assessed by measuring dermal penetration. Specifically: (a) the dermal penetration of each of the chemicals aged in soil was compared to the respective pure chemicals (without soil) and to the chemicals added freshly to soil; and b) the effects of soil composition (percent sand, clay, organic matter) on dermal penetration were examined.

2. MATERIALS AND METHODS

2.1 Chemicals

BaP, generally labeled with tritium $[^3]H(G)$, having a specific activity of 50 Ci/mmol and radiochemical purity of 99%, was obtained from American Radiolabeled Chemicals, Inc., St. Louis, MO. Naphthalene (1,4,5,8-$^{14}$C) was custom synthesized by E.I. DuPont deNemours and Co., Inc., New England Nuclear Research Products, Boston, MA. The compound had a specific activity of 15 mCi/mmol and radiochemical purity of 99%. Non-radioactive BaP with $\geq$ 96% purity was purchased from Sigma-Aldrich, St. Louis, MO.

2.2 Soils

Studies were conducted on two different soils that are representative of soil types widely distributed in the United States (USDA, 1972, 1977). The Atsion soil consists of 90% sand, 8% silt, 2% clay, 4.4% organic matter; has a pH of 4.2; and was collected from the Cohansley sand formation near Chatsworth in south-central New Jersey. The Keyport soil contains 50% sand, 28% silt, 22% clay, 1.6% organic matter; has a pH of 5; and was collected from the Woodbury formation near Moorestown in southwestern New Jersey. Soil particle size distribution was as follows: Atsion soil = 50-100 µm (22.2%), 100-250 µm (76.3%), $> 250$ µm (1.5%); Keyport soil = 50-100 µm (17%), 100-250 µm (65.3%), 250-500 µm (13.6%), $> 500$ µm (4.1%). Soil analyses were performed by the Soil Testing Laboratory at Rutgers Cooperative Extension Resource Center, Rutgers University, New Brunswick, NJ. Organic matter content was measured by a modified Walkley and Black (1934) dichromate oxidation method.

2.3 Chemical Aging in Soil

Individual chemicals were added to each of the soils that were previously autoclaved and hydrated to 11% (w/w) with sterile distilled-deionized water. This is the maximum amount of water that could be used to lightly moisten the soils without there being an excess of water when each chemical was added to the soils. The final concentration of $^3$H-BaP tracer (400 ng/g soil) together with unlabeled BaP was 1.67 mg/g soil. The final concentration of $^{14}$C naphthalene was 200 ug/g soil. After each chemical was mixed thoroughly with the soils to ensure uniform distribution of chemical, treated soils were added to Teflon-sealed vials and aged in the dark at room temperature for three months.
2.4 Animal Model

Whole pig skin was obtained from the costo-abdominal areas of euthanized (40-60 lb) male Yorkshire pigs (Cook College Farm, Rutgers University, New Brunswick, NJ). The pig has been widely accepted as an animal model for studying human percutaneous absorption of a large variety of chemicals under various experimental conditions (Bartek et al., 1972; Reifenrath and Hawkins, 1986) because of the well documented histological (Monteiro-Riviere and Stromberg, 1985), physiological, biochemical, and pharmacological similarities between pig skin and human skin (Qiao and Riviere, 2000). Skin was transported to the laboratory in ice-cold HEPES buffered (25 mM) Hank’s balanced salt solution (HHBSS), pH 7.4, containing gentamycin sulfate (50 mg/l) (Collier et al., 1989) after which it was immediately prepared for diffusion cells according to Bronaugh and Stewart (1985).

2.5 In Vitro Dermal Penetration Studies

Excised skin was cut to a thickness of 200 µm with a dermatome (Padgett Electro-Dermatome Model B, Padgett Instruments Inc., Kansas City, MO) and circular pieces were mounted into Teflon flow-through diffusion cells (Crown Bio Scientific, Inc., Somerville, NJ). The exposed skin surface area (0.64 cm²) was maintained at a temperature of 32°C. The dermal side of each skin sample was perfused with HHBSS containing 10% fetal bovine serum (Sigma/Aldrich) at a flow rate of 3 ml/h and aerated continuously with oxygen (Collier et al., 1989). Each chemical was applied separately to the stratum corneum surface of the skin in vehicle (acetone for BaP, ethanol for naphthalene), immediately after the addition of 30 mg of soil or after aging in 30 mg of each of the two soils. The chemical doses per cm² of skin surface area were: 78 ug BaP and 3.3 ug naphthalene. After skin was treated and diffusion cells were capped, charcoal tubes (SKC Inc., Eighty-Four, PA) were attached to the upper chambers of the diffusion cells to trap any chemical volatilizing from the skin surface. Volatilization losses were detected by measuring radioactivity in glacial acetic extracts of charcoal.

Receptor fluid (perfusate) was collected in scintillation vials containing 10 ml of Formula-989 liquid scintillation cocktail (Packard Instruments Co., Inc., Meriden, CT) up to 16 h postdosing. After 16 h of exposure to chemical alone or in soil, loosely adsorbed chemical was washed from the surface of the skin with soap and water (once with 1 ml of a 1% aqueous soap solution and twice with 1 ml of distilled-deionized water). Skin samples were completely solubilized in Solvable (Packard) for 8 h at 50°C to determine the amount of radioactivity remaining in skin. Radioactivity in all samples was counted by liquid scintillation spectrometry (LS 7500, Beckman Instruments, Inc., Fullerton, CA). Sample quench was corrected by using the H-ratio method.

2.6 Statistical Analysis

All data were reported as the mean ± standard error of the mean (SEM). Statistical differences between treatment groups were determined by one-way analysis of variance (ANOVA) followed by Scheffe’s test. The level of significance was p < 0.05.
3. RESULTS AND DISCUSSION

The effect of soil type and aging on dermal penetration is compared for BaP and naphthalene in Table 1 (Atsion soil) and Table 2 (Keyport soil). Total penetration represents the sum of chemical penetrating into receptor fluid and the amount in skin that potentially can penetrate into receptor fluid with time (Chu et al., 1996). Table 1 indicates a significantly lower total penetration (10.9 fold less) for pure naphthalene than for pure BaP concurrent with a 91% loss of pure naphthalene dose due to volatilization. Of the pure BaP dose that penetrated (76.0%), greater than 99% was bound to skin with the remainder appearing in the receptor fluid. The reverse occurred with pure naphthalene, where significantly less of the penetrated dose was bound to skin and significantly more appeared in the receptor fluid versus pure BaP.

Table 1. Comparison of Dermal Penetration of Benzo(a)pyrene and Naphthalene Following Contact with Atsion Soil

<table>
<thead>
<tr>
<th></th>
<th>Pure B(a)P</th>
<th>Immediate B(a)P</th>
<th>Aged B(a)P</th>
<th>Pure Naphthalene</th>
<th>Immediate Naphthalene</th>
<th>Aged Naphthalene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor Fluid</td>
<td>0.2 ± 0.1a</td>
<td>5.1 ± 0.2b</td>
<td>0.1 ± 0.0</td>
<td>5.1 ± 1.0b</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Bound to Skin</td>
<td>75.8 ± 3.2</td>
<td>2.0 ± 0.2b</td>
<td>3.7 ± 0.5</td>
<td>2.0 ± 0.5b</td>
<td>3.7 ± 0.5</td>
<td>0.2 ± 0.0b</td>
</tr>
<tr>
<td>Total Penetration</td>
<td>76.0 ± 3.2</td>
<td>7.0 ± 0.2b</td>
<td>32.1 ± 1.1</td>
<td>7.2 ± 1.2</td>
<td>32.1 ± 1.1</td>
<td>85.7 ± 1.7</td>
</tr>
<tr>
<td>Volatilization</td>
<td>---</td>
<td>91.0 ± 2.0</td>
<td>---</td>
<td>84.8 ± 1.8</td>
<td>84.8 ± 1.8</td>
<td>85.7 ± 1.7</td>
</tr>
<tr>
<td>Decontamination</td>
<td>24.2 ± 2.2</td>
<td>0.2 ± 0.0</td>
<td>46.4 ± 2.4</td>
<td>8.4 ± 1.8</td>
<td>46.4 ± 2.4</td>
<td>19.0 ± 0.3</td>
</tr>
<tr>
<td>Soil</td>
<td>---</td>
<td>---</td>
<td>57.3 ± 1.7</td>
<td>45.6 ± 1.7</td>
<td>57.3 ± 1.7</td>
<td>11.9 ± 0.3</td>
</tr>
</tbody>
</table>

\(^{a}\)Percent initial dose (mean ± SEM) for n=9-13 replicates per treatment from 3 pigs.

\(^{b}\)Significantly different from respective B(a)P treatment (p < 0.05).

Immediate addition of Atsion soil to BaP reduced the total penetration and amount bound to skin by \(\geq 88\%\) versus pure compound (\(\leq 8.5\%\) versus \(\leq 76\%\) initial dose)(Table 1). Aging further reduced these values to 3.7\% of initial dose. On the other hand, immediate addition of Atsion soil to naphthalene produced no change in total penetration and amount bound to skin versus pure compound (\(\leq 7\%\) initial dose), however, aging in soil reduced by \(\geq 90\%\) the amounts in these categories to \(\leq 0.3\%\) initial dose (significantly lower versus BaP). For both BaP and naphthalene, immediate addition of Atsion soil increased the amount found in skin wash (35.6 and 8.4\% initial dose, respectively) versus treatment with pure compound (24.2 and 0.2\% initial dose, respectively) as well as resulted in retention of chemical by soil (57.3 and 45.6\%, respectively). Furthermore, for naphthalene, immediate addition of Atsion soil also reduced volatilization to 32.1\% of initial dose versus pure compound (91\%). Aging versus immediate addition to Atsion soil did not alter the total amounts of BaP in skin wash and soil (94\% initial dose). However, aging versus immediate addition to Atsion soil reduced total amounts of naphthalene in skin wash and soil (13 versus 54\%, respectively) as well as increased volatilization (86 versus 32\%, respectively).

Immediate addition of Keyport soil reduced the total penetration and amount bound to skin of BaP by \(\geq 95\%\) versus pure compound (Table 2), a reduction greater than that achieved with Atsion soil (Table 1). The amounts of naphthalene in the same categories were also reduced by \(\geq 24\%\) versus pure compound, a reduction not achieved with Atsion soil. Immediate addition of Keyport soil also reduced the amount of naphthalene appearing in receptor fluid versus pure compound, but to a value significantly greater than that of BaP. Moreover, naphthalene volatilization was reduced to 13.8\% of initial dose by immediate addition of Keyport soil, a
value 2.3 fold lower than that achieved with Atsion soil. As with Atsion soil, more of each compound was retained in skin wash and in soil following immediate addition of Keyport soil with the amount of naphthalene retained in Keyport soil (74.8% of initial dose) 1.6 fold greater than that in Atsion soil. Aging in Keyport soil further reduced amount bound to skin and total penetration amount for each compound; the total penetration achieved for naphthalene (<0.1% of initial dose) was significantly lower than that achieved for BaP (<1.8% of initial dose). As with Atsion soil, aging in Keyport soil did not alter total amounts of BaP in skin wash and soil (>98% initial dose) versus immediate addition to soil. However, aging in versus immediate addition to Keyport soil reduced total amounts of naphthalene in skin wash and soil (9 versus 76%, respectively) as well as increased volatilization (91 versus 14%, respectively).

Table 2. Comparison of Dermal Penetration of Benzo(a)pyrene and Naphthalene Following Contact with Keyport Soil

<table>
<thead>
<tr>
<th>Pure</th>
<th>Immediate</th>
<th>Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor Fluid</td>
<td>B(a)P</td>
<td>Naphthalene</td>
</tr>
<tr>
<td>Bound to Skin</td>
<td>0.2 ± 0.1a</td>
<td>5.1 ± 0.2a</td>
</tr>
<tr>
<td>Total Penetration</td>
<td>75.8 ± 3.2</td>
<td>2.0 ± 0.2b</td>
</tr>
<tr>
<td>Volatilization</td>
<td>76.0 ± 3.2</td>
<td>7.0 ± 0.2b</td>
</tr>
<tr>
<td>Decontamination</td>
<td>24.2 ± 2.2</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Soil</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

a Percent initial dose (mean ± SEM) for n=9-13 replicates per treatment from 3 pigs.
b Significantly different from respective B(a)P treatment (p < 0.05).

Dermal bioavailability is represented by total penetration, which is the sum of chemical penetrating into receptor fluid and amount in skin that potentially can penetrate into receptor fluid with time. Dermal bioavailability factors (total penetration of pure compound/total penetration of compound added immediately to or aged in soil) were calculated to aid in determining dermal exposure limits for each compound in soil (Table 3). The immediate addition of either soil reduced dermal bioavailability of BaP with Keyport being more effective than Atsion soil (21.7 versus 8.9, respectively). On the other hand, immediate addition of Atsion soil had no effect on dermal bioavailability of naphthalene (1.0), while immediate addition of Keyport soil resulted in only a moderate reduction (1.3). Aging in soil was more effective than immediate addition of soil in reducing dermal bioavailability of both pure compounds. Aging in Atsion soil was approximately equally effective for BaP and naphthalene (1.0), while immediate addition of Keyport soil resulted in only a moderate reduction (1.3). Aging in soil was more effective than immediate addition of soil in reducing dermal bioavailability of both pure compounds. Aging in Atsion soil was approximately equally effective for BaP and naphthalene (20.5 versus 23.3 ratio, respectively). On the other hand, aging in Keyport versus Atsion soil was more than twice as effective for BaP (42.2 versus 20.5 ratio) and more than three times as effective for naphthalene (23.3 versus 70 ratio). As a result, the effect of aging in reducing dermal bioavailability of BaP was nearly two-fold; Atsion soil was only slightly more effective than Keyport soil. On the other hand, the effect of aging in reducing dermal bioavailability of naphthalene was 10 to 28 fold greater than for BaP with Keyport two-fold more effective than Atsion soil (53.8 versus 23, respectively).
Table 3. Dermal Bioavailability of Pure Polycyclic Aromatic Hydrocarbons Versus Polycyclic Aromatic Hydrocarbons Added Immediately to or Aged in Soil

<table>
<thead>
<tr>
<th>Soil</th>
<th>Pure/Immediate</th>
<th>Pure/Aged</th>
<th>Effect of Aging (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B(a)P</td>
<td>Naphthalene</td>
<td>B(a)P</td>
</tr>
<tr>
<td>Atsion</td>
<td>8.9(^a)</td>
<td>1.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Keyport</td>
<td>21.7</td>
<td>1.3</td>
<td>42.2</td>
</tr>
</tbody>
</table>

\(^a\) Ratio of percent total penetration for \(n = 9-13\) replicates per treatment from 3 pigs.

\(^b\) Data represent fold decrease with aging compared to immediate treatment.

Volatilization was responsible for substantially reducing (by 91%) the dose of pure naphthalene available for penetration, making it the predominant factor in decreasing total penetration more than ten-fold versus BaP. Although immediate addition of either soil reduced volatilization, retention of naphthalene by soil and appearance in skin wash achieved the same total reduction of dose seen with pure compound. Consequently, immediate addition of soil produced no (Atsion soil) or only a moderate reduction (25%) (Keyport soil) in total naphthalene penetration. Due to greater water (less lipid) solubility than BaP, the majority of naphthalene that penetrated skin appeared in receptor fluid than was bound to skin following treatment with pure compound or immediate addition of soil. On the other hand, aging in either soil not only achieved volatilization losses for naphthalene equal to those of pure compound, but also further reduced naphthalene dose due to retention by soil. This greater total reduction in naphthalene dose (>99%) with aging in either soil resulted in significantly less total penetration and decreased the risk from dermal exposure.

Stronger surface adsorption of naphthalene to the mineral component of clay is indicated by 60% more compound retained by the higher clay content Keyport soil following immediate addition of soil. Clays, which typically have high surface areas, can enhance sorption through weak physical interactions and can impede chemical mass transfer due to clay aggregation and clay interlayers (Ake et al., 2001; Pu et al., 2004). However, naphthalene interactions with either soil were reversible as evidenced following aging when compound volatilized in amounts equal to that which volatilized in the absence of soil. While aging in either soil only accounted for 9-12% of the initial dose, this amount added to that lost due to volatilization and to skin wash was sufficient to significantly reduce total penetration to <0.3%.

Soil adsorption following immediate addition of soil was the predominant factor in decreasing total penetration of BaP by >89% of the pure compound and in reducing the risk from dermal exposure. This decrease in penetration was primarily in amount of compound bound to skin, a site for BaP metabolism to carcinogenic products (Ng et al., 1992). Therefore, soil adsorption of BaP is important in reducing risk from dermal exposure to the compound. Stronger adsorption to the mineral component of clay is indicated by the greater decrease in total penetration (>95%) achieved following immediate contact with the higher clay content Keyport soil, a decrease only achieved with further aging in Atsion soil. On the other hand, a greater additional reduction in total penetration occurred after aging in the higher organic content Atsion soil. These findings are consistent with soil mineral matter acting as an adsorbent in the sorption of nonionic organic compounds in soil and soil organic matter acting primarily as a partition medium (Chiou et al., 2000; Haderlein and Schwarzenbach, 1993; Chiou and Shoup, 1985; Gschwend and Wu, 1985; Karickhoff et al., 1979).
4. CONCLUSIONS

This study revealed that while immediate addition of either soil reduced volatilization of naphthalene, only soil with higher clay content moderately reduced dermal penetration. It was only after aging in soil that naphthalene penetration was reduced 70 fold by soil with higher clay content and 23 fold by soil with higher organic content. On the other hand, BaP dermal penetration was reduced >88% following immediate addition of either soil, with further reductions occurring with aging. Thus, it can be concluded that soil matrix and aging in soil decreased the dermal bioavailability of naphthalene and BaP in quantitatively different ways. The data presented in this paper highlight the need to incorporate bioavailability data into the health risk assessment of dermal exposure to soils contaminated with BaP or naphthalene.

5. REFERENCES


NJDHSS (New Jersey Department of Health and Senior Services) 1998. Hazardous Substance Fact Sheet: Benzo(a)pyrene.


