Supporting Information

Novel Nitro-PAH Formation from Heterogeneous Reactions of PAHs with NO₂, NO₃/N₂O₅, and OH Radicals: Prediction, Laboratory Studies and Mutagenicity

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Synthesis of NPAH standards

Synthesis of standards that were not commercially available was accomplished through direct nitration of the parent PAH with nitric acid in acetic anhydride following conditions provided by Cho et al.\textsuperscript{1} Nitration of benzo[k]fluoranthene provided 7-nitrobenzo[k]fluoranthene\textsuperscript{2}.\textsuperscript{3} and 3,7-dinitrobenzo[k]fluoranthene as major and minor compounds respectively. Nitration of benzo[ghi]perylene provided 7-nitrobenzo[ghi]perylene and 5-nitrobenzo[ghi]perylene.\textsuperscript{4} The authors reported a 60:40 ratio of 5-nitrobenzo[ghi]perylene to 7-nitrobenzo[ghi]perylene.\textsuperscript{4} These compounds were characterized by 1D $^1$H and $^{13}$C NMR, 2D $^1$H-$^1$H Correlation Spectroscopy (COSY), 2D $^1$H-$^{13}$C Heteronuclear Single-Quantam Correlation and Multiple-Bond Correlation (HSQC and HMBC) NMR, Infrared, GCMS, and High Resolution Mass Spectrometry. The structure of 3,7-dinitrobenzo[k]fluoranthene was elucidated using the techniques described above along with 1D Nuclear Overhauser Effect (NOE) NMR spectroscopy.
OH radical Exposure. OH radicals were generated by the photolysis of methyl nitrite (CH$_3$ONO) at wavelength of $> 300$ nm in the presence of added NO.$^{5,6}$

\[
\begin{align*}
\text{CH}_3\text{ONO} + h\nu & \rightarrow \text{CH}_3\text{O} + \text{NO} \\
\text{CH}_3\text{O} + \text{O}_2 & \rightarrow \text{HCHO} + \text{HO}_2 \\
\text{HO}_2 + \text{NO} & \rightarrow \text{OH} + \text{NO}_2
\end{align*}
\]

Approximately 1 ppm of CH$_3$ONO and NO were flushed into the chamber every hour, leading to estimated average OH radical concentration in the chamber of $2 \times 10^7$ molecule cm$^{-3}$ ($\sim$0.8 ppt). The chamber was operated in the flush mode to avoid the build-up of NO$_2$ and HNO$_3$ in the chamber. However, a minor amount of HNO$_3$ was expected to form and could have nitrated the PAHs or possibly catalyzed nitration by NO$_2$. Irradiations were carried out at 20% of the maximum light intensity for 140 minutes.

$\text{NO}_3/\text{N}_2\text{O}_5$ Exposure. The $\text{NO}_3/\text{N}_2\text{O}_5$ exposure was carried out in the dark and NO$_3$ radicals were generated by the thermal decomposition of N$_2$O$_5$.$^{7,8}$:

\[
\text{N}_2\text{O}_5 \rightarrow \text{NO}_2 + \text{NO}_3 \quad (1)
\]

The generated NO$_3$ also reacts with NO$_2$ to form N$_2$O$_5$:

\[
\text{NO}_2 + \text{NO}_3 \rightarrow \text{N}_2\text{O}_5 \quad (2)
\]

Under ambient conditions, NO$_2$, NO$_3$ and N$_2$O$_5$ are present at equilibrium concentrations and the NO$_3$ concentration can be calculated based on the rate constants of reactions (1) and (2).$^9$ One addition of approximately 0.44 and 0.75 ppm of N$_2$O$_5$ and NO$_2$, respectively, was made every hour, with a total of two additions over the entire 165 minutes of exposure, by flushing into the chamber with a stream of N$_2$. The chamber was continually flushed. The amount of NO$_2$ added was proportional to the N$_2$O$_5$ concentration in order to control the NO$_3$ formation.$^8$ This resulted in an estimated average NO$_3$ concentration of $\sim 660$ ppt over the course of exposure.
\textit{NO}_2\textit{ Exposure.} The NO\textsubscript{2} experiment was conducted in the dark and operated with the chamber in the flush mode. NO\textsubscript{2} was generated by oxidation of NO with O\textsubscript{2} and introduced to the chamber. The average NO\textsubscript{2} concentration was \(\sim 4.9\) ppm over the entire 238 minutes of exposure.
**Deuterium Isotope Effect on Mutagenicity**

The results from deuterium isotope effect mutagenicity studies for BaP/BaP-d\textsubscript{12}, 6-NO\textsubscript{2}-BaP/6-NO\textsubscript{2}-BaP\textsubscript{d}_{11}, PYR/PYR-d\textsubscript{10} and 1-NO\textsubscript{2}-PYR/1-NO\textsubscript{2}-PYR-d\textsubscript{9} are shown in Figures SI.9A-D and Figures SI.10A-D. ANOVA analysis was carried out to determine statistical significance of differences between deuterated and non-deuterated pairs. There was no statistically significant deuterium isotope effect (ANOVA, P > 0.05) for the parent BaP and BaP-d\textsubscript{12}, and PYR and PYR-d\textsubscript{10} in the direct acting mutagenicity assay (Figure SI.9A and SI.10A). However, a statistically significant deuterium isotope effect (ANOVA, P < 0.05) was observed for 6-NO\textsubscript{2}-BaP and 6-NO\textsubscript{2}-BaP-d\textsubscript{11}, and 1-NO\textsubscript{2}-PYR and 1-NO\textsubscript{2}-PYR-d\textsubscript{9} (Figures SI.9C and SI.10C). While 6-NO\textsubscript{2}-BaP exhibited a weak direct-acting mutagenicity, the activity of 6-NO\textsubscript{2}-BaP-d\textsubscript{11} was comparable to the background response. However, 1-NO\textsubscript{2}-PYR and 1-NO\textsubscript{2}-PYR-d\textsubscript{9} were mutagenic. In the Salmonella assay without metabolic activation, the metabolism of NPAHs proceeds through nitroreduction to form DNA adducts.\textsuperscript{10} Isomeric NPAHs with lower reduction potentials have been shown to be direct-acting mutagens and their reduction potentials indicate the electron affinity of NPAHs.\textsuperscript{11} A study on unsubstituted PAHs found that the deuterated PAHs had higher reduction potentials.\textsuperscript{12} Therefore, the decreased direct-acting mutagenicity of 6-NO\textsubscript{2}-BaP-d\textsubscript{11}, compared to 6-NO\textsubscript{2}-BaP, may be because of its higher reduction potential, inhibiting the nitroreduction process.

In the Salmonella assay with metabolic activation, no statistically significant deuterium isotope effect was observed for the parent BaP/BaP-d\textsubscript{12} and PYR/PYR-d\textsubscript{10} (ANOVA, P > 0.05) (Figures SI.9B and SI.10B). The S9-mediated metabolism of aromatic compounds were proposed to occur via 1) arene oxidation or 2) nonconcerted addition of an iron(IV) oxyl species.\textsuperscript{13} Both pathways are followed by the so-called “NIH shift”, involving a shift of hydrogen or deuterium to an adjacent position during hydroxylation reaction.\textsuperscript{13}
Because the substitution of deuterium for hydrogen did not result in different mutagenic activity for deuterated and non-deuterated pairs, it suggested that a step prior to the ring oxidation may be the rate-limiting step. However, a statistically significant deuterium isotope effect was observed for 6-NBaP/6-NBaPd_{11} and 1-NO\textsubscript{2}-PYR/1-NO\textsubscript{2}-PYR-d_{9} (ANOVA, P < 0.05) and substitution of deuterium for hydrogen lowered the mutagenicity (Figures SI.9D and SI.10D). It should be noted that, while 6-NO\textsubscript{2}-BaP-d_{11} was not mutagenic in the assays with S\textsubscript{9}, 1-NO\textsubscript{2}-PYR-d_{9} was mutagenic but induced lower colony counts than the non-deuterated analog. In the presence of metabolic activation, more metabolic pathways, including nitroreduction, ring-oxidation followed by nitroreduction, and a ring-oxidation followed by nitroreduction and esterification, can be involved in metabolizing NPAHs in an S9-mediated assay. If the ring oxidation was the only metabolic pathway responsible for converting 6-NO\textsubscript{2}-BaP/6-NO\textsubscript{2}-BaPd_{11}, and 1-NO\textsubscript{2}-PYR/1-NO\textsubscript{2}-PYR-d_{9} to a mutagenic form, the same result as the parent BaP/BaP-d_{12} and PYR/PYR-d_{10} would have been expected. And if the nitroreduction alone was the major metabolic pathway, the deuterium isotope effect would not be expected from 1-NO\textsubscript{2}-PYR/1-NO\textsubscript{2}-PYR-d_{9}, because the deuterium isotope effect was not apparent in the absence of metabolic activation. However, in the case of 6-NBaP/6-NBaPd_{11}, the deuterium isotope effect was observed in both assays (with and without metabolic activation). This suggested that several co-metabolic pathways, possibly selective for each NPAH, may be involved in the metabolism of nitro products when exogenous bioactivation is presence.
Table SI.1: Free energies ($\Delta G_{\text{rxn}}$) of OH-PAH adducts computed using density functional theory (B3LYP) and the 6-31G(d) basis set compared to NPAH isomers identified in a previous gas-phase OH-radical chamber study.

<table>
<thead>
<tr>
<th>Parent PAH</th>
<th>Numbering Scheme</th>
<th>OH-PAH-Adduct $\Delta G_{\text{rxn}}$ (Kcal/mol)</th>
<th>Theoretical NPAH formed in gas phase</th>
<th>Chamber NPAH measured (%yield)\textsuperscript{15}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pyrene</td>
<td><img src="image" alt="Pyrene Numbering Scheme" /></td>
<td><img src="image" alt="Pyrene OH-PAH Adduct" /></td>
<td><img src="image" alt="Pyrene NPAH" /></td>
<td>2-nitropyrene (~0.5%) 4-nitropyrene (~0.06%)</td>
</tr>
<tr>
<td>2. Fluoranthene</td>
<td><img src="image" alt="Fluoranthene Numbering Scheme" /></td>
<td><img src="image" alt="Fluoranthene OH-PAH Adduct" /></td>
<td><img src="image" alt="Fluoranthene NPAH" /></td>
<td>2-nitrofluoranthene (~3%) 7-nitrofluoranthene (~1%) 8-nitrofluoranthene (~0.3%)</td>
</tr>
</tbody>
</table>
**Table SI.2:** Computed dipole moments of NPAHs identified in the chamber studies, using density functional theory (B3LYP) and the 6-31G(d) basis set, and predicted GC retention orders.

<table>
<thead>
<tr>
<th>NPAH</th>
<th>Computed Dipole Moment (Debye)</th>
<th>Predicted Retention order</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-NBaP</td>
<td>4.85</td>
<td>1</td>
</tr>
<tr>
<td>1-NBaP</td>
<td>6.06</td>
<td>2</td>
</tr>
<tr>
<td>3-NBaP</td>
<td>6.16</td>
<td>3</td>
</tr>
<tr>
<td>7-NBkF</td>
<td>4.02</td>
<td>1</td>
</tr>
<tr>
<td>1-NBkF</td>
<td>4.68</td>
<td>2</td>
</tr>
<tr>
<td>8-NBkF</td>
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<td>3-NBkF</td>
<td>5.94</td>
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</tr>
<tr>
<td>9-NBkF</td>
<td>6.61</td>
<td>5</td>
</tr>
<tr>
<td>7-NBghiP</td>
<td>4.51</td>
<td>1</td>
</tr>
<tr>
<td>4-NBghiP</td>
<td>5.75</td>
<td>2</td>
</tr>
<tr>
<td>5-NBghiP</td>
<td>6.03</td>
<td>3</td>
</tr>
</tbody>
</table>
Table SI.3: Estimated percent nitro PAH product formation relative to the amount of unexposed parent PAH. Calculated from \((\Sigma \text{area NPAHs in TIC following exposure})/(\text{area PAH in TIC prior to exposure})\) using EI TICs and normalizing for dilution volumes and amounts injected.

<table>
<thead>
<tr>
<th></th>
<th>NO\textsubscript{2}</th>
<th>NO\textsubscript{3}/N\textsubscript{2}O\textsubscript{5}</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaP-d\textsubscript{12}</td>
<td>90%</td>
<td>41%</td>
<td>20%</td>
</tr>
<tr>
<td>BkF-d\textsubscript{12}</td>
<td>a</td>
<td>30%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>BghiP-d\textsubscript{12}</td>
<td>0%</td>
<td>4%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>DaiP-d\textsubscript{14}</td>
<td>23%</td>
<td>3%</td>
<td>0%</td>
</tr>
<tr>
<td>DalP</td>
<td>19%</td>
<td>4%</td>
<td>0%</td>
</tr>
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</table>

a: unable to determine fraction due to a significant loss during sample preparation
Table SI.4: C-C-N-O dihedral angles of NPAHs computed using density functional theory (B3LYP) and the 6-31G(d) basis set.

<table>
<thead>
<tr>
<th>NPAHs</th>
<th>Angle</th>
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<tbody>
<tr>
<td>1-NBaP</td>
<td>22.4</td>
</tr>
<tr>
<td>3-NBaP</td>
<td>24.7</td>
</tr>
<tr>
<td>6-NBaP</td>
<td>54.7</td>
</tr>
<tr>
<td>1-NBkF</td>
<td>19.7</td>
</tr>
<tr>
<td>3-NBkF</td>
<td>3.6</td>
</tr>
<tr>
<td>7-NBkF</td>
<td>51.4</td>
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<tr>
<td>8-NBkF</td>
<td>24.6</td>
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<tr>
<td>9-NBkF</td>
<td>0.0</td>
</tr>
<tr>
<td>4-NBghiP</td>
<td>26.2</td>
</tr>
<tr>
<td>5-NBghiP</td>
<td>25.1</td>
</tr>
<tr>
<td>7-NBghiP</td>
<td>53.2</td>
</tr>
<tr>
<td>5-NDaiP</td>
<td>55.5</td>
</tr>
<tr>
<td>6-NDaiP</td>
<td>53.7</td>
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</table>
**Figure S1.1.** Overlaid full scan NCI chromatograms of unexposed BaP-d\textsubscript{12} and exposed BaP-d\textsubscript{12} with A) NO\textsubscript{2}, B) NO\textsubscript{3}/N\textsubscript{2}O\textsubscript{5}, and C) OH radicals. Inset chromatograms are “zoomed in” versions of full chromatograms, with largest peak shown offscale. All chromatograms are NCI full scan. A m/z ion in bold indicates a base peak. (--- Unexposed BaP-d\textsubscript{12} , ——Exposed BaP-d\textsubscript{12})

<table>
<thead>
<tr>
<th>A. NO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z 264</td>
</tr>
<tr>
<td>1. 308  6-NBaP-d\textsubscript{11}</td>
</tr>
<tr>
<td>2. 308  1-NBaP-d\textsubscript{11}</td>
</tr>
<tr>
<td>3. 308  3-NBaP-d\textsubscript{11}</td>
</tr>
<tr>
<td>4. 440  ?</td>
</tr>
<tr>
<td>5. 483  ?</td>
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<table>
<thead>
<tr>
<th>B. NO\textsubscript{3}/N\textsubscript{2}O\textsubscript{5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z 264</td>
</tr>
<tr>
<td>1. 308  6-NBaP-d\textsubscript{11}</td>
</tr>
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<td>2. 308  1-NBaP-d\textsubscript{11}</td>
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<tr>
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<tr>
<td>4. 440  ?</td>
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<td>6. 315, 191, 176</td>
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<th>C. OH radicals</th>
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<tbody>
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<td>3. 308, 3-NBaP-d\textsubscript{11}</td>
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<td>4. 440  ?</td>
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Figure S1.2. Overlaid full scan NCI chromatograms of unexposed BkF-d12 and exposed BkF-d12 with A) NO₂, B) NO₃/N₂O₅, and C) OH radicals. Inset chromatograms are “zoomed in” versions of full chromatograms, with largest peak shown offscale. All chromatograms are NCI full scan. A m/z ion in bold indicates a base peak. (--- Unexposed BkF-d12, ----Exposed BkF-d12)

### A. NO₂

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<tr>
<td>1</td>
<td>308</td>
<td>7-NEBF-d₁₁</td>
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<td>3-NEBF-d₁₁</td>
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<tr>
<td>3</td>
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### B. NO₃/N₂O₅

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<td>7-NEBF-d₁₁</td>
</tr>
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### C. OH radicals

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<td>318</td>
<td>1-NEBF-d₁₁</td>
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<tr>
<td>5</td>
<td>318</td>
<td>1-NEBF-d₁₁</td>
</tr>
<tr>
<td>6</td>
<td>292</td>
<td>?</td>
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<tr>
<td>7</td>
<td>427</td>
<td>?</td>
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</table>
Figure SI.3: Free energies ($\Delta G_{\text{rxn}}$) of OH-3-NO$_2$-BkF adduct computed using density functional theory (B3LYP) and the 6-31G(d) basis set.
Figure SI.4. Overlaid full scan NCI chromatograms of unexposed BghiP-d_{12} and exposed BghiP-d_{12} with A) NO\textsubscript{2}, B) NO\textsubscript{3}/N\textsubscript{2}O\textsubscript{5}, and C) OH radicals. Inset chromatograms are “zoomed in” versions of full chromatograms, with largest peak shown offscale. All chromatograms are NCI full scan. A m/z ion in bold indicates a base peak. (---- Unexposed BghiP-d_{12}, —— Exposed BghiP-d_{12})

### A. NO\textsubscript{2}

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<td>?</td>
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### B. NO\textsubscript{3}/N\textsubscript{2}O\textsubscript{5}

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<td>2-N\textsubscript{E}ghiP-d\textsubscript{41}</td>
</tr>
<tr>
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<td>4-N\textsubscript{E}ghiP-d\textsubscript{41}</td>
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<td>332</td>
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### C. OH radicals

<table>
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</tr>
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<tbody>
<tr>
<td>4</td>
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</table>
Figure S1.5. Overlaid full scan NCI chromatograms of unexposed DaiP-d_{14} and exposed DaiP-d_{14} with A) NO_{2}, B) NO_{3}/N_{2}O_{5}, and C) OH radicals. Inset chromatograms are “zoomed in” versions of full chromatograms, with largest peak shown offscale. All chromatograms are NCI full scan. A m/z ion in bold indicates a base peak. (--- Unexposed DaiP-d_{14}, - - - Exposed DaiP-d_{14})

A. NO_{2}

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B. NO_{3}/N_{2}O_{5}

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C. OH radicals

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</table>
Figure SI.6. Overlaid full scan NCI chromatograms of unexposed DalP and exposed DalP with A) NO₂, B) NO₃/N₂O₅, and C) OH radicals. Inset chromatograms are “zoomed in” versions of full chromatograms, with largest peak shown offscale. All chromatograms are NCI full scan. A m/z ion in bold indicates a base peak. ( ⬇️ Unexposed DalP, ⬆️ Exposed DalP)

### A. NO₂

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### B. NO₃/N₂O₅

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### C. OH radicals

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<td>6-NDalP</td>
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</table>
**Figure S1.7:** Dose response profiles of 7-NBkF, 3,7-DNBkF, 5-NBghiP and 7-NBghiP in A. TA98 (-S9) B. TA98 (+S9).

<table>
<thead>
<tr>
<th>Compound</th>
<th>TA 98 (-S9) (\text{rev/nmol})</th>
<th>TA (+S9) (\text{rev/nmol})</th>
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<tr>
<td>7-NO₂-BkF</td>
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<td>&lt; 1</td>
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<tr>
<td>3,7-NO₂-BkF</td>
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<td>513</td>
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<tr>
<td>5-NO₂-BghiP</td>
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<tr>
<td>7-NO₂-BghiP</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
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</table>
**Figure SI.8:** Mean (± standard error) of A. direct- and B. indirect-acting mutagenicities (revertants/nmol) of filter extracts. All extracts were tested in triplicate for mutagenic activity.
Figure S1.9: Mean (±95% confidence interval) direct- and indirect-acting mutagenic activities of BaP vs BaP-d_{12} and 6-NBaP vs 6-NBaP-d_{11}. 

A. 

B. 

C. 

D.
**Figure SI.10**: Mean (±95% confidence interval) direct- and indirect-acting mutagenic activities of PYR vs PYR-d$_{10}$ and 1-NP vs 1-NP-d$_9$.  

A. Direct-acting mutagenic activity (revertants)

B. Indirect-acting mutagenic activity (revertants)

C. Direct-acting mutagenic activity (revertants)

D. Indirect-acting mutagenic activity (revertants)
References