**Global Tracking of Transformation Products of Environmental Contaminants**

**by 2H-labeled Stable Isotope-Assisted Metabolomics**

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**Supplemental Method**

## Reagents and soil

Crude soil (silty loam) was collected at a depth of 0–20 cm from South-Central University for Nationalities, Wuhan, China. Basic properties of the natural crude soil were as follows as our previous report: soil texture, silty loam; soil order: cambisols; pH, 5.7; total organic carbon, 21.3 g/kg; available N, 70.5 mg/kg; available P, 7.83 mg/kg; available K, 55.7 mg/kg 1.

Milli-Q water was obtained from a Millipore purification system (EQ7000, Waters-Millipore Corporation, Milford, MA). Hexane (HEX, HPLC grade), acetone (ACE, HPLC grade), acetonitrile (MeCN, HPLC grade), ammonium acetate (NH4OAc, HPLC grade), methanol (MeOH, HPLC grade), ethyl acetate (EtOAc, HPLC grade), ethoxyethane (ETH, HPLC grade), formic acid (FA, HPLC grade), hydrogen chloride (HCl, 34-37 % solution in water, trace metal grade), anhydrous sodium sulfate (Na2SO4, 99.7%), 5% Pt/C, 10% Pd/C and D2O (99.9%) were purchased from Sigma-Aldrich, St. Louis.

Pyrene (AR grade, 97%), naphthalene (AR grade, 98%), fluorene (AR grade, 97%), anthracene (AR grade, 97%), O-Terphenyl (AR grade, 99%), dichlorvos (AR grade, 97%), naphthalene-*d*8 (CP grade，≥98%), 6PPD (98%, GC grade) were purchased from Aladdin Industrial Co., Ltd.

Atrazine-*d*5 (98%), dichlorvos-*d*6 (98.0%), pyrene-*d*10 (98%), anthracene-*d*10 (98%), and fluorene-*d*10 (98%), were purchased from Cambridge Isotope Laboratories, Tewksbury, MA. Sulfamethoxazole-d4 (99.7%), fluorene-13C6 (98%) and sulfadiazine-13C6 (98%) were purchased from MTAR Research Chemicals Inc., USA. Additionally, atrazine (97%, RHAWN) and sulfamethoxazole (HPLC grade, 98%, Shanghai YUANYE) were purchased from local distributor.

## Quantitative analysis of pyrene and pyrene-*d*10 by GC-MS

Quantitative analysis of pyrene and pyrene-*d*10 in soil extracts was accomplished by Trace 1300 Series GC coupled with TSQ9000EVO triple quadrupole (Thermo) with a TG-5MS GC column (30m x 0.25mm x 0.25μm, Thermo), and anthracene-*d*10 were added into blank and mixed samples as internal standard. The temperature gradient used was as follows: from 60 to 290 ℃ at a heating rate of 8 ℃/min, and from 290 to 300℃ at 30℃/min, finally holding for 15 min. A 1 μL aliquot of the samples was introduced in splitless injection. S/SL temperature, 300℃. The transfer line between the GC and the TSQ9000 was kept at 280℃. The mass spectrometer operated in the electron impact (EI) ionization mode (+70eV) with a source temperature of 280 ℃ in full scan mode with a scan range of 50−650 m/z.

## Untargeted recovery of 8 Isotopologue Pairs (7+6PPD) from Soil Extract

The column was a Thermo HypersilGoldC18 (250 × 4.6 mm, 5 μm). Mass spectrometry was detected via profile mode (scan range, m/z 60−900) with a resolving power of 140 000 fwhm (at m/z 200) and an automatic gain control setting of 3 × 106 with a maximum injection time of 200 ms. The heated electrospray ionization (ESI) source was operated using the following settings: sheath gas flow rate, 40 au; auxiliary gas flow rate, 30 au; spray voltage, 3.8 kV; capillary temperature, 320 oC; aux gas heater temp, 380 oC. The column used for separation was a Thermo HypersilGoldC18 (250 × 4.6 mm, 5 μm).

For untargeted recovery of 7 isotopologue pairs (dichlorvos, atrazine, sulfamethoxazole, naphthalene, pyrene, fluorene, anthracene) from soil extract, following chromatographic condition was used in UPLC-ESI-HRMS analysis: 5 μL of samples was injected into UPLC-ESI-HRMS system. UPLC solvents were A, water in 0.1% FA, and B, MeCN with 0.1% FA. UPLC were performed at 1 mL/min at 25 oC with the following linear gradient (minutes, %B): 0, 5%; 4, 5%; 8, 95%; 26, 95%; 28, 5%; 30, 5%.

For untargeted recovery of 6PPD isotopologue pairs from soil extract experiment, methanol was used as an organic phase in UPLC for a better ionization efficiency in ESI, which is different for the other 7 isotopologue pairs. Following chromatographic condition was used: 5 μL of samples was injected into the UPLC-ESI-HRMS system. UPLC solvents were A, water with 3.8 mM NH4OAc, and B, MeOH. UPLC were performed at 1 mL/min at 25 oC with the following linear gradient (minutes, %B): 0, 10%; 2, 10%; 12, 100%; 22, 100%; 25, 10%; 30, 10%.

Mass spectrum raw data were firstly formatted to .mzXML by ProteoWizard (3.0.20353x86\_64) MSConvertGUI(64-bit) as the following setting: Output format mzXML, Binary encoding precise 64-bit, Write index yes, Use zilib compression yes, TPP compatibility yes, Filter peakPicking vendor msLevel=1-, Filter polarity positive.

For untargeted recovery of 8 isotopologue pairs (7+6PPD) from soil extract, features lists were obtained by MZmine2 as follows:

mzXML files were imported into MZmine2 (2.53) with following key setting: Mass detection, RT auto range, MS level 1, Polarity +, Spectrum type any, centroid model, noise level 5000; ADAP Chromatogram, Min group size in # of scans, 5, Group intensity threshold, 5000, Min highest intensity 5000, m/z tolerance 5 ppm; Chromatogram deconvolution, Wavelets (ADAP), S/N threshold 5, S/N estimator Intensity window SN, min feature height 5000, coefficient/are threshold 50, peak duration range 0-2, RT wavelet range 0-0.4; Isotopic peaks grouper, m/z tolerance 5 ppm, RT tolerance 0.05, Monotonic shape yes, Maximum charge 2, Representative isotope Most intense; Feature list row filters, peak duration range 0.04-5; Retention time calibration, m/z tolerance 5 ppm, RT tolerance 3.0, minmum standard intensity 1.0E5; Join aligner, m/z tolerance 5 ppm, Weight for m/z and RT, 75 and 25, Retention time tolerance 2.5; Duplicate peak filter, NEW AVERAGE, m/z tolerance 5 ppm, RT tolerance, 0.15 min; Feature list rows filter, Minimum peaks in a row, 10; Standard compound normalizer, Nearest standard, Peak measurement type peak height, m/z vs RT balance 1, Standard compounds m/z 172.0978 at 12.55 (fluorene-13C6). For untargeted recovery of 6-PPD isotopologue pairs from soil extract, Standard compounds m/z 257.0798 at 8.90 (sulfadiazine-13C6).

Features information was exported in .csv format with m/z, RT and height information from MZmine2. Then, after removing blank data and adjusting the sequence as follows: Mix1:3 (6 replicates) and Mix3:1 (6 replicates), it was imported into 2H-SIAM(V1.0) with the following setting: Enable F2 and F3, Mass tolerance 7 ppm, RT tolerance 1 min, Number of labeled atom 3-10, Mass difference of atoms between labeled or not (Da) 1.006174, Ratio for F1, F2 and F3, 0.3333, 3 and 3, Tolerance for F1, F2 and F3, 0.3, 0.3 and 0.5.

## GC-MS qualitative study of 2H-labeled 6PPD

A qualitative study of 6PPD, first and third round of H/D exchange products was accomplished by GC-MS (Thermo, Trace 1300, TSQ9000EVO triple quadrupole) coupled with TG-5MS GC column (30m x 0.25mm x 0.25μm, Thermo). The temperature gradient used was as follows: from 60 to 290 ℃ at a heating rate of 8 ℃/min, and from 290 to 300℃ at 30℃/min, finally holding for 15 min. A 1μL aliquot of the samples was introduced in splitless injection. S/SL temperature, 300℃. The transfer line between the GC and the TSQ9000 was kept at 280℃. The mass spectrometer operated in the electron impact (EI) ionization mode (+70eV) with a source temperature of 280 ℃ in full scan mode with a scan range of 50−650 m/z.

## 600M NMR determination of the 6PPD-*d*9 structure

6PPD-*d9* (~2.5 mg) was dissolved in 0.5 mL of DMSO, and the solution was transferred into a 5 mm NMR tube for NMR analysis. NMR analysis was performed on a Bruker Ascend 600 MHz spectrometer equipped with a 5 mm probe head (PADUL 13C). All channels were tuned and matched for each sample before the respective acquisitions, which was performed at room temperature (298 K). The one-dimensional (ID) 1H NMR spectra were collected using the parameters shown below. PULPROG(zg30), TD(32768), NS(8), DS(0), SWH(9014.423 Hz), FIDRES(0.195125 Hz), AQ(2.5625076 sec), RG(128), DW(78.200 usec), DE(6.50 usec), TE(296.3), D1(1.00000000 sec), TD0(1). CHANNEL f1: NUC1(1H), P1(13.10 usec), PL1(1.80 dB), PL1W(8.92857742 W), SFO1(600.1700153 MHz), SI(32768), SF(600.1700153 MHz), WDW(EM), SSB(0), LB(0.30Hz), GB(0).

## Untargeted Recovery of TPs of Pyrene from Soil by 2H-SIAM

UPLC-ESI-HRMS study

For untargeted recovery of TPs of pyrene from the soil, following chromatographic condition was used in UPLC-ESI-HRMS analysis: 5 μL of samples was injected into the UPLC-ESI-HRMS system. UPLC solvents were A, water in 0.1% FA, and B, MeCN with 0.1% FA. UPLC were performed at 1 mL/min at 25 oC with the following linear gradient (minutes, %B): 0, 5%; 4, 5%; 8, 95%; 26, 95%; 28, 5%; 30, 5%.

Mass spectrometry was detected via profile mode (scan range, m/z 60−900) with a resolving power of 140 000 fwhm (at m/z 200) and an automatic gain control setting of 3 × 106 with a maximum injection time of 200 ms. The heated electrospray ionization (ESI) source was operated using the following settings: sheath gas flow rate, 40 au; auxiliary gas flow rate, 20 au; spray voltage, 3.8 kV; capillary temperature, 325 oC.

Mass spectrum raw data were firstly formatted to .mzXML by ProteoWizard (3.0.20353x86\_64) MSConvertGUI(64-bit) as the following setting: Output format mzXML, Binary encoding precise 64-bit, Write index yes, Use zilib compression yes, TPP compatibility yes, Filter peakPicking vendor msLevel=1-, Filter polarity positive.

mzXML files were imported into MZmine2 (2.53) and the features list was obtained by the following models and settings:

Mass detection, RT auto range, MS level 1, Polarity +, Spectrum type any, centroid model, noise level 5000; ADAP Chromatogram, Min group size in # of scans, 5, Group intensity threshold, 5000, Min highest intensity 5000, m/z tolerance 5 ppm; Chromatogram deconvolution, Wavelets (ADAP), S/N threshold 5, S/N estimator Intensity window SN, min feature height 5000, coefficient/are threshold 100, peak duration range 0-2, RT wavelet range 0-0.4; Isotopic peaks grouper, m/z tolerance 5 ppm, RT tolerance 0.05, Monotonic shape yes, Maximum charge 2, Representative isotope Most intense; Feature list row filters, peak duration range 0.04-5; Retention time calibration, m/z tolerance 5 ppm, RT tolerance 3.0, minmum standard intensity 1.0E5; Join aligner, m/z tolerance 5 ppm, Weight for m/z and RT, 75 and 25, Retention time tolerance 2.5; Duplicate peak filter, NEW AVERAGE, m/z tolerance 5 ppm, RT tolerance, 0.15 min; Feature list rows filter, Minimum peaks in a row, 10; Standard compound normalizer, Nearest standard, Peak measurement type peak height, m/z vs RT balance 1, Standard compounds m/z 188.1405 at 16.14 (anthracene-d10).

Features list was exported as .csv format with m/z, RT and height by MZmine2. Then, after removing blank data and adjusting the sequence as follows: Mix1:3 (6 replicates) and Mix3:1 (6 replicates), it was imported into 2H-SIAM(V1.0) with the following setting: Enable F2 and F3, Mass tolerance 7 ppm, RT tolerance 1 min, Number of labeled atom 3-10, Mass difference of atoms between labeled or not (Da) 1.006174, Ratio for F1, F2 and F3, 0.3333, 3 and 3, Tolerance for F1, F2 and F3, 0.3, 0.3 and 0.5.

UPLC-ESI-Q-HRMS study

To further confirm the structures of the target compound, the MS fragment (MS2) was conducted in the mode of higher energy collisional dissociation (HCD) to obtain precursor/daughter ion pairs information. The MS1 precursors were operated in quadrupole mode with a 0.4 m/z isolation window. MS2 fragmentations were scanned with orbitrap at a resolution of 140 000 with a nominal collision energy of 40.

GC-EI-HRMS study

The chromatographic separation was achieved with a TraceGOLD-5MS column (30 m x 0.25 mm x 0.25um film, Thermo) using the following temperature program: 60℃ (holding time 1 min), up to 290℃ at 8℃/min, and up to 310℃ with a rate of 30℃/min (46 min). A 2 μL aliquot of the samples was introduced in splitless injection. The transfer line between the GC and the Q Exactive was kept at 270℃. The mass spectrometer operated in the electron impact (EI) ionization mode (+70eV) with a source temperature of 300℃ in full scan mode with a scan range of 50−750 m/z.

Mass spectral data were acquired in full scan mode (50-750 m/z) and the AGC target was set to 1x106. The resolving power of HRMS is 60 000 fwhm (at m/z 200). Metabolites were ionized using electron impact (EI) with high-energy (70 eV).

Mass spectrum raw data were formatted to .mzXML by ProteoWizard (3.0.20353x86\_64) MSConvertGUI(64-bit) as the following setting: Output format mzXML, Binary encoding precise 64-bit, Write index yes, Use zilib compression yes, TPP compatibility yes, Filter peakPicking vendor msLevel=1-, Filter polarity positive.

mzXML files were imported into MZmine2 (2.53) and the features list was obtained by following models and setting: Mass detection, RT auto range, MS level 1, Polarity +, Spectrum type any, centroid model, noise level 5000; ADAP Chromatogram, Min group size in # of scans, 5, Group intensity threshold, 5000, Min highest intensity 5000, m/z tolerance 5 ppm; Chromatogram deconvolution, Wavelets (ADAP), S/N threshold 5, S/N estimator Intensity window SN, min feature height 5000, coefficient/are threshold 100, peak duration range 0-2, RT wavelet range 0-0.4; Isotopic peaks grouper, m/z tolerance 5 ppm, RT tolerance 0.05, Monotonic shape yes, Maximum charge 2, Representative isotope Most intense; Feature list row filters, peak duration range 0.04-5; Retention time calibration, m/z tolerance 5 ppm, RT tolerance 1.0, minmum standard intensity 1.0E6; Join aligner, m/z tolerance 5 ppm, Weight for m/z and RT, 75 and 25, Retention time tolerance 1; Duplicate peak filter, NEW AVERAGE, m/z tolerance 5 ppm, RT tolerance, 0.1 min; Feature list rows filter, Minimum peaks in a row, 6; Standard compound normalizer, Nearest standard, Peak measurement type peak height, m/z vs RT balance 1, Standard compounds m/z 188.1404 at 18.14 (anthracene-*d*10).

Feature list information was exported in .csv format with m/z, RT and height information from MZmine2. Then, after removing blank data and adjusting the sequence as follows: Mix1:3 (3 replicates) and Mix3:1 (3 replicates), it was imported into 2H-SIAM(V1.0) with the following setting: Mass tolerance 10 ppm, RT tolerance 0.5 min, Number of labeled atom 3-10, Mass difference of atoms between labeled or not (Da) 1.006174, Ratio for F1, F2 and F3, 0.3333, 3 and 3, Tolerance for F1, F2 and F3, 0.3, 0.3 and 0.5.

图表, 箱线图

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Figure S1 Quantification of pyrene and pyrene-*d*10 by GC-MS.Quantitative analysis of pyrene and pyrene-*d*10, from aliquots of samples stored in -80oC and aliquots of samples at the end of the treatments, were determined by GC-MS (Thermo, Trace 1300, TSQ9000EVO triple quadrupole) with a TG-5MS GC column (30 m × 0.25 mm, 0.25 μm, Thermo), and anthracene-*d*10 were added into samples as internal standard.The results are presented at 6 independent biological experiments, and data were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan test, where different letters indicate significant differences at P < 0.05.

图形用户界面, 应用程序

描述已自动生成

Figure S2 GUI interface of 2H-SIAM(V1.0). 2H-SIAM(1.0) was written for Win10, based on Visual Basic .NET.

图形用户界面

低可信度描述已自动生成

Figure S3 Proposed data processing workflow for 2H-SIAM. Raw data from UPLC-ESI-HRMS or GC-EI-HRMS were transformed to .mzXML files and imported into MZmine2 for feature detection, chromatogram builds, deconvolution, align, peak filter and normalization. The features list was exported by MZmine2 and imported into 2H-SIAM(V1.0) for isotopologue pair searching and screening by triple filters. Additionally, from Step3 to Step 6 could be replaced by the other algorithm, for instance, XCMS, XCMS online, MS-DIAL, MetaboAnalyst, et al.

图形用户界面, 应用程序

描述已自动生成

Figure S4 Algorithm for 2H-SIAM(1.0). When a features list (.csv format) is imported into 2H-SIAM(V1.0), it starts with the calculation of means of replicates. Then, it tracks isotopologue pairs with indicated isotope label, m/z and RT tolerance. The potential natural compounds are denoted as M, and the potential isotope-labeled compounds are denoted as M'. The mean intensities of the features M and M' from the Mix1:3 and Mix3:1 samples, that is MMix1:3, MMix3:1, M'Mix1:3 and M'Mix3:1, are used for the calculation of fn. Then 3 defined quantitative filters F1, F2 and F3 are used to track features with isotopologue as potential TPs. For instance, In the case of R1 = 0.33, Tol.1 =0.3, Filter 1 will constitute a range between 0.11 and 1.1. If the calculated f1 follows into this range, the feature pairs will pass this filter, and be recorded in the output file of 2H-SIAM(1.0), and further filters will be further evaluated.



Figure S5 EIC of 3 isotopologue pairs from soil extract. EIC of 3 typical isotopologue pairs from Mix1:3 which contain 10 ppm natural contaminants and 30 ppm 2H-labeled contaminants.



Figure S6 EIC and isotope pattern of isotopologue pairs of M221, M232 and M233 @ 12.8 min (UPLC-ESI-HRMS). Data were extracted from the Mix3:1 sample.



Figure S7 EIC and isotope pattern of isotopologue pairs of M262 and M218 (UPLC-ESI-HRMS). Data were extracted from the Mix3:1 sample.



Figure S8 EIC and isotope pattern of isotopologue pairs of M235 and M225 (UPLC-ESI-HRMS). Data were extracted from the Mix3:1 sample.

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Figure S9 EIC and isotope pattern of isotopologue pairs of M231 and M244 (UPLC-ESI-HRMS). Data were extracted from the Mix3:1 sample.



Figure S10 EIC of isotopologue pairs of M211, M219 and M247 (UPLC-ESI-HRMS).Data were extracted from the Mix3:1 sample.

**图示

描述已自动生成**

Figure S11 Identification of M262 from GC-EI-HRMS data. (a) EIC of isotopologue pair M262 and M'262-*d*8 from Mix1:3; (b) Normalized height of M262 and M'262-*d*8 features used for triple filters of 2H-SIAM(V1.0); (c) Fragments of M262 and M'262-*d*8 extracted from GC-EI-HRMS.

图表

低可信度描述已自动生成

Figure S12 Identification of M218 from GC-EI-HRMS data. (a) EIC of isotopologue pair M218 and M'218-*d*9 from Mix1:3; (b) Normalized height of M218 and M'218-*d*9 features used for triple filters of 2H-SIAM(V1.0); (c) Fragments of M218 and M'218-*d*9 extracted from GC-EI-HRMS.

图表

描述已自动生成

Figure S13 Identification of M232 from GC-EI-HRMS data. (a) EIC of isotopologue pair M232 and M'232-*d*9 from Mix1:3; (b) normalized height of M232 and M'232-*d*9 features used for triple filters of 2H-SIAM(V1.0); (c) fragments of M232 and M'232-*d*9 extracted from GC-EI-HRMS.

图示

描述已自动生成

## Figure S14 EIC of phthalic acid and its possible isotopologue (**UPLC-ESI-HRMS)**.

## Table S1 Comparison of Algorithms for Stable Isotope-Assisted Metabolomics.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Algorithms | Data Processing | Quantitative  Filters | Isotope  Labels | MS Platform | Scans or  features -based\* | GUI Interface | Year | References |
| NTFD | Embedded | 1 | Any | GC-MS | Scan | Yes | 2013 | 2 |
| MzMatch-ISO | XCMS | 1 | Any | LC-MS | Features | Yes | 2013 | 3 |
| ALLocator | XCMS | 1 | 13C | LC-MS | Scan | Yes | 2014 | 4 |
| X13CMS | XCMS | 1 | Any | Any | Features | No | 2014 | 5 |
| Credentialing features | XCMS | 3 | 13C | Any | Features | No | 2014 | 6 |
| HiTIME | Embedded | 1 | Any | LC-MS | Scan | Yes | 2015 | 7 |
| geoRge | XCMS | 1 | Any | Any | Features | No | 2015 | 8 |
| MetExtract II | Embedded | 1 | Any | LC-MS | Scan | Yes | 2017 | 9 |
| 2H-SIAM(V1.0) | Any# | 1 or 3 | Any | Any | Features | Yes | 2022 |  |

\*, refers to compare the intensity of isotopologue pairs based on scans of the mass spectrum or extracted features; #, refers to any data processing algorithms which could provide features list from raw data, for instance, XCMS, MZmine2, MS-DIAL, MetaboAnalyst, and other commercial algorithms.

## Table S2 Functional Parameters in 2H-SIAM(1.0).

|  |  |
| --- | --- |
| Name | Explanation or Proposed Setting |
| Enable F2 and F3 | enable the choice of only 1 filter or 3 filters |
| Mass tolerance (ppm) | mass error for the mass spectrum; QExactive 10, Q-tof 40, Triple quadruple, 1000 |
| RT tolerance (min) | define as ± RT tolerance, 1 or 0.5 min, depending on dataset |
| Number of labeled atoms | Indicate the numbers of isotope labels to be detected |
| Mass difference | defines mass difference of the label to be used, e.g., 13C or 2H. For 2H, it is 1.006174 |
| Ratio of F1, F2, F3 | theoretic ratio for each filter |
| Tolerance of F1, F2, F3 | for F1 and F2, set tolerance at 0.1-0.3; For F3, set tolerance at 0.3-0.5 |

## Table S3 Selected 14 annotated feature pairs (pyrene and 13 TPs) detected by 2H-SIAM pipeline.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Isotopologue pairs | | Detected Ions | |  | Annotation\* |  | | |  | 2H-SIAM and Filters | | | |
| m/z | RT (min) | Height | Name | Theoretic | error | Ion | Confidence  Level | Pair | F1 | F2 | F3 |
| m/z | ppm | 0.1-1.1 | 0.9-10 | 1.5-6 |
| M202 |  | 202.0777 | 18.4 | 1336 | pyrene | 202.0777 | 0.0 | [M]+ | L1 | √ | 1.01 | 3.50 | 2.64 |
|  | M'202-*d*10 | 212.1405 | 17.9 |  | pyrene-*d*10 | 212.1405 | 0.1 | [M]+ |  |  |  |  |  |
| M233 |  | 233.0597 | 13.6 | 464 | 4,5-pyrenequinone | 233.0597 | 0.0 | [M+H]+ | L2a | √ | 0.60 | 6.98 | 4.83 |
|  | M'233-*d*8 | 241.1099 | 13.5 |  | 4,5-pyrenequinone-*d*8 | 241.1099 | -0.1 | [M+H]+ |  |  |  |  |  |
| M233 |  | 233.0597 | 12.8 | 36.6 | 1,6-pyrenequinone | 233.0597 | 0.0 | [M+H]+ | L3 | √ | 0.11 | 1.34 | 4.65 |
|  | M'233-*d*8 | 241.1099 | 12.8 |  | 1,6-pyrenequinone-*d*8 | 241.1099 | -0.1 | [M+H]+ |  |  |  |  |  |
| M221 |  | 221.0598 | 14.6 | 68.4 | 2H-naphtho[2,1,8-def ]chromen-2-one | 221.0597 | 0.4 | [M+H]+ | L2a | √ | 0.31 | 3.08 | 4.57 |
|  | M'221-*d*8 | 229.1100 | 14.4 |  | 2H-naphtho[2,1,8-def ]chromen-2-one-*d*8 | 229.1099 | 0.3 | [M+H]+ |  |  |  |  |  |
| M232 |  | 232.0883 | 19.5 | 15.4 | 1-methoxypyrene | 232.0883 | 0.2 | [M]+ | L2a | √ | 0.32 | 2.37 | 4.28 |
|  | M'232-*d*9 | 241.1447 | 19.1 |  | 1-methoxypyrene-d9 | 241.1448 | -0.2 | [M]+ |  |  |  |  |  |
| M262 |  | 262.0988 | 17.5 | 59.0 | 1,6-dimethoxypyrene | 262.0988 | -0.1 | [M]+ | L2a | √ | 0.38 | 2.30 | 2.94 |
|  | M'262-*d*8 | 270.1491 | 17.3 |  | 1,6-dimethoxypyrene-d8 | 270.1490 | 0.0 | [M]+ |  |  |  |  |  |
| M218 |  | 218.0727 | 14.3 | 402 | 1-hydroxypyrene | 218.0726 | 0.4 | [M]+ | L2b | √ | 0.74 | 6.29 | 4.95 |
|  | M'218-*d*9 | 227.1291 | 14.2 |  | 1-hydroxypyrene-*d*9 | 227.1291 | 0.0 | [M]+ |  |  |  |  |  |
| M219 |  | 219.0805 | 12.5 | 13.6 | pyrene-1,2-oxide | 219.0804 | 0.3 | [M+H]+ | L4 | √ | 0.28 | 2.80 | 1.60 |
|  | M'219-*d*10 | 229.1435 | 12.6 |  | pyrene-1,2-oxide-*d*10 | 229.1432 | 1.3 | [M+H]+ |  |  |  |  |  |
| M235 |  | 235.0754 | 18.3 | 36 | Pyreno[4,5-B:9,10-B']Bisoxirene, 3b,4a,7b,8a-Tetrahydro- | 235.0754 | 0.2 | [M+H]+ | L3 | √ | 1.07 | 9.09 | 3.77 |
|  | M'235-*d*10 | 245.1382 | 17.9 |  | Pyreno[4,5-B:9,10-B']Bisoxirene, 3b,4a,7b,8a-Tetrahydro-*d*10 | 245.1381 | 0.3 | [M+H]+ |  |  |  |  |  |
| M211 |  | 211.0754 | 12.9 | 13.5 | 3,4-dihydroxyphenanthrene | 211.0754 | 0.2 | [M+H]+ | L4 | √ | 0.75 | 1.14 | 1.8 |
|  | M'211-*d*7 | 218.1176 | 12.2 |  | 3,4-dihydroxyphenanthrene-*d*7 | 218.1193 | -7.8 | [M+H]+ |  |  |  |  |  |
| M225 |  | 225.0369 | 13.6 | 94 | phenanthro[4,5-bcd]thiophene, 4-oxide | 225.0369 | 0.2 | [M+H]+ | L3 | √ | 0.54 | 4.31 | 4.84 |
|  | M'225-*d*8 | 233.0871 | 13.5 |  | phenanthro[4,5-bcd]thiophene, 4-oxide-*d*8 | 233.0871 | 0.1 | [M+H]+ |  |  |  |  |  |
| M231 |  | 231.0805 | 12.8 | 125 | 1-pyrenecarbaldehyde | 231.0804 | 0.3 | [M+H]+ | L4 | √ | 0.11 | 1.24 | 4.32 |
|  | M'231-*d*8 | 239.1306 | 12.8 |  | 1-pyrenecarbaldehyde-*d*8 | 239.1307 | -0.2 | [M+H]+ |  |  |  |  |  |
| M247 |  | 247.0754 | 12.1 | 6.9 | 1-pyrenecarboxylic acid | 247.0754 | 0.2 | [M+H]+ | L5 | √ | 0.38 | 1.5 | 1.7 |
|  | M'247-*d*10 | 257.1356 | 12.0 |  | 1-pyrenecarboxylic acid-*d*10 | 257.1381 | -9.8 | [M+H]+ |  |  |  |  |  |
| M244 |  | 244.1121 | 18.4 | 25.9 | 1-ethynylpyrene | 244.1121 | -2.1 | [M+NH4]+ | L3 | √ | 0.88 | 4.66 | 3.18 |
|  | M'244-*d*9 | 253.1685 | 17.9 |  | 1-ethynylpyrene-*d*9 | 253.1686 | 1.2 | [M+NH4]+ |  |  |  |  |  |

\* isomer may exist, as untargeted mass spectrometry is blind to stereochemistry and often regiochemistry; height values are normalized height and given as the mean of Mix1:3 samples; RT, average retention time given by MZmine2 after alignment.

## Table S4 Selected annotated feature pairs detected by GC-EI-HRMS.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Isotopologue pairs | Detected Ions | |  | Annotation\* | | | | | 2H-SIAM and Filters | | | |
| Detected | RT | Height\* | Name | Theoretic | error | Ion | Confidence | Pair | F1 | F2 | F3 | |
| m/z | min | m/z | ppm | Level | 0.1-1.1 | 0.9-10 | 1.5-6 | |
| M202 | 202.0776 | 22.2 | 820 | pyrene | 202.0777 | -0.5 | [M]+ | L1 | √ | 0.94 | 5.53 | 3.34 | |
| M'202-*d*10 | 212.1404 | 22.1 |  | pyrene-*d*10 | 212.1405 | -0.3 | [M]+ |  |  |  |  |  | |
| M220 | 220.0519 | 24.0 | 0.26 | 2H-naphtho[2,1,8-def ]chromen-2-one | 220.0519 | 0.1 | [M]+ | L2a | √ | 0.38 | 2.90 | 3.09 | |
| M'220-*d*8 | 228.1022 | 23.9 |  | 2H-naphtho[2,1,8-def ]chromen-2-one -*d*8 | 228.1021 | 0.5 | [M]+ |  |  |  |  |  | |
| M262 | 262.0989 | 25.5 | 0.06 | 1,6-Dimethoxypyrene# | 262.0988 | 0.3 | [M]+ | L2a | √ | 0.22 | 1.81 | 2.98 | |
| M'262-*d*8 | 270.1491 | 25.5 |  | 1,6-Dimethoxypyrene-*d*8# | 270.1490 | 0.20 | [M]+ |  |  |  |  |  | |
| M218 | 218.0726 | 26.1 | 0.05 | 1-hydroxypyrene# | 218.0726 | -0.1 | [M]+ | L2b | √ | 1.00 | 6.93 | 2.82 | |
| M'218-*d*9 | 227.1293 | 26.1 |  | 1-hydroxypyrene-*d*9 | 227.1291 | 0.8 | [M]+ |  |  |  |  |  | |
| M232 | 232.0884 | 25.3 | 0.01 | 1-methoxypyrene# | 232.0883 | 0.6 | [M]+ | L2a | √ | 0.30 | 2.44 | 3.00 | |
| M'232-*d*9 | 241.1448 | 25.2 |  | 1-methoxypyrene-*d*9 | 241.1448 | 0.17 | [M]+ |  |  |  |  |  | |

\*, isomer may exist; normalized height.

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