Supplementary Material: tobacco-specific and combustion pollutants in settled house dust in Malta

Details of the extraction, cleaning and concentration of PAHs, Nicotine, Nicotelline and TSNAs from settled house dust (SHD).

Extraction of PAHs
Approximately 200 mg of sieved SHD were weighed and placed in a 16 × 125 culture tube. 50 μL of Nicotine-d₄ internal standard and 50 μL of Nicotelline + TSNA internal standard mix were prepared in advance in 0.5 mL 1M H₂SO₄ per sample. Similarly, 50 μL of PAH internal standard mix was prepared in 0.5 mL DCM/Pentane (50:50) per sample. Each dust sample was spiked with the abovementioned internal standard mixes and 1.5 mL of water and 5 mL of DCM/Pentane(50:50) were added. The mix was vortexed for 30 s and sonicated for 30 min at 25 °C. After vortexing for 30 s, the tubes were centrifuged at 3700 rpm for 4 min and then frozen in an acetone-dry ice bath.

The organic phase (this is Fraction 1) was transferred in a 13 × 100 tube and kept for PAHs analysis.

Fraction 1: PAHs
This fraction was blown to dryness at 25 °C under a gentle stream of nitrogen and reconstituted with 500 μL of hexane. 300 mg of anhydrous sodium sulfate as a drying agent were added to an Agilent Bond Elut 10 mL, 1 mg silica column. The silica column was prewashed with 3 mL MeOH followed by 3 mL acetone, conditioned with 3mL hexane/DCM (50:50) followed by 6 mL hexane, loaded with 500 μL of reconstituted extract in hexane and eluted with 6 mL of hexane/DCM (70:30) in a 13 × 100 tube. 200 μL of m-xylene were spiked in the collected eluent and blown down under a gentle stream of nitrogen to a final extract volume of 200 μL which was...
transferred with a glass Pasteur pipette in a GC vial with a 250 μL insert. 2 μL were injected in a GC-MS.

**Extraction of Nicotelline + TSNAs**

After Fraction 1 was removed, 1 mL of 45% potassium carbonate + 5% Na₂EDTA were added to the remaining aqueous phase followed by 8 mL of DCM/Pentane/EtOAc/IPA (40:40:15:5). After vortexing for 1 min and centrifuging at 3700 rpm for 4 min, the two phases were placed in an acetone-dry ice bath.

At this stage the organic phase was split as follows:

**Fraction 2:** In a 13 × 100 tube containing 0.5 mL of 1M H₂SO₄, 3 mL of the organic phase were poured, for **Nicotine** to be extracted from it.

**Fraction 3:** In another 13 × 100 tube containing 0.5 mL of 1M H₂SO₄, the remaining 5 mL of the organic phase were poured, for **Nicotelline + TSNAs** to be extracted from it.

Both Fractions 2 & 3 were vortexed for 1 min, centrifuged at 3700 rpm for 4 min and frozen in an acetone-dry ice bath after which the organic phase was discarded.

**Fraction 2: Nicotine**

The remaining aqueous phase was neutralized with 0.5 mL 50% K₂CO₃/2N NH₄OH and 0.5 mL 90:10 Toluene/Butanol were added. The two phases were vortexed for 1 min, centrifuged at 3700 rpm for 3 min and frozen in an acetone-dry ice bath. The 0.5 mL organic phase was poured in an EDTA deactivated GC vial and injected 2 μL in GC-MS.

**Fraction 3: Nicotelline + TSNAs**

The remaining aqueous phase was neutralized with 0.5 mL 45% K₂CO₃/5% Na₂EDTA and 5 mL 40:40:15:5 DCM/Pent/EtAc/IPA were added. The mixture was vortexed for 1 min, centrifuged at 3700 rpm for 3 min and frozen in an acetone-dry ice bath. The organic phase was poured in a 13 × 100 tube and spiked with 100 μL of 1% HCl in MeOH and dried at 60 °C under a gentle nitrogen flow. Reconstitution of analytes was done with 200 μL 0.1 M ammonium formate in 20% MeOH. 30 μL were injected in
Supplementary Table 1. Characteristics and statistics of districts in Malta

| District | Name          | Population | Area\(^a\) (% | Type                              |
|----------|---------------|------------|----------------|----------------------------------|
| 1        | Northern Harbour | 131,056 | 7.6 | Urban                           |
| 2        | Northern       | 69,467 | 23.3 | Urban background/Rural           |
| 3        | Southern Harbour | 80,170 | 8.3 | Urban/ Harbour                   |
| 4        | Western        | 59,817 | 22.9 | Urban background/Rural           |
| 5        | South Eastern  | 68,044 | 17.2 | Quarries/ industrial/ Trans-shipment hub |

\(^a\)Percentage area occupied by district in relation to the surface area of Malta.

Supplementary Table 2. Precision and accuracy for determination of nicotine, nicotelline, TSNAs and PAHs in analyte spiked clay soil\(^a\) used as QC samples

| Pollutant | LOQ (ng/g) | Actual (ng/g) | Measured (ng/g) | SD (ng/g) | % RSD | % Accuracy | % Recovery |
|-----------|------------|---------------|-----------------|-----------|-------|------------|------------|
| Nicotine  | 1.4        | 1000.0        | 976.9           | 20.7      | 2.1   | -2.3       | 97.7       |
|           |            | 333.3         | 351.3           | 0.5       | 0.1   | 5.4        | 105.4      |
|           |            | 111.1         | 115.2           | 0.5       | 0.4   | 3.7        | 103.7      |
|           |            | 37.0          | 38.0            | 0.3       | 0.7   | 2.7        | 102.7      |
|           |            | 12.3          | 12.4            | 0.0       | 0.3   | 0.3        | 100.3      |
|           |            | 4.1           | 4.3             | 0.1       | 1.5   | 4.8        | 104.8      |
|           |            | 1.4           | 1.2             | 0.0       | 1.6   | -14.5      | 85.5       |
| Nicotelline | 0.36      | 100.0         | 91.4            | 7.6       | 8.3   | -8.6       | 91.4       |
|           |            | 33.3          | 32.1            | 2.6       | 8.3   | -3.7       | 96.3       |
|           |            | 11.1          | 11.8            | 1.2       | 9.9   | 5.8        | 105.8      |
|      | 3.7 | 4.0 | 0.2 | 5.5 | 8.8 | 108.8 |
|------|-----|-----|-----|-----|-----|-------|
| 1.2  | 1.3 | 0.1 | 6.3 | 6.4 | 106.4|
| 0.4  | 0.4 | 0.0 | 6.2 | -4.9| 95.1 |

**NNK** 0.14

|      | 100.0 | 100.3 | 0.4 | 0.4 | 0.3 | 100.3 |
|------|-------|-------|-----|-----|-----|-------|
| 33.3 | 30.4  | 3.7   | 12.3| -8.7| 91.3 |
| 11.1 | 10.9  | 0.4   | 3.5 | -2.2| 97.8 |
| 3.7  | 3.7   | 0.2   | 6.3 | -0.2| 99.8 |
| 1.2  | 1.2   | 0.1   | 9.4 | 0.7 | 100.7|
| 0.4  | 0.4   | 0.0   | 5.8 | 3.5 | 103.5|
| 0.1  | 0.1   | 0.0   | 9.5 | 4.2 | 104.2|

**NNN** 0.02

|      | 100.0 | 79.5  | 18.0 | 22.6 | -20.5| 79.5 |
|------|-------|-------|------|------|------|------|
| 33.3 | 26.9  | 0.6   | 2.2  | -19.3| 80.7 |
| 11.1 | 10.5  | 0.8   | 7.7  | -5.4 | 94.6 |
| 3.7  | 3.8   | 0.2   | 4.8  | 2.5  | 102.5|
| 1.2  | 1.3   | 0.0   | 3.6  | 3.2  | 103.2|
| 0.4  | 0.4   | 0.0   | 7.1  | -5.2 | 94.8 |

**NAB** 1.23

|      | 100.0 | 92.9  | 4.9  | 5.2  | -7.1 | 92.9 |
|------|-------|-------|------|------|------|------|
| 33.3 | 34.1  | 0.9   | 2.6  | 2.3  | 102.3|
| 11.1 | 11.4  | 0.7   | 6.2  | 2.8  | 102.8|
| 3.7  | 3.9   | 0.1   | 2.8  | 4.9  | 104.9|
| 1.2  | 1.2   | 0.1   | 7.1  | 1.0  | 101.0|

**NAT** 0.02

|      | 100.0 | 100.2 | 4.2  | 4.2  | 0.2  | 100.2|
|------|-------|-------|------|------|------|------|
| 33.3 | 33.3  | 0.1   | 0.3  | -0.2 | 99.8 |
| 11.1 | 11.0  | 0.3   | 3.1  | -1.2 | 98.8 |
| 3.7  | 3.7   | 0.0   | 1.0  | 1.2  | 101.2|
| 1.2  | 1.2   | 0.0   | 1.0  | 0.8  | 100.8|
| 0.4  | 0.4   | 0.0   | 2.1  | 1.9  | 101.9|
| 0.1  | 0.1   | 0.0   | 1.2  | -2.0 | 98.0 |
|       | 0.05 | 0.0 | 0.0 | 0.9 | -2.1 | 97.9 |
|-------|------|-----|-----|-----|------|------|
|       | 0.02 | 0.0 | 0.0 | 1.8 | -0.2 | 99.8 |
| Ph    | 0.82 | 200.0 | 172.2 | 23.2 | 13.5 | -13.9 | 86.1 |
|       | 66.7 | 66.1 | 1.4 | 2.1 | -0.9 | 99.1 |
|       | 22.2 | 22.6 | 0.9 | 4.0 | 1.7 | 101.7 |
|       | 7.4 | 6.8 | 0.1 | 0.7 | -8.2 | 91.8 |
|       | 2.5 | 2.5 | 0.2 | 8.6 | 0.6 | 100.6 |
|       | 0.8 | 0.9 | 0.0 | 4.3 | 3.7 | 103.7 |
| An    | 0.09 | 200.0 | 179.6 | 18.1 | 10.1 | -10.2 | 89.8 |
|       | 66.7 | 66.0 | 1.5 | 2.3 | -0.9 | 99.1 |
|       | 22.2 | 23.5 | 0.9 | 4.0 | 5.7 | 105.7 |
|       | 7.4 | 6.8 | 0.3 | 4.6 | -7.6 | 92.4 |
|       | 2.5 | 2.4 | 0.1 | 5.3 | -3.5 | 96.5 |
|       | 0.8 | 0.9 | 0.0 | 2.4 | 8.0 | 108.0 |
| Fluo  | 2.47 | 200.0 | 182.1 | 13.8 | 7.6 | -8.9 | 91.1 |
|       | 66.7 | 65.6 | 0.4 | 0.6 | -1.6 | 98.4 |
|       | 22.2 | 23.6 | 0.2 | 1.0 | 6.4 | 106.4 |
|       | 7.4 | 7.1 | 0.2 | 2.2 | -3.6 | 96.4 |
|       | 2.5 | 2.4 | 0.1 | 2.7 | -1.2 | 98.8 |
| Pyr   | 7.41 | 200.0 | 186.8 | 16.8 | 9.0 | -6.6 | 93.4 |
|       | 66.7 | 66.5 | 4.1 | 6.2 | -0.3 | 99.7 |
|       | 22.2 | 23.9 | 1.2 | 4.9 | 7.5 | 107.5 |
|       | 7.4 | 6.9 | 0.3 | 4.6 | -6.8 | 93.2 |
|       | 2.5 | 2.5 | 0.1 | 2.3 | 1.7 | 101.7 |
| B(a)A | 0.09 | 200.0 | 184.5 | 11.4 | 6.2 | -7.7 | 92.3 |
|       | 66.7 | 64.9 | 2.0 | 3.2 | -2.7 | 97.3 |
|       | 22.2 | 23.9 | 0.4 | 1.7 | 7.4 | 107.4 |
|       | 7.4 | 6.8 | 0.2 | 3.6 | -7.7 | 92.3 |
|       | 2.5 | 2.3 | 0.1 | 4.3 | -4.9 | 95.1 |
|     | 0.3  | 0.3  | 0.0  | 1.1  | 10.7 | 110.7 |
|-----|------|------|------|------|------|-------|
|     | 0.1  | 0.1  | 0.0  | 12.3 | 2.8  | 102.8 |
| Chry| 2.47 | 200.0| 181.73| 18.01| 9.91 | -9.14 |
|     | 66.67| 64.11| 4.64  | 7.23 | -3.84| 96.16 |
|     | 22.22| 24.40| 1.79  | 7.34 | 9.82 | 109.82|
|     | 7.41 | 7.01 | 0.27  | 3.85 | -5.39| 94.61 |
|     | 2.47 | 2.52 | 0.13  | 5.01 | 2.10 | 102.10|
| B(b)F|2.47 | 200.0| 181.5 | 14.6 | 8.0  | -9.2  |
|     | 66.7 | 62.6 | 4.0   | 6.4  | -6.1 | 93.9  |
|     | 22.2 | 23.7 | 1.0   | 4.4  | 6.7  | 106.7 |
|     | 7.4  | 6.9  | 0.2   | 3.4  | -7.2 | 92.8  |
|     | 2.5  | 2.5  | 0.1   | 5.4  | 1.6  | 101.6 |
| B(a)P|0.09 | 200.0| 185.8 | 12.0 | 6.4  | -7.1  |
|     | 66.7 | 64.5 | 3.1   | 4.8  | -3.2 | 96.8  |
|     | 22.2 | 24.2 | 1.6   | 6.6  | 8.9  | 108.9 |
|     | 7.4  | 6.8  | 0.3   | 3.9  | -7.6 | 92.4  |
|     | 2.5  | 2.4  | 0.2   | 9.4  | -1.3 | 98.7  |
| IndP|2.47 | 200.0| 187.1 | 9.9  | 5.3  | -6.5  |
|     | 66.7 | 66.0 | 1.4   | 2.2  | -1.0 | 99.0  |
|     | 22.2 | 26.4 | 2.5   | 9.4  | 18.9 | 118.9 |
|     | 7.4  | 7.0  | 0.2   | 3.4  | -5.8 | 94.2  |
|     | 2.5  | 2.5  | 0.1   | 3.6  | -0.4 | 99.6  |
|     | 0.8  | 1.0  | 0.1   | 13.1 | 15.5 | 115.5 |
| D(ah)A|0.09| 200.0| 195.2 | 4.8  | 2.5  | -2.4  |
|      | 66.7 | 66.0 | 1.6   | 2.4  | -1.0 | 99.0  |
|      | 7.4  | 7.2  | 0.3   | 4.4  | -2.5 | 97.5  |
|      | 2.5  | 2.6  | 0.1   | 3.7  | 5.7  | 105.7 |
|      | 0.3  | 0.3  | 0.0   | 3.3  | -5.1 | 94.9  |
An aqueous solution of nicotelline, nicotine, TSNAs and PAHs containing the specified amounts was added to 200 mg powdered, dried clay soil. Samples were extracted and analyzed as described for the SHD in the experimental section. For all compounds, results are based on the mean of four replicates. LOQ: Limit of quantitation (ng/g); SD: standard deviation; RSD: relative standard deviation.

**Supplementary Table 3. Equations for typical standard curves**

| Pollutant | Concentration range (ng/g) | Slope<sup>a</sup> | Intercept | Correlation coefficient ($r^2$) |
|-----------|---------------------------|-------------------|-----------|-------------------------------|
| Nicotine  | 0-200,000                 | 0.191056          | 0.00698   | 0.9986                        |
| Nicotelline | 0-200                      | 0.35566           | 0.00184   | 0.9982                        |
| NNK       | 0-200                      | 0.206839          | -0.00759  | 0.9992                        |
| NNN       | 0-200                      | 0.144304          | -0.00012  | 1.0000                        |
| NAT       | 0-200                      | 0.133022          | 1.99774   | 0.9993                        |
| NAB       | 0-200                      | 0.786727          | 0.145523  | 0.9976                        |
| Ph        | 0-0-10,000                 | 0.227839          | 0.062935  | 0.9975                        |
| An        | 0-200                      | 0.016556          | 0.004256  | 0.9903                        |
| Fluo      | 0-200                      | 0.037721          | 0.054607  | 0.9979                        |
| Pyr       | 0-200                      | 0.035267          | 0.038995  | 0.9991                        |
| BaA       | 0-200                      | 0.035974          | 0.013190  | 0.9939                        |
| Chry      | 0-200                      | 0.032483          | 0.005760  | 0.9940                        |
| BbF       | 0-200                      | 0.034311          | 0.003298  | 0.9938                        |
| BaP       | 0-200                      | 0.025556          | 0.012023  | 0.9998                        |
|       |    IndP    |    DahA    |   BghiP    |
|-------|-----------|-----------|------------|
|       | 0.030388  | 0.012978  | 0.9998     |
|       | 0.047156  | -0.006188 | 0.9992     |
|       | 0.037242  | 0.013107  | 0.9993     |

*a Equations were determined by linear regression: response ratio = a × (amount ratio) + intercept.*
### Supplementary Table 4. Descriptive statistics for all pollutants (in ng/g) of outdoor deposited house dust collected in Malta

| Pollutant | Valid n | Detection frequency (%) | Mean  | SD    | Min | Max (ng/g) | Q1  | Q2  | Q3  |
|-----------|---------|-------------------------|-------|-------|-----|-----------|-----|-----|-----|
| Nicotine  | 16      | 80                      | 3692  | 11428 | 75  | 46,364    | 213 | 580 | 1414|
| Nicotelline | 20    | 100                     | 2     | 4     | 0   | 17        | 0   | 1   | 2   |
| NNK       | 20      | 100                     | 0.5   | 0.9   | 0.0 | 2.9       | 0.1 | 0.2 | 0.3 |
| NNN       | 20      | 100                     | 0.4   | 1.0   | 0.0 | 4.3       | 0.0 | 0.1 | 0.2 |
| NAT       | 20      | 100                     | 0.2   | 0.4   | 0.0 | 2.1       | 0.0 | 0.0 | 0.1 |
| NAB       | 20      | 100                     | 0.2   | 0.2   | 0.0 | 0.7       | 0.1 | 0.1 | 0.3 |
| Σ TSNA    | 20      | 100                     | 1.3   | 2.3   | 0.1 | 9.9       | 0.2 | 0.5 | 0.9 |
| Ph        | 20      | 100                     | 135   | 81    | 24  | 326       | 82  | 110 | 180 |
| An        | 20      | 100                     | 13    | 10    | 1   | 37        | 6   | 10  | 21  |
| Fluo      | 20      | 100                     | 178   | 124   | 32  | 506       | 83  | 152 | 246 |
| Pyr       | 18      | 90                      | 171   | 110   | 33  | 463       | 104 | 135 | 207 |
| Pollutant | Smoking 20 | Smoking 100 | Q1 | Q2 | Q3 |
|-----------|------------|-------------|----|----|----|
| BaA       | 20         | 100         | 57 | 37 | 5  |
| Chry      | 20         | 100         | 110| 73 | 15 |
| BbF       | 20         | 100         | 87 | 62 | 16 |
| BaP       | 20         | 100         | 78 | 50 | 11 |
| IndP      | 20         | 100         | 74 | 49 | 13 |
| DahA      | 20         | 100         | 18 | 14 | 2  |
| BghiP     | 20         | 100         | 92 | 61 | 14 |
| ΣPAH      | 20         | 100         | 1074| 717| 133 |

Q1, Q2, Q3 are the 25th, 50th and 75th percentiles respectively.

**Supplementary Table 5.** Mann Whitney U test for all pollutants in SHD to check statistical difference across smoking and non-smoking houses

| Pollutant | Smoking 20 | Smoking 100 | Mann-Whitney U | Wilcoxon |
|-----------|------------|-------------|----------------|----------|
| BaA       | 8.00       | 0.00        | 0.00           | 0.00     |
| Chry      | 128.0      | 0.00        | 0.00           | 0.00     |
| BbF       | 88.0       | 0.00        | 0.00           | 0.00     |
| BaP       | 112.0      | 0.00        | 0.00           | 0.00     |
| IndP      | 157.0      | 0.00        | 0.00           | 0.00     |
| DahA      | 51.0       | 0.00        | 0.00           | 0.00     |
| BghiP     | 15.7       | 0.00        | 0.00           | 0.00     |
| ΣPAH      | 51.0       | 0.00        | 0.00           | 0.00     |

Test statistics

| Pollutant | Nicotine | Nicotine | NN | NN | NA | NA | ΣTS | ΣTS | Ph | Ph | An | An | Fluo | Fluo | Pyr | Pyr | BaA | BaA | Chry | Chry | BbF | BbF | BaP | BaP | IndP | IndP | DahA | DahA | BghiP | BghiP | ΣPAH | ΣPAH |
|-----------|----------|----------|----|----|----|----|-----|-----|----|----|----|----|------|------|-----|-----|-----|-----|------|------|-----|-----|-----|-----|------|------|-----|-----|------|------|------|------|
| Mann-Whitney U | 8.00 | 0.00 | 128.0 | 0.00 | 88.0 | 0.00 | 131.0 | 131.0 | 99.5 | 99.5 | 112.0 | 112.0 | 121.0 | 121.0 | 51.0 | 51.0 | 46.0 | 46.0 | 65.0 | 65.0 | 45.0 | 45.0 | 48.0 | 48.0 | 92.0 | 92.0 | 54.0 | 54.0 | 81.0 | 81.0 | 101.0 | 101.0 | 74.0 | 74.0 |
| Wilcoxon  | 74.0   | 0.00   | 869.0 | 0.00 | 873.0 | 0.00 | 829.0 | 829.0 | 840.0 | 840.0 | 853.0 | 853.0 | 226.0 | 226.0 | 106.0 | 106.0 | 101.0 | 101.0 | 143.0 | 143.0 | 111.0 | 111.0 | 103.0 | 103.0 | 158.0 | 158.0 | 90.0 | 90.0 | 172.0 | 172.0 | 192.0 | 192.0 | 179.0 | 179.0 |
Supplementary Table 6. Kruskal-Wallis H test for all pollutants in SHD to check statistical difference across the different districts in Malta

| Test statistics\(^{a,b}\) |
|---------------------------|
| Nicoti | Nicotell | NN | NN | NA | NA | ∑TS | Ph | An | Flu | Pyr | Ba | Chr | Bb | BaP | Ind | Dah | Bgh | ∑PA |

\(^a\)Grouping variable: smoker; \(^b\)not corrected for ties.
|     | ne  | ine  | K     | N     | T     | B     | NA    | o     | A     | y     | F     | P     | A     | iP    | H     |
|-----|-----|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Kruskal-Wallis H | 3.941 | 4.262 | 5.9   | 4.0   | .93   | .99   | 5.579 | 4.1   | 1.7   | 5.4   | 3.9   | 3.1   | 3.8   | 4.8   | 4.5   | 3.5   | 3.39  | 4.55  | 4.06  |
| df  | 4   | 4    | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     |
| Asymp. | 0.414 | 0.372 | 0.2   | 0.4   | 0.9   | 0.9   | 0.233 | 0.3   | 0.7   | 0.2   | 0.4   | 0.5   | 0.4   | 0.3   | 0.3   | 0.4   | 0.49  | 0.33  | 0.39  |
| Sig. | 0.00 | 0.05 | 0.20  | 0.11  | 0.07  | 0.66  | 0.08  | 0.74  | 0.48  | 0.17  | 0.31  | 0.30  | 0.07  | 0.31  | 0.68  | 0.05  | 0.06  | 0.07  |

*Kruskal Wallis test; bGrouping variable: district.

Supplementary Table 7. Spearman rho correlation plot for nicotine, nicotelline, TSNA and PAHs
Supplementary Table 8. Nicotine, $\sum$ TSNA and $\sum$ PAH concentrations (in ng/g) and % load of pollutant in indoor/outdoor and smoker/non-smokers’ SHD

| Place                     | N  | Nicotine (%) | $\sum$ TSNA (%) | $\sum$ PAH (%) |
|---------------------------|----|--------------|-----------------|----------------|
| Indoor                    | 54 | 18246        | 26              | 3169           |
|                           |    | 85.1         | 0.1             | 14.8           |
| Outdoor                   | 20 | 3692         | 1.3             | 1074           |
|                           |    | 77.4         | 0.1             | 22.5           |
| Indoor-smokers            | 16 | 37366        | 40              | 762            |
|                           |    | 97.9         | 0.1             | 2.0            |
| Indoor-non-smokers        | 30 | 864          | 20              | 4256           |
|                           |    | 16.8         | 0.4             | 82.8           |

All concentrations are mean values (in ng/g).