NORMAN Interlaboratory study (ILS) on passive sampling of emerging pollutants

STUDY RESULTS: challenging substances – Steroid hormones, PFOS/PFOA, Bisphenol A, Triclosan

Chemical Monitoring On Site (CM Onsite) organised by NORMAN Association and JRC in support of CIS WFD

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Irstea, freshwater systems, ecology and pollution research unit, Lyon, France

Final Workshop on Norman ILS on passive sampling, 29-30 Oct., Ispra, Italy
Design of the exercise

**Steroid hormones: 15 laboratories**
17-alpha-Estradiol (17 \( \alpha \)E2)
17-alpha-Ethinylestradiol (17 \( \alpha \)EE2)
17-beta-Estradiol (17 \( \beta \)E2)
Estriol (E3)
Estrone (E1)
➔ Cemagref (Irstea) as ref. lab.

**Triclosan (TCL): 8 laboratories**
➔ UK Environment Agency as ref. lab.

**Bisphenol A (BPA): 11 laboratories**
➔ UK Environment Agency as ref. lab.

**Fluorinated surfactants : 9 laboratories**
PFOA, PFOS
➔ European Commission
DG Joint Research Centre as ref. lab.

➔ Campaigns on site : in June-July 2011
Unstability of steroids in water (4°C, white glass containers)

- αEE2 not degraded after 72h
- αE2 et βE2 degraded → E1
Preliminary study on stability for steroids (2)

- Spiking level ~10 ng/L
- Matrix: wastewater treatment plant effluent
- Storage in amber glass containers at -20°C during 7 days
- Defrosting at 4°C

Stability of steroids when waters are frozen and stored in amber glass containers and defrosted at 4°C
WATER SAMPLING PROTOCOL - field samples
Steroid hormones, PFOS/PFOA, Bishpenol A, Triclosan

**FIELD SAMPLES**

**Automatic sampler (on site):**
Collect 100 mL/h x 24h = 2400 ml/day

**Transport to RECETOX:**
Transfer 24h composite water sample every day from 12x1 L autosampler cylinders to a clean 2.5 L amber glass bottle, homogenise and transport on ice to the laboratory

**Filter through Whatman GF/F**

**min. 2000 mL/day**

- **570 mL/day**
  - Triclosan AND Bishpenol A
    - bottle A
      - 2x1 L; glass bottle
      - Store @ 4°C
      - 2000 mL / 7-day composite sample Send weekly to UK EA
    - bottle B
      - 2x1 L; glass bottle
      - Store @ 4°C
      - 2000 mL / 7-day composite sample Send weekly to UK EA

- **570 mL/day**
  - PFOA/PFOS
    - bottle C
      - 2L; Nalgene
      - Store @ 4°C
      - 2000 mL / 7-day composite sample Send weekly to DG JRC IES
    - bottle D
      - 2L; Nalgene
      - Store @ 4°C
      - 2000 mL / 7-day composite BACKUP, store at RECETOX

- **340 mL/day**
  - Steroid hormones
    - bottle E
      - 2x1 L; glass bottle
      - Store @ -20°C
      - 1200 mL / 7-day composite sample Send weekly to Cemagref Lyon store at RECETOX
    - bottle F
      - 2x1 L; glass bottle
      - Store @ -20°C
      - 1200 mL / 7-day composite BACKUP store at RECETOX
WATER SAMPLING PROTOCOL- blank samples
Steroid hormones, PFOS/PFOA, Bishpenol A, Triclosan

BLANK SAMPLES
1000 mL Milliq water/day
Filter through Whatman GF/F

285 ml/day
- bottle Blank A
  2L; glass bottle
  Store @ 4°C
  2000 ml / 7-day composite BLANK
  triclosan and bisphenol A
  Send weekly to UK EA

285 ml/day
- bottle Blank B
  2L; Nalgene
  Store @ 4°C
  2000 ml / 7-day composite BLANK
  PFOS/PFOA
  Send weekly to DG JRC IES

170 ml/day
- bottle Blank C
  1L; glass bottle
  Store @ -20°C
  1200 ml / 7-day composite BLANK
  Steroids
  Send weekly to Cemagref Lyon
Self assessed level of expertise in analysis of target compound groups in passive samplers

<table>
<thead>
<tr>
<th>Laboratory Steroid hormones</th>
<th>Fluorinated surfactants</th>
<th>Triclosan</th>
<th>Bisphenol A</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>A</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>19</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>20</td>
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<td>C</td>
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<tr>
<td>21</td>
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<td>23</td>
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<td>A</td>
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<td>26</td>
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<td>36</td>
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<td>C</td>
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<td>37</td>
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<td>39</td>
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<td>43</td>
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<td>44</td>
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<td>C</td>
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<td></td>
</tr>
<tr>
<td>49</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>50</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A- expert  
B-some experience  
C-limited experience
Categories of participant samplers

<table>
<thead>
<tr>
<th>Sampler</th>
<th>Abbreviation</th>
<th>Steroid hormones</th>
<th>Fluorinated surfactants</th>
<th>Triclosan</th>
<th>Bisphenol A</th>
</tr>
</thead>
<tbody>
<tr>
<td>POCIS pharmaceutical</td>
<td>POCIS</td>
<td>26, 39, 49</td>
<td>29, 37, 39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empore Disk</td>
<td>ED</td>
<td>19</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>POCIS, pesticide</td>
<td>POCIP</td>
<td>33</td>
<td>23, 39, 45</td>
<td></td>
<td></td>
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<tr>
<td>Silicone rubber material</td>
<td>SR</td>
<td>43</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Speedisks</td>
<td>SPEED</td>
<td>43</td>
<td></td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Modified POCIS</td>
<td>POCIM</td>
<td>36</td>
<td>19, 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard SPMD (length 1m)</td>
<td>SPMD</td>
<td>23</td>
<td>23, 23</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Polyoxymethylene</td>
<td>POM</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Low density polyethylene</td>
<td>LDPE</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results for steroid hormones
Example: 17 beta Estradiol

**QC**: the median and geometric mean of participant within the uncertainty range stated by the central laboratory

- **Provided sampler, ng/cm²**
  - **Median**: 0.035
  - **s**: 0.099
  - **Geomean**: 0.060
  - **n**: 6
  - **Outliers**: 0

- **Participant sampler, Cw in ng/L**
  - **Median**: 1.33
  - **s**: 5.4
  - **Geomean**: 0.66
  - **n**: 4
  - **Outliers**: 0

- **Spot samples**
  - **Period 1**: 0.54
  - **Period 2**: 0.58

- **LOD**

**Laboratory number**

- **Set value**
- **Expanded Uncertainty (k = 2)**
- **2 SD of log₂ transformed data**
- **Outliers**

**Example**: 17 beta Estradiol

- **QC**: the median and geometric mean of participant within the uncertainty range stated by the central laboratory

- **Only 4 lab for participant sampler (in ng/L)**

- **TWAC < LOQ**

Laboratory number

- **Standard solution, µg/mL**
- **Median**: 0.0204
- **s**: 0.011
- **Geomean**: 0.022
- **n**: 13
- **Outliers**: 2
- **s excl. outl**: 0.0050
- **Refvalue**: 0.02
- **Exp. Unc**: 0.00

**Log₂ scale**

- **Set value expanded Uncertainty (k = 2)**
- **2 SD of log₂ transformed data**
- **Outliers**

**Water sample mean**
Example 17 beta Estradiol – Samplers comparison

**Uptake (ng/cm²)**

- **Provided sampler**
  - 2
  - 1
  - 0.5
  - 0.25
  - 0.13
  - 0.063
  - 0.031
  - 0.016
  - 0.0078
  - 0.0039
  - 0.002

- **Participant sampler**
  - 2
  - 1
  - 0.5
  - 0.25
  - 0.13
  - 0.063
  - 0.031
  - 0.016
  - 0.0078
  - 0.0039
  - 0.002

**Cw (ng/L)**

- **Provided sampler**
  - 64
  - 32
  - 16
  - 8
  - 4
  - 2
  - 1
  - 0.5
  - 0.25
  - 0.13
  - 0.063
  - 0.031
  - 0.016
  - 0.0078
  - 0.0039
  - 0.002

- **Participant sampler**
  - 64
  - 32
  - 16
  - 8
  - 4
  - 2
  - 1
  - 0.5
  - 0.25
  - 0.13
  - 0.063
  - 0.031
  - 0.016
  - 0.0078
  - 0.0039
  - 0.002

**LOD**

Composite water sample (2 per exposure)
Standard solution

- 4 replicates of sample injection to the instrumental system
- Injections spread over the analysis sequences (at least 4 other sample injections are made between individual injections of this solution)

- Reference concentration of steroids within the range comprised by the participant results (median + 2 standard deviations excluding outliers)
- Except for 17-αEE2, the median and geometric mean of participant results were within the uncertainty range stated by the central laboratory.
- Outlier results were reported by 3 laboratories (20, 23 and 36).
Provided sampler (= POCIS pharm without PRC)

Median value expanded uncertainty \( (k = 2) \)

Analysis of triplicates of POCIS exposed for 14 d

Conc. in field blank close to LOD \(< 10\% \text{ Conc. in exposed samplers} \)

Because of very low water concentration (see table right), < 6 lab. were able to measure steroids (except estrone) above their LOQs in provided samplers

<table>
<thead>
<tr>
<th>Sample/Compound</th>
<th>(bottle C1)</th>
<th>(bottle C2)</th>
<th>(bottle E1)</th>
<th>(bottle E2)</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-alpha-Estradiol</td>
<td>&lt;1.30</td>
<td>&lt;0.90</td>
<td>&lt;1.05</td>
<td>&lt;0.90</td>
<td>ng/L</td>
</tr>
<tr>
<td>17-alpha-Ethinylestradiol</td>
<td>&lt;17.25</td>
<td>&lt;14.2</td>
<td>&lt;10.00</td>
<td>&lt;11.98</td>
<td>ng/L</td>
</tr>
<tr>
<td>17-beta-Estradiol</td>
<td>0.70</td>
<td>&lt;0.45</td>
<td>0.50</td>
<td>0.58</td>
<td>ng/L</td>
</tr>
<tr>
<td>Estriol</td>
<td>&lt;2.90</td>
<td>&lt;2.75</td>
<td>&lt;7.45</td>
<td>&lt;8.33</td>
<td>ng/L</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;1.10</td>
<td>&lt;0.85</td>
<td>&lt;0.85</td>
<td>&lt;0.73</td>
<td>ng/L</td>
</tr>
</tbody>
</table>

Water concentrations
Participant sampler, Cw

Median value expanded uncertainty ($k = 2$)

Weekly spot samples

LOD in spot samples

ng/L

Median value expanded uncertainty ($k = 2$)

0 outliers

ng/L

Weekly spot samples

LOD in spot samples

n=2  n=3  n=4  n=1  n=8

17αE2  17αEE2  17βE2  E3  E1
Ratio of water concentrations provided / participant sampler

\[ \frac{C_{w(NPS)}}{C_{w(PPS)}} \]

NPS – provided passive sampler; PPS – participant passive sampler
# Variability of reported results (excluding outliers)

<table>
<thead>
<tr>
<th>Steroid hormones</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within laboratory</td>
</tr>
<tr>
<td></td>
<td>Min.</td>
</tr>
<tr>
<td>Variability:</td>
<td></td>
</tr>
<tr>
<td>Matrix analysed:</td>
<td></td>
</tr>
<tr>
<td>Standard solution</td>
<td>11%</td>
</tr>
<tr>
<td>NPS amount</td>
<td>53%</td>
</tr>
<tr>
<td>NPS water concentration</td>
<td>48%</td>
</tr>
<tr>
<td>Provided sampler</td>
<td></td>
</tr>
<tr>
<td>PPS amount</td>
<td>3%</td>
</tr>
<tr>
<td>PPS water concentration</td>
<td>3%</td>
</tr>
<tr>
<td>Participant sampler</td>
<td></td>
</tr>
</tbody>
</table>

NPS – provided passive sampler; PPS – participant passive sampler

- High variabilities because concentrations in passive samplers close to LOQs
- Analysis of steroids in complex environmental matrixes is challenging
Variability of reported results

<table>
<thead>
<tr>
<th>NPS – provided passive sampler; PPS – participant passive sampler</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Standard solution</th>
<th>NPS amount</th>
<th>NPS water concentration</th>
<th>PPS amount</th>
<th>PPS water concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-alpha-Estradiol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-alpha-Ethinylestradiol</td>
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</tr>
<tr>
<td>17-beta-Estradiol</td>
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<td></td>
</tr>
<tr>
<td>Estriol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Within laboratory Mean vs. Between laboratory
Conclusions for steroids

- Standard solution:
  - Acceptable variability with exception of 17-α-EE2 (between lab. variability of 53%)
  - Instrumental methods was not expected to cause excessive variability in reported data

- Analysis of steroids in passive samplers = a challenge
  - High within laboratory variability explained by concentrations close to LOQs + matrix effect + non-homogeneity of sampling

- A direct comparison of passive sampling data with spot sampling not possible since spot sample data below LOQ. However, no contradiction between passive sampling and spot sampling results

- Passive sampling method allows measurement of concentrations lower than spot sampling but the interlaboratory precision is not sufficient
Results for PFOS and PFOA
Example:

**PFOS**

- **QC**: the median and geometric mean of participant not within the uncertainty range stated by the central laboratory (not for PFOA)

* Only 2 lab for participant sampler (in ng/L) passive sampler calibration is scarce (no calibration data available)
Example PFOS - Samplers comparison

Uptake (ng/cm²)

Cw (ng/L)

Water sample mean > 7 ng/L

LOD
**Sample/Compound** | **Bottle C1 (20.6.-26.6.)** | **Bottle C2 (27.6.-4.7.)** | **units**
--- | --- | --- | ---
PFOA | 27.5 | 36.0 | ng/L
PFOS | 5.7 | 8.5 | ng/L
Variability of reported results

<table>
<thead>
<tr>
<th>Fluorinated surfactants</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within laboratory</td>
</tr>
<tr>
<td></td>
<td>Min.</td>
</tr>
<tr>
<td>Variability: Matrix analysed:</td>
<td></td>
</tr>
<tr>
<td>Standard solution</td>
<td>2%</td>
</tr>
<tr>
<td>NPS amount</td>
<td>15%</td>
</tr>
<tr>
<td>NPS water concentration</td>
<td>5%</td>
</tr>
<tr>
<td>PPS amount</td>
<td>18%</td>
</tr>
<tr>
<td>PPS water concentration</td>
<td>20%</td>
</tr>
</tbody>
</table>

NPS – provided passive sampler; PPS – participant passive sampler

- An excellent within laboratory variability of analysis of individual compounds in standard solution (2%)

- In provided samplers for sampler uptake: a good within laboratory variability (15 to 25%) and acceptable between variability (<51%)

- In all samplers: no possible to evaluate a between laboratory variability for water concentration (n=2)
Variability of reported results

**PFOA**

- Within laboratory Mean
- Between laboratory

**PFOS**

- Within laboratory
- Between laboratory
Results for BPA and TCL
**Example:**

**BPA**

* QC : the median and geometric mean of participant > reference value (idem for TCL)

- Participant sampler (in ng/L) :
  - Only 3 lab, because calibration is scarce (no calibration data available)
  - median value < LOD < water sample mean

---

![Chart showing standard solution, provided sampler, and participant sampler results](chart.png)

**Spot samples**
- Period 1: 198
- Period 2: 171
- LOD: 75

**Participant Sampler**
- Cw in ng/L
  - Median: 4.8
  - s: 10.9
  - GeomMean: 5.5
  - n: 3
  - Outliers: 0

**Provided Sampler**
- Uptake ng/cm2
  - Median: 6.4
  - s: 7.7
  - GeomMean: 3.9
  - n: 6
  - Outliers: 0

**Standard solution, µg/mL**
- Median: 0.258
- s: 0.29
- Geomean: 0.29
- n: 6
- Outliers: 0

**Composite water sample**
- (2 per exposure)
- mean value

> provided sampler, ng/cm2

> participant sampler, Cw in ng/L

> composite water sample

> water sample mean

> outlier colour

> sampler type

> repeatability (± 2 × SD)

> median

> - 2 × stand. dev. of log₂ transf. data

> + expanded uncertainty with k = 2

> reference value

> - 2 × stand. dev. of log₂ transf. data

> - expanded uncertainty with k = 2

> provided sampler, uptake ng/cm²
Example BPA - Samplers comparison

Composite water sample
(2 per exposure)

Uptake (ng/cm²)

Cw (ng/L)

Provided sampler
Participant sampler

Example BPA - Samplers comparison

Composite water sample
(2 per exposure)

Uptake (ng/cm²)

Cw (ng/L)

Provided sampler
Participant sampler

LOD

POM

ED
Standard solution, µg/mL

Provided sampler, ng/cm²

Participant sampler, Cw in ng/L

Water composite samples

<table>
<thead>
<tr>
<th>Sample/Compound</th>
<th>Bottle A1 (20.6.-26.6.)</th>
<th>Bottle A2 (27.6.-4.7.)</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclosan</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>ng/L</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>206</td>
<td>122</td>
<td>ng/L</td>
</tr>
</tbody>
</table>
# Variability of reported results

<table>
<thead>
<tr>
<th>Compound:</th>
<th>Bisphenol A</th>
<th>Triclosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variability:</td>
<td>Coefficient of variation (%)</td>
<td>Coefficient of variation (%)</td>
</tr>
<tr>
<td>Matrix analysed:</td>
<td>Within laboratory</td>
<td>Between laboratory</td>
</tr>
<tr>
<td>Mean</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Standard solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provided sampler</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPS amount</td>
<td>8%</td>
<td>1%</td>
</tr>
<tr>
<td>NPS water concentration</td>
<td>14%</td>
<td>5%</td>
</tr>
<tr>
<td>Participant sampler</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPS amount</td>
<td>31%</td>
<td>10%</td>
</tr>
<tr>
<td>PPS water concentration</td>
<td>33%</td>
<td>6%</td>
</tr>
</tbody>
</table>

NPS – provided passive sampler; PPS – participant passive sampler

- A good within laboratory variability in standard solution (mean CV at 8 and 3% for BPA and TCL)
- In provided samplers for sampler uptake: a good within laboratory variability (mean CV at 19 and 15% for BPA and TCL) and relatively high between variability (>98%)
- In all samplers: no reasonable to evaluate a between laboratory variability for water concentration (n= 3 for BPA, 2 pour TCL)
Variability of reported results

**Bisphenol A**

**Triclosan**
Conclusions for PFOS/PFOA, BPA and TCL

- Few results on TWA concentration in water - passive sampler calibration is scarce (no calibration data available)

- In provided samplers for sampler uptake (ng/cm$^2$):
  - For perfluorinated compounds - a good within laboratory variability (15 to 25%) and acceptable between variability (<51%)
  - For BPA and TCL - a good within laboratory variability (mean CV at 19 and 15% for BPA and TCL) and relatively high between variability (>98%)
Thank you for your attention
Example: Estrone

### Standard Solution, µg/mL

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>0.0217</td>
</tr>
<tr>
<td>s</td>
<td>0.021</td>
</tr>
<tr>
<td>Geomean</td>
<td>0.022</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
</tr>
<tr>
<td>Outliers</td>
<td>2</td>
</tr>
<tr>
<td>s excl. outl</td>
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<tr>
<td>Refvalue</td>
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<td>Exp. Unc</td>
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### Provided Sampler uptake, ng/cm²

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Median</td>
<td>0.053</td>
</tr>
<tr>
<td>s</td>
<td>0.084</td>
</tr>
<tr>
<td>Geomean</td>
<td>0.063</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
</tr>
<tr>
<td>Outliers</td>
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</tr>
<tr>
<td>s excl. outl</td>
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</table>

### Participant Sampler, Cw in ng/L

<table>
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<tr>
<th>Metric</th>
<th>Value</th>
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<tbody>
<tr>
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<tr>
<td>s</td>
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<td>GeomMean</td>
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<td>n</td>
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</tr>
<tr>
<td>Outliers</td>
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</tr>
</tbody>
</table>

Spot samples:
- Period 1
- Period 2
- LOD

Composite water sample (2 per exposure)

Repeatability (± 2 × SD)

Mean value

Outlier colour

Provided sampler type

Reference value

Expanded uncertainty with k = 2

+ 2 × stand. dev. of log₂ transf. data

Median

- 2 × stand. dev. of log₂ transf. data

+ expanded uncertainty with k = 2

Median

- expanded uncertainty with k = 2

Estrone
Estrone - Samplers comparison

**Uptake in ng/cm²**

<table>
<thead>
<tr>
<th>SPEED</th>
<th>SR</th>
<th>POM</th>
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<td>1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
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**Cw in ng/L**

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Example:
17-alpha-Ethinylestradiol
17-alpha-Ethinylestradiol - samplers comparison

**Uptake in ng/cm²**

**Cw in ng/L**

- **Provided sampler**
- **Participant sampler**

- POM