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Final report

# Integrated strategy for the assessment of aquatic and terrestrial bioaccumulation

## Supporting Information

by:

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On behalf of the German Environment Agency

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# 1 Substance data endpoints

## 1.1 O-terphenyl

**Table 1: Tier 3 – OECD TG 321 HYBIT BCF - o-terphenyl**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Literature |
| Source detail | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 |
| Comment | Male *H. azteca* | Female *H. azteca* |
| Results | | |
| $BCF_k$ | 2617 | 5636 |
| $BCF_{SS}$ | 2619 | 4539 |
| $BCF_{kL}$ | 10145 (5% lipid)<br>6086 (3% lipid) | 11549 (5% lipid)<br>6929 (3% lipid) |
| $BCF_{SSL}$ | 10149 (5% lipid)<br>6090 (3% lipid) | 9300 (5% lipid)<br>5580 (3% lipid) |
| Comment | Co-exposure with HCB | Co-exposure with HCB |
| Validity criteria (TG 321) | | |
| Water temp. | N/A | N/A |
| Dissolved $O_2$ | N/A | N/A |
| TWA stable | Yes | N/A |
| Water conc. < solubility | Yes | Yes |
| Mortality | N/A | N/A |
| [NPOC] | N/A | N/A |
| Comment | Study was performed prior to TG development<br><br>Selected for data comparison | Study was performed prior to TG development<br><br>Not selected for data comparison (female *H. azteca*) |

**Table 2: Tier 3 – OECD TG 319 A/B IVIVE BCF - o-terphenyl**

| | | Value 1 |
|---|---|---|
| | Source | Literature |
| | Source details | Fay et al. 2014 |
| | Comment | Laboratory comparison data |

| | | Value 1 |
|---|---|---|
| | Hep. materials | |
| | Donor species | Rainbow trout |
| | Hepatic material | Cryopreserved hepatocytes |
| | Protein or cell concentration | 2 x $10^6$ cells/mL |
| | Cell viability | > 80% |
| | [Characterization of S9 / HEP] | Yes (as fresh hepatocytes) |
| | Test setup | |
| | Incubation temperature | 11°C |
| | Assay duration | 240 min |
| | Start conc. | 2µM |
| | Incubation volume | 1mL |
| | Inactive controls | HI-Hepatocytes |
| | Inactive controls < 20% loss? | Yes |
| | Time points sampled | 7 |
| | Comment | |
| | Results | |
| | Log-linear? | No |
| | Rates | N/A |
| | Replicates | 2 x 3 replicates |
| | IVIVE BCF | N/A |
| | IVIVE model | N/A |
| | Comment | Selected for data comparison |

**Table 3: Tier 4 – OECD TG 305 fish in vivo BCF - o-terphenyl**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Registration data | Literature | Literature |
| Source details | ECHA registration dossier EC number 262-967-7 | Böhm et al. 2017 | Schlechtriem et al. 2019 |
| Comment | | | Not primary source, CERI et al. 1992 |
| Results | | | |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| $BCF_k$ | | 12040 | |
| $BCF_{SS}$ | | N/A | Log BCF 2.7 (≙ 501)<br>Log BCF 3.5 (≙ 3162) |
| $BCF_{kL}$ | 12882 | 10060 | |
| $BCF_{SSL}$ | | N/A | |
| Comment | Bluegill fish (Lepomis macrochirus<br>Growth corrected $BCF_{kL}$ | Rainbow trout | Common carp |

## 1.2 Methoxychlor

**Table 4: Tier 3 – OECD TG 321 HYBIT BCF - methoxychlor**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 |
| Comment | Male *H. azteca* | Mixed sex *H. azteca* | Male *H. azteca* |
| Results | | | |
| $BCF_k$ | 7830 | 18471 | 6205 |
| $BCF_{SS}$ | 5170 | 7983 | 6663 |
| $BCF_{kL}$ | 15660 (5% lipid)<br>9396 (3% lipid) | 28329 (5% lipid)<br>16997 (3% lipid) | N/A |
| $BCF_{SSL}$ | 10340 (5% lipid)<br>6204 (3% lipid) | 12244 (5% lipid)<br>7346 (3% lipid) | N/A |
| Comment | Co-exposure with B[a]P | Co-exposure with B[a]P | $^{14}C$-exposure, TRR data, no lipid data |
| Validity criteria (TG 321) | | | |
| Water temp. | N/A | N/A | N/A |
| Dissolved $O_2$ | N/A | N/A | N/A |
| TWA stable | N/A | N/A | N/A |
| Water conc. < solubility | Yes | Yes | Yes |
| Mortality | N/A | N/A | N/A |
| [NPOC] | N/A | N/A | N/A |
| Comment | Study was performed prior to TG development | Study was performed prior to TG development | Study was performed prior to TG development |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| | Selected for data comparison | Not selected for data comparison (mixed sex *H. azteca*) | Not selected for data comparison (no lipid normalization; TRR-data) |

**Table 5: Tier 3 - OECD TG 319 A/B IVIVE BCF - methoxychlor**

| | Value 1 | Value 2 | Value 3 | Value 4 |
|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature |
| Source details | Fay et al. 2014 | Nichols et al. 2018a | Nichols et al. 2018a | Lee et al. 2022 |
| Comment | Laboratory comparison data | Ring trial data | Ring trial data | |
| **Hep. material** | | | | |
| Donor species | Rainbow trout | Rainbow trout | Rainbow trout | Rat |
| Hepatic material | Cryopreserved hepatocytes | Cryopreserved hepatocytes | Hepatic S9 | S9 |
| Protein or cell concentration | 2 x $10^6$ cells/mL | 2 x $10^6$ cells/mL | 1 mg/mL | 1 mg/mL |
| Cell viability | > 80% | > 80% | / | - |
| [Characterization of S9 / HEP] | Yes (as fresh hepatocytes) | Yes | Yes | Yes |
| **Test setup** | | | | |
| Incubation temperature | 11°C | | | 37°C |
| Assay duration | 240 min | 240 min | 240 min | 7 min |
| Start conc. | 2µM | 0.32 µM | 0.32 µM | 0.5 µM |
| Incubation volume | 1mL | 1mL | 1mL | 4 mL |
| Inactive controls | HI-Hepatocytes | HI-Hepatocytes | HI-S9 | HI-S9 |
| Inactive controls < 20% loss? | Yes | Yes | Yes | Yes |
| Time points sampled | 7 | 7 | 7 | 7 |
| Comment | | | | |
| Results | | | | |
| Log-linear? | Yes | Yes | Yes | Yes |
| Rates (± SD) | $CL_{in\ vitro,\ int}$: 0.065 ± 0.038 mL/h/$10^6$ cells. | $CL_{in\ vitro,\ int}$: 0.08 ± 0.02 mL/h/$10^6$ cells. | $CL_{in\ vitro,\ int}$: 0.32 ± 0.03 mL/h/mg protein | $k_{met}$: 0.252 ± 0.00478 SE $h^{-1}$ |
| Replicates | 3 x 3 | | | |

| | Value 1 | Value 2 | Value 3 | Value 4 |
|---|---|---|---|---|
| IVIVE BCF (± SD) | 4195 ± 535 | 3835 ± 398 ($f_U$=$f_U$, P/$f_{U,HEP}$)<br>446 ± 84 ($f_U$ = 1) | 3359 ± 113 ($f_U$=$f_U$, P/$f_{U,S9}$)<br>423 ± 18 ($f_U$ = 1) | BMF: 0.012 ± 0.00073 SE kg lipid/kg |
| IVIVE model | Nichols et al. 2013 | Nichols et al. 2013 | Nichols et al. 2013 | Lee et al. 2022 IVIVE-B model |
| Comment | Laboratory comparison data<br>BCF data taken from literature<br>Selected for data comparison | Ring trial data<br>BCF data taken from literature<br>Selected for data comparison | Ring trial data<br>BCF data taken from literature<br>Selected for data comparison | Selected for data comparison |

**Table 6: Tier 4 - OECD TG 305 fish in vivo BCF - methoxychlor**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Thesis Ina Bischof, 2018 | Thesis Ina Bischof, 2018 | UNEP 2020 |
| Comment | | | Draft Risk Profile (POPS) |
| **Results** | | | |
| $BCF_k$ | | N/A | 667 – 8300 |
| $BCF_{SS}$ | 2686 ± 1260<br>3854 ± 1158 (high steady-state values) | 1188 ± 207 | |
| $BCF_{kL}$ | | N/A | |
| $BCF_{SSL}$ | 1479 ± 694<br>2122 ± 638 (high steady-state values) | 621 ± 108 | |
| Comment | Rainbow trout<br>Unsteady uptake pattern | Common Carp<br>Decline on body burden during uptake | Different experiments and fish species |
| **Validity criteria (TG 305)** | | | |
| Water temp. | | | |
| Dissolved $O_2$ | | | |
| TWA stable | No, peak at start of exposure | No, peak at start of exposure | |
| Water conc. < solubility | | | |
| Mortality | | | |
| [NPOC] | | | |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Comment | | | |

## 1.3 β-HCH (β- hexachlorocyclohexane)

**Table 7: Tier 3 - OECD TG 321 HYBIT BCF - β-HCH**

| | Value 1 |
|---|---|
| Source | This project |
| Source details | *Cf*. chapter A.2.2 |
| Comment | |
| **Results** | |
| $BCF_k$ | |
| $BCF_{SS}$ | |
| $BCF_{kL}$ | |
| $BCF_{SSL}$ | |
| Comment | |
| Validity criteria (TG 321) | |
| Water temp. | |
| Dissolved $O_2$ | |
| TWA stable | |
| Water conc. < solubility | |
| Mortality | |
| [NPOC] | |
| Comment | Selected for data comparison |

**Table 8: Tier 3 - OECD TG 319 A/B IVIVE BCF - β-HCH**

| | Value 1 | Value 2 |
|---|---|---|
| Source | This project | Literature |
| Source details | *Cf*. chapter A.4.2 & A.6.2 | Lee et al. 2022 |
| Comment | | |
| **Hep. materials** | | |
| Donor species | Rainbow trout | Rat |
| Hepatic material | Cryopreserved hepatocytes | S9 |
| Protein or cell concentration | | 1 mg/mL |
| Cell viability | | - |

| | Value 1 | Value 2 |
|---|---|---|
| [Characterization of S9 / HEP] | | Yes |
| **Test setup** | | |
| Incubation temperature | | 37°C |
| Assay duration | | 90 min |
| Start conc. | | 0.25 µM |
| Incubation volume | | 4 mL |
| Inactive controls | | Yes, HI-S9 |
| Inactive controls < 20% loss? | | |
| Time points sampled | | 7 |
| Comment | | |
| **Results** | | |
| Log-linear? | | Yes |
| Rates (± SD) | | $k_{met}$ = 0.00103 ±0.000751 SE $h^{-1}$, n = 3), |
| Replicates | | 3 |
| IVIVE BCF | | BMF: 2.5 ± 0.96 SE kg lipid/kg lipid |
| IVIVE model | | Lee et al. 2022, IVIVE-B model |
| Comment | Selected for data comparison | Selected for data comparison |

**Table 9: Tier 4 - OECD TG 305 fish in vivo BCF - β-HCH**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | OECD QSAR Tool | Yamato et al. 1983 | UNEP 2007 |
| Comment | | | Risk Profile |
| **Results** | | | |
| $BCF_k$ | 293 ± 86 | | |
| $BCF_{SS}$ | | 1043 | 1460 |
| $BCF_{kL}$ | | | |
| $BCF_{SSL}$ | | | |
| Comment | Rainbow trout, hatching | Guppies | Zebra fish |

## 1.4 Pentachlorobenzene

**Table 10: Tier 3 - OECD TG 321 HYBIT BCF - pentachlorobenzene**

| | | **Value 1** |
|---|---|---|
| | Source | Literature |
| | Source details | Landrum et al. 2009 |
| | Comment | |
| | **Results** | |
| | $BCF_k$ (±SD) | 1913 ± 222 at 0.019 µM/L<br>1452 ± 193 at 0.036 µM/L<br>1874 ± 221 at 0.078 µM/L<br>2164 ± 314 at 0.12 µM/L<br>2258 ± 264 at 0.20 µM/L<br>1139 ± 118 at 0.32 µM/L<br>2143 ± 230 at 0.42 µM/L<br>871 ± 78 at 0.47 µM/L<br>666 ± 59 at 0.56 µM/L<br>780 ± 64 at 0.57 µM/L<br>577 ± 65 at 0.78 µM/L |
| | $BCF_{SS}$ | N/A |
| | $BCF_{kL}$ | N/A |
| | $BCF_{SSL}$ | N/A |
| | Comment | Multiple beaker exposure, lipid content based on dry weight |
| | **Validity criteria (TG 321)** | |
| | Water temp. | N/A |
| | Dissolved $O_2$ | N/A |
| | TWA stable | N/A |
| | Water conc. < solubility | N/A |
| | Mortality | N/A |
| | [NPOC] | N/A |
| | Comment | Study performed prior to TG development<br>Authors suspect that the test substance is not biotransformed by *H. azteca*<br>Authors suspect that higher concentrations lead to narcotic effects that explain lower BCFs at higher water concentrations<br>Selected for data comparison:<br>Median BCF of all tests at < 0.45µM/L: 1913 |

**Table 11: Tier 3 - OECD TG 319 A/B IVIVE BCF - pentachlorobenzene**

| | | Value 1 |
|---|---|---|
| | Source | Literature |
| | Source details | Laue et al. 2014 |
| | Comment | |
| | **Hep. material** | |
| | Donor species | Rainbow trout |
| | Hepatic material | S9 |
| | Protein or cell concentration | 1 mg/mL |
| | Cell viability | - |
| | [Characterization of S9 / HEP] | N/A |
| | Test setup | |
| | Incubation temperature | 12°C |
| | Assay duration | 120 min |
| | Start conc. | 0.4 µM |
| | Incubation volume | 0.2 mL |
| | Inactive controls | Yes (HI-S9) |
| | Inactive controls < 20% loss? | Yes |
| | Time points sampled | 5 |
| | Comment | |
| | **Results** | |
| | Log-linear? | Yes |
| | Rates | $CL_{IN\ VITRO,\ INT}$: 0 mL/h/mg protein |
| | Replicates | 3 |
| | IVIVE BCF | 6308 $f_U$ calc<br>6308 $f_U$ = 1 |
| | IVIVE model | Nichols et al. 2013 |
| | Comment | Selected for data comparison |

**Table 12: Tier 4 - OECD TG 305 fish in vivo BCF - pentachlorobenzene**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Literature |
| Source details | Laue et al. 2020 | OECD QSAR Toolbox |

| | Value 1 | Value 2 |
|---|---|---|
| Comment | | |
| Results | | |
| $BCF_k$ | | |
| $BCF_{SS}$ | | Values from approx. 4000 – 23000<br>18 values for rainbow trout<br>39 values for sheepshead minnow<br>5 values for common carp<br>13 values for guppy<br>10 values for other speicies |
| $BCF_{kL}$ | | |
| $BCF_{SSL}$ | 8596 (from Yakata et al. 2006), Carp<br>7689 (from MITI database), Carp<br>7647 (from Oliver and Niimi 1983), Rainbow trout | |
| Comment | | |

## 1.5 Pentachlorophenol

**Table 13: Tier 3 - OECD TG 321 HYBIT BCF - Pentachlorophenol**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Nuutinen et al. 2003 |
| Comment | Uptake and depuration determined in separate experiments |
| Results | |
| $BCF_k$ | 132 |
| $BCF_{SS}$ | N/A |
| $BCF_{kL}$ | N/A |
| $BCF_{SSL}$ | N/A |
| Comment | |
| Validity criteria (TG 321) | |
| Water temp. | N/A |
| Dissolved $O_2$ | N/A |
| TWA stable | N/A |
| Water conc. < solubility | N/A |
| Mortality | N/A |
| [NPOC] | N/A |
| Comment | Selected for data comparison |

**Table 14: Tier 3 - OECD TG 319 A/B IVIVE BCF - Pentachlorophenol**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Chen et al. 2016 |
| Comment | |
| Hep. material | |
| Donor species | Rainbow trout |
| Hepatic material | S9 |
| Protein or cell concentration | 1 mg/mL |
| Cell viability | - |
| [Characterization of S9 / HEP] | Yes |
| Test setup | |
| Incubation temperature | 11°C |
| Assay duration | 120 min |
| Start conc. | 0.5 µM |
| Incubation volume | 0.2 mL |
| Inactive controls | Yes |
| Inactive controls < 20% loss? | Yes |
| Time points sampled | 3 |
| Comment | 1% solvent in incubation! |
| Results | |
| Log-linear? | Yes |
| Rates | Slope: Not significantly different from 0 |
| Replicates | N/A |
| IVIVE BCF | N/A |
| IVIVE model | N/A |
| Comment | Selected for data comparison |

**Table 15: Tier 4 - OECD TG 305 fish in vivo BCF- Pentachlorophenol**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | OECD QSAR Toolbox |
| Comment | ECOTOX database |

| | Value 1 |
|---|---|
| Results | |
| $BCF_k$ | Values from 195 – 479 (Rainbow trout, 5 values)<br>Broad data basis for different species |
| $BCF_{SS}$ | |
| $BCF_{kL}$ | |
| $BCF_{SSL}$ | |
| Comment | |

## 1.6 PCB 153

**Table 16: Tier 3 - OECD TG 321 HYBIT BCF- PCB 153**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 |
| Comment | Male *H. azteca* | Female *H. azteca* | Male *H. azteca* |
| Results | | | |
| $BCF_k$ | 153615 | 268562 | 100475 |
| $BCF_{SS}$ | N/A | N/A | N/A |
| $BCF_{kL}$ | N/A | N/A | 258956 (5% lipid)<br>155373 (3% lipid) |
| $BCF_{SSL}$ | N/A | N/A | N/A |
| Comment | Co-exposed with DB[a,h]anthracene<br>No lipid data available<br>6 days uptake, no steady-state | Co-exposed with DB[a,h]anthracene<br>No lipid data available<br>6 days uptake, no steady-state | Co-exposed with PCB 77<br>12 days uptake<br>No steady state reached |
| Validity criteria (TG 321) | | | |
| Water temp. | N/A | N/A | N/A |
| Dissolved $O_2$ | N/A | N/A | N/A |
| TWA stable | N/A | N/A | N/A |
| Water conc. < solubility | Yes | Yes | Yes |
| Mortality | N/A | N/A | N/A |
| [NPOC] | | | |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Comment | Study performed prior to TG development<br>Not selected for data comparison (no lipid normalization) | Study performed prior to TG development<br>Not elected for data comparison (female *H. azteca*) | Study performed prior to TG development<br>Selected for data comparison |

**Table 17: Tier 3 - OECD TG 319 A/B IVIVE BCF- PCB 153**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Trowell et al. 2018 |
| Comment | Mixture incubation |
| Hep. Material | |
| Donor species | Rainbow trout |
| Hepatic material | Fresh hepatocytes |
| Protein or cell concentration | N/A<br>25 mg/mL (ca. 6.3 x $10^6$ cells/mL) |
| Cell viability | > 85% |
| [Characterization of S9 / HEP] | |
| Test setup | |
| Incubation temperature | 13°C |
| Assay duration | 300 min |
| Start conc. | |
| Incubation volume | 0.5 mL |
| Inactive controls | Yes |
| Inactive controls < 20% loss? | |
| Time points sampled | 6 |
| Comment | 1% solvent in incubations |
| Results | |
| Log-linear? | Yes |
| Rates | $k_d$: Not significantly different from 0 |
| Replicates | |
| IVIVE BCF | 7218 |
| IVIVE model | Trowell et al. 2018 |
| Comment | Selected for data comparison |

**Table 18: Tier 4 - OECD TG 305 fish in vivo BCF- PCB 153**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Schlechtriem et al. 2017 | Trowell et al. 2018 | Schlechtriem et al. 2019 |
| Comment | | Not primary source | Not primary source |
| Results | | | |
| $BCF_k$ | 18539 | 8913 - 740000 | Log BCF = 5.7 (≙ 501187) |
| $BCF_{SS}$ | N/A | | |
| $BCF_{kL}$ | 12526<br>$BCF_{kgL}$: 230287 | | |
| $BCF_{SSL}$ | N/A | | |
| Comment | Rainbow trout | Cohen and Clay 1994; Gerhart and Carlson 1978; Gobas and MacKay 1987; Hickox and Denton 2000; Jimenez et al. 1987; Johnsen et al. 1989; Leversee et al. 1983; Levine et al. 1997; Lu et al. 1977; McCarthy and Jimenez 1985; Spacie et al. 1983<br>In Trowell et al. 2018 | Opperhuizen & Shrap 1987<br>Fox et al. 1994<br>In Schlechtriem et al. 2019 |

## 1.7 Fluorene

**Table 19: Tier 3 - OECD TG 321 HYBIT BCF - fluorene**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Lee et al. 2002 |
| Comment | |
| Results | |
| $BCF_k$ | 266 at 295 µg/L<br>255 at 403 µg/L<br>303 at 532 µg/L<br>315 at 1055 µg/L |
| $BCF_{SS}$ | N/A |
| $BCF_{kL}$ | N/A |
| $BCF_{SSL}$ | N/A |
| Comment | $^{14}$C-TRR-based analyses |

| | Value 1 |
|---|---|
| | Steady state BCFs are not reported, but it is mentioned that there is no significant difference between kinetic BCFs and steady-state BCFs |
| Validity criteria (TG 321) | |
| Water temp. | N/A |
| Dissolved $O_2$ | N/A |
| TWA stable | No (linear decline) |
| Water conc. < solubility | N/A |
| Mortality | N/A |
| [NPOC] | N/A |
| Comment | Study performed prior to TG development<br>Selected for data comparison:<br>Median BCF 285 |

**Table 20: Tier 3 - OECD TG 319 A/B IVIVE BCF- fluorene**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Literature |
| Source details | Fay et al. 2017 | Nichols et al. 2013 |
| Comment | | |
| Hep. material | | |
| Donor species | Rainbow trout | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes | S9 |
| Protein or cell concentration | 2 x $10^6$ cells/mL | 1 mg/mL |
| Cell viability | Not in all runs (2 runs only approx. 50%) | |
| [Characterization of S9 / HEP] | Yes | Yes |
| **Test setup** | | |
| Incubation temperature | 11°C | 11°C |
| Assay duration | 90 min | |
| Start conc. | 0.93 µM | 0.77 - 08 µM |
| Incubation volume | 1.6 mL | 0.2 mL |
| Inactive controls | HI-Hepatocytes | HI-S9 |

| | Value 1 | Value 2 |
|---|---|---|
| Inactive controls < 20% loss? | Yes | |
| Time points sampled | 6 | |
| Comment | | |
| **Results** | | |
| Log-linear? | Yes | |
| Rates | $CL_{in\ vitro,\ int}$: 0.3 ± 0.11 mL/h/$10^6$ cells (median rate) | $CL_{in\ vitro,\ int}$: 0.36 ± 0.01 ml/h/mg protein Mean ± SD of all values for both genders (N = 6) |
| Replicates | 9 (for each lot 1 experiment) | |
| IVIVE BCF | Not reported 374 (own calculation) | |
| IVIVE model | Not reported Krause & Goss 2020, one compartment (own calculation) | |
| Comment | Selected for data comparison | Selected for data comparison |

**Table 21: Tier 4 - OECD TG 305 fish in vivo BCF- fluorene**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Literature |
| Source details | OECD QSAR Toolbox | Carlson et al. 1978 in Veith et al. 1989 |
| Comment | | |
| Results | | |
| $BCF_k$ | 9 values from 58 – 724 | 1288 (reported as log BCF) |
| $BCF_{SS}$ | | |
| $BCF_{kL}$ | | |
| $BCF_{SSL}$ | | |
| Comment | Rainbow trout | Fathead minnow |

## 1.8 Phenanthrene

**Table 22: Tier 3 – OECD TG 321 HYBIT BCF - phenanthrene**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Lee et al. 2002 |
| Comment | |
| Results | |
| $BCF_k$ | 440 at 87 µg/L<br>456 at 144 µg/L<br>473 at 223 µg/L<br>504 at 343 µg/L |
| $BCF_{SS}$ | N/A |
| $BCF_{kL}$ | N/A |
| $BCF_{SSL}$ | N/A |
| Comment | $^{14}$C-TRR-based analyses<br>Steady state BCFs are not reported, but it is mentioned that there is no significant difference between kinetic BCFs and steady-state BCFs |
| Validity criteria (TG 321) | |
| Water temp. | N/A |
| Dissolved $O_2$ | N/A |
| TWA stable | No (linear decline) |
| Water conc. < solubility | N/A |
| Mortality | N/A |
| [NPOC] | N/A |
| Comment | Study performed prior to TG development<br><br>Selected for data comparison:<br>Median BCF: 465 |

**Table 23: Tier 3 – OECD TG 319 A/B IVIVE BCF- phenanthrene**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Fay et al. 2017 | Nichols et al. 2018b | Nichols et al. 2013 |
| Comment | | Method optimization | |
| Hep. material | | | |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Donor species | Rainbow trout | Rainbow trout | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes | S9 | S9 |
| Protein or cell concentration | 2 x $10^6$ cells/mL | 1 mg/mL | 1 mg/mL |
| Cell viability | Not in all runs (2 runs only approx. 50%) | - | - |
| [Characterization of S9 / HEP] | Yes | Yes | Yes |
| **Test setup** | | | |
| Incubation temperature | 11°C | 11°C | 11°C |
| Assay duration | 50 min | 20 – 120 min | |
| Start conc. | 1.5 µM | Several | 1.26 - 1.29 µM |
| Incubation volume | 1.6 mL | N/A | 0.2 mL |
| Inactive controls | HI-Hepatocytes | N/A | HI-S9 |
| Inactive controls < 20% loss? | Unclear (*cf.* Figure 1) | N/A | |
| Time points sampled | 6 | 7 | |
| Comment | | | |
| **Results** | | | |
| Log-linear? | Yes | Yes | |
| Rates | $CL_{in\ vitro,\ int}$: 0.21 ± 0.04 mL/h/$10^6$ cells (median rate) | $CL_{in\ vitro,\ int}$: 2.1 mL/h/mg protein<br><br>Fitted value | $CL_{in\ vitro,\ int}$: 0.58 mL/h/mg protein<br><br>Mean ± SD of all values for both genders (N = 6) |
| Replicates | 9 (for each lot 1 experiment) | Several under different conditions | |
| IVIVE BCF | Not reported | N/A | |
| IVIVE model | Not reported<br>Krause & Goss 2020, one compartment (own calculation) | N/A | |
| Comment | Selected for data comparison | $K_M$ value determined: 0.52 µM | Selected for data comparison |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| | | Not selected for data comparison (fitted value) | |

**Table 24: Tier 4 – OECD TG 305 fish in vivo BCF- phenanthrene**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Dossier data |
| Source details | OECD QSAR Toolbox | SVHC Support Document, ECHA 2018 |
| Comment | | |
| Results | | |
| $BCF_k$ | 708 (reported as log BCF)<br>1288 (reported as log BCF) | 2229 - 6118 |
| $BCF_{SS}$ | | |
| $BCF_{kL}$ | | |
| $BCF_{SSL}$ | | |
| Comment | Values for carp and fathead minnow | Several fish species |

## 1.9 Anthracene

**Table 25: Tier 3 – OECD TG 321 HYBIT BCF - anthracene**

| | Value |
|---|---|
| Source | Literature |
| Source details | Landrum & Scavia 1983 |
| Comment | $^{14}$C-TRR-based values<br>Comparison of influence of sediment on kinetics |
| Results | |
| $BCF_k$ | 2089 ± 355 (no substratum, no biotransformation-correction) |
| $BCF_{SS}$ | 1800 ± 329 (no substratum, biotransformation-correction, determined at sampling at the end of the uptake phase) |
| $BCF_{kL}$ | N/A |
| $BCF_{SSL}$ | N/A |
| Comment | Authors state that no steady state was reached |
| Validity criteria (TG 321) | |
| Water temp. | N/A |
| Dissolved $O_2$ | N/A |
| TWA stable | N/A |

| | Value |
|---|---|
| Water conc. < solubility | N/A |
| Mortality | N/A |
| [NPOC] | N/A |
| Comment | Study was performed prior to TG development<br>Data selected for comparison |

**Table 26: Tier 3 – OECD TG 319 A/B IVIVE BCF- anthracene**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Literature |
| Source details | Fay et al. 2017 | Nichols et al. 2013 |
| Comment | | |
| Hep. Material | | |
| Donor species | Rainbow trout | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes | S9 |
| Protein or cell concentration | 2 x $10^6$ cells/mL | 1.0 mg/ml S9 protein |
| Cell viability | Not in all runs (2 runs only approx. 50%) | N/A |
| [Characterization of S9 / HEP] | Yes | Yes |
| Test setup | | |
| Incubation temperature | 11°C | 11°C |
| Assay duration | 50 min | |
| Start conc. | 0.14 µM | 0.14 - 0.15 µM |
| Incubation volume | 1.6 mL | 0.2 mL |
| Inactive controls | HI-Hepatocytes | HI-S9 |
| Inactive controls < 20% loss? | Unclear (cf. Figure 1) | |
| Time points sampled | 6 | |
| Comment | | |
| Results | | |
| Log-linear? | Yes | |
| Rates | $CL_{in\ vitro,\ int}$: 0.32 ± 0.10 mL/h/$10^6$ cells (median rate) | $CL_{in\ vitro,\ int}$: 0.49 ± 0.04 (ml/h/mg protein) |
| Replicates | 9 (for each lot 1 experiment) | |

| | Value 1 | Value 2 |
|---|---|---|
| IVIVE BCF | Not reported<br>647 (own calculation) | |
| IVIVE model | Not reported<br>Krause & Goss 2020, one compartment (own calculation) | |
| Comment | <br><br>Data selected for comparison | Mean data (male and female *H. azteca* mixed)<br>Data selected for comparison |

**Table 27: Tier 4 – OECD TG 305 fish in vivo BCF- anthracene**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Database entry | Database entry | Dossier data |
| Source details | OECD QSAR Toolbox | OECD QSAR Toolbox | SVHC Support Document, ECHA 2008 |
| Comment | | | |
| Results | | | |
| BCF | 191 – 724 (reported as log BCF) | 676 - 3890 | 900 - 6000 |
| Comment | Rainbow trout | Fish species, other than rainbow trout | Several fish species, “Val.” category = 2. “De Maagd et al. 1996 value was not considered: ECHA 2018 concludes that this study is not category 2, but 3. |

## 1.10 Pyrene

**Table 28: Tier 3 – OECD TG 321 HYBIT BCF - pyrene**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | This project | Literature | Literature |
| Source details | *Cf*. chapter A.2.1 | Schlechtriem et al. 2019 | Lee et al. 2002 |
| Comment | | Male *H. azteca* | |
| Results | | | |
| $BCF_k$ | | 5870 | 5165 at 9.4 µg/L<br>3792 at 29.6 µg/L<br>3730 at 40.9 µg/L<br>2323 at 66.8 µg/L |
| $BCF_{SS}$ | | 5427 | N/A |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| $BCF_{kL}$ | | N/A | N/A |
| $BCF_{SSL}$ | | N/A | N/A |
| Comment | | $^{14}$C-TRR based data | $^{14}$C-TRR-based analyses<br>Steady state BCFs are not reported, but it is mentioned that there is no significant difference between kinetic BCFs and steady-state BCFs |
| Validity criteria (TG 321) | | | |
| Water temp. | | N/A | N/A |
| Dissolved $O_2$ | | N/A | N/A |
| TWA stable | | N/A | N/A |
| Water conc. < solubility | | Yes | N/A |
| Mortality | | N/A | N/A |
| [NPOC] | | N/A | N/A |
| Comment | Data selected for comparison | Study conducted prior to TG development<br>Data not selected for comparison (no lipid data, TRR-based values) | Study conducted prior to TG development<br>Data not selected for comparison (no lipid data, TRR-based values) |

**Table 29: Tier 3 – OECD TG 319 A/B IVIVE BCF - pyrene**

| | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 |
|---|---|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature | Literature | Literature |
| Source details | Bourgeois et al. 2022 | Connors et al. 2013 | Fay et al. 2017 | Laue et al. 2020 | Laue et al. 2023 | Laue et al. 2023 |
| Comment | | | | | | |
| Hep. material | | | | | | |
| Donor species | Rainbow trout | Rainbow trout | Rainbow trout | Rainbow trout | Rainbow trout | Common carp |
| Hepatic material | Cryopreserved hepatocytes | S9 | Cryopreserved hepatocytes | S9 | S9 | S9 |
| Protein or cell concentration | $1.35 \times 10^6$ cells/ mL | 1 mg/mL | $2 \times 10^6$ cells/mL | 1 mg/mL | | |
| Cell viability | 86% | - | | - | | |

| | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 |
|---|---|---|---|---|---|---|
| [Characterization of S9 / HEP] | | | Yes | Yes | Yes | Yes |
| Test setup | | | | | | |
| Incubation temperature | N/A | 11°C | 11°C | 12°C | 12°C | |
| Assay duration | 150 min | | 50 min | 3 min<br>30 min<br>60 min | | |
| Start conc. | 5 µg/L (≙ 0,025 µM/L) | 0.38 µM | 0.025 µM/L | 0.025 µM<br>0.2 µM<br>1 µM | | |
| Incubation volume | 5 mL | 0.2 mL | 1.6 mL | 02. mL | | |
| Inactive controls | Yes, HI-Heps | Yes, HI-S9 | Yes, HI-hepatocytes | Yes, SI-S9 | Yes, SI-S9 | |
| Inactive controls < 20% loss? | Yes | Yes | Yes | Yes | | |
| Time points sampled | 6 | 7 | 6 | 6 | | |
| Comment | | | | | | |
| Results | | | | | | |
| Log-linear? | Yes | Yes | Yes | Yes | | |
| Rates | $CL_{IN\ VIVO,INT,HEP}$: 1.03 ± 0.12 mL/h/$10^6$ cells | $CL_{IN\ VITRO,\ INT}$: 472 mL/h/g liver | $CL_{IN\ VITRO,INT,HEP}$: 3.1 ± 1.,0 mL/h/$10^6$ cells | in vitro intrinsic clearance: 16.77 (0.025µM) 6.51 (0.2µM) 2.63 (1µM) mL/h/mg protein | $CL_{IN\ VITRO,\ INT}$: 17.09 mL/h/mg protein<br><br>$CL_{IN\ VITRO,\ INT}$: 12.15 mL/h/mg protein | $CL_{IN\ VITRO,\ INT}$: 1.01 mL/h/mg protein |
| Replicates | 3 | 2 | 9 (single runs from 9 different fish) | | | |
| IVIVE BCF | N/A | N/A | N/A | | | |
| IVIVE model | N/A | N/A | N/A | | | |
| Comment | | | | | | |

| | Value 7 | Value 8 | Value 9 | Value 10 | Value 11 | Value 12 |
|---|---|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature | This project | Literature |
| Source details | Nichols et al. 2018a | Nichols et al. 2018a | Mingoia et al. 2010 | Zercher et al. 2024 | *Cf*. chapter A.5.4 & A.6.1 | Lee et al. 2022 |
| Comment | Ring trial summary | Ring trial summary | Evaluation of cryopreservation on metabolic capacity | | | |
| Hep. material | | | | | | |
| Donor species | Rainbow trout | Rainbow trout | Rainbow trout | Rainbow trout | Rainbow trout | Rat |
| Hepatic material | Cryopreserved hepatocytes | S9 | Hepatocytes | S9 | Cryopreserved hepatocytes | S9 |
| Protein or cell concentration | 2 x $10^6$ cells/mL | 1 mg/mL | 2×$10^6$ cells/mL | 1 mg/mL | | 1 mg/mL |
| Cell viability | | | | - | | - |
| [Characterization of S9 / HEP] | Yes | Yes | Yes | Yes | | Yes |
| Test setup | | | | | | |
| Incubation temperature | 11°C | 11°C | 11°C | 13°C | | 37°C |
| Assay duration | 50 minutes | 14 minutes | 240 min | 14 min | | 7 min |
| Start conc. | 0.025 µM | 0.025 µM | 1 µM | 0.025µM | | 0.05µM |
| Incubation volume | 1 mL | 1 mL | | 0.5 mL | | 4 mL |
| Inactive controls | Yes, HI-hepatocytes | No | | Yes, room-temperature inactivated S9 | | Yes, HI-S9 |
| Inactive controls < 20% loss? | Yes | N/A | | | | Yes |
| Time points sampled | 7 | 7 | 7 | 6 | | 7 |

| | Value 7 | Value 8 | Value 9 | Value 10 | Value 11 | Value 12 |
|---|---|---|---|---|---|---|
| Comment | | | 1% solvent in incubation | | | |
| Results | | | | | | |
| Log-linear? | | | No (*cf*. Fig S1) | Yes | | Yes |
| Rates | $CL_{IN\ VITRO,INT}$: 1.86 ± 0.77 (Lot 1) 2.36 ± 0.72 (Lot 2) 1.50 ± 0.28 (Lot 3) 1.51 ± 0.22 (Lot 4) 1.47 ± 0.50 (Lot 5) ml/h/$10^6$ cells | $CL_{IN\ VITRO,INT}$: 16.38 ± 2.21 (Lot 1) 21.72 ± 4.99 (Lot 2) 27.14 ± 4.11 (Lot 3) 20.43 ± 9.05 (Lot 4) 19.22 ± 7.52 (Lot 5) ml/h/mg protein | $CL_{inv\ itro\ int}$: 0.036 ml/h/$10^6$ cells (mean) 0.036±0.003 0.036±0.003 0.041±0.005 0.036±0.004 0.052±0.005 0.047±0.004 0.064±0.006 0.055±0.003 0.018±0.005 0.014±0.004 0.014±0.008 | In vitro $Cl_{int}$: 5.75 mg/h/mL | | $k_{met}$: 0.139 ± 0.00500 SE $h^{-1}$ |
| Replicates | 6 labs | 6 labs | 11 | 2 | | 37 |
| IVIVE BCF | 709 ± 78 ($f_U$ = $f_U$, P/$f_U$, HEP) 213 ± 2 ($f_U$ = 1.0) | 316 ± 21 ($f_U$ = $f_U$, P/$f_U$, S9) 204 ± 1 ($f_U$ = 1.0) | 1974±133 2076±198 1794±161 1951±164 1508±107 1618±108 1273±101 1424±51.2 3124±495 3505±238 3430±1086 | 3350 | | BMF =0.022 ± 0.00078 SE kg lipid/kg lipid |
| IVIVE model | Nichols et al. 2013 | Nichols et al. 2013 | Han et al. 2007 | BAT Tool V. 2.0.2 | | Lee et al. 2022, IVIVE-B model |
| Comment | | | Three batches compared, each batch analyzed at 4 different times. Batch 3 shows lower activity. | Contains S9 depletion data for additional fish species | Data selected for comparison | Data selected for comparison |

**Table 30: Tier 4 – OECD TG 305 fish in vivo BCF - pyrene**

| | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 |
|---|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature | Database entry |
| Source details | Laue et al. 2020 | Nichols et al. 2018a | Zercher et al. 2024 | Zercher et al. 2024 | OECD QSAR Toolbox |
| Comment | Not primary source | Not primary source, Johnsson et al. 2004 | Not primary source, Ren et al. 2021 | Not primary source, US EPA 2013 | |
| Results | | | | | |
| BCF | 50 | 78 (average of 4 values) | 22.8 | 1044 - 1351 | 50 – 977 (reported as log BCF) |
| Comment | Sheepshead minnow | Sheepshead minnows | | | Carp and guppy data |

## 1.11 Benzo[a]pyrene (B[a]P)

**Table 31: Tier 3 – OECD TG 321 HYBIT BCF - B[a]P**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Literature |
| Source details | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 |
| Comment | Co-exposed with methoxychlor<br>Male *H. azteca* | Co-exposed with methoxychlor<br>Mixed sex *H. azteca* |
| Results | | |
| $BCF_k$ | 3259 | 3329 |
| $BCF_{SS}$ | 3034 | 3029 |
| $BCF_{kL}$ | 6518 (5% lipid)<br>3911 (3% lipid) | 5106 (5% lipid)<br>3064 (3% lipid) |
| $BCF_{SSL}$ | 6068 (5% lipid)<br>3641 (3% lipid) | 4646 (5% lipid)<br>2787 (3% lipid) |
| Comment | | |
| Validity criteria (TG 321) | | |
| Water temp. | N/A | N/A |
| Dissolved $O_2$ | N/A | N/A |
| TWA stable | N/A | N/A |
| Water conc. < solubility | Yes | Yes |
| Mortality | N/A | N/A |

| | Value 1 | Value 2 |
|---|---|---|
| [NPOC] | N/A | N/A |
| Comment | Study performed prior to TG development<br>Data selected for comparison | Study performed prior to TG development<br>Data not selected for comparison (mixed sex *H. azteca*) |

**Table 32: Tier 3 – OECD TG 319 A/B IVIVE BCF- B[a]P**

| | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 |
|---|---|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature | Literature | Literature |
| Source details | Fay et al. 2014 | Fay et al. 2017 | Han et al. 2007 | Han et al. 2009 | Mingoia et al. 2010 | Nichols et al. 2018b |
| Comment | Laboratory comparison data | | | | Activity comparison after cryo-storage | Method optimization |
| Hep. material | | | | | | |
| Donor species | Rainbow trout | Rainbow trout | Rainbow trout | Rainbow trout | Rainbow trout | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes | Cryopreserved hepatocytes | Fresh hepatocytes | S9 | Fresh hepatocytes Cryopreserved hepatocytes | S9 |
| Protein or cell concentration | 2 x $10^6$ cells/mL | 2 x $10^6$ cells/mL | 2 x $10^6$ cells/mL | 2 mg/mL | 2 x $10^6$ cells/mL | 1 mg/mL |
| Cell viability | < 80% | Not in all runs (2 runs only approx. 50%) | 95% | - | 98.2 - 99.5% | - |
| [Characterization of S9 / HEP] | Yes (as fresh hepatocytes) | Yes | No | Yes | Yes (fresh and cryopreserved) | Yes |
| Test setup | | | | | | |
| Incubation temperature | 11°C | 11°C | 10°C | 10°C | 10°C | 11°C |
| Assay duration | 240 min | 240 min | 120 min | 240 min | 240 | 2 – 180 min |

| | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 |
|---|---|---|---|---|---|---|
| Start conc. | 0.5µM | 0.16 µM | 2 µM | 2 µM | 4 µM | Several |
| Incubation volume | 1mL | 1.6 mL | N/A | N/A | N/A | N/A |
| Inactive controls | HI-Hepatocytes | HI-Hepatocytes | HI-Hepatocytes | N/A | N/A | N/A |
| Inactive controls < 20% loss? | Yes | Yes | Yes | N/A | N/A | N/A |
| Time points sampled | 7 | 6 | 7 | 7 | 7 | 7 |
| Comment | | | | pH 7.4! | Solvent concentration of 1% (ACN) | |
| Results | | | | | | |
| Log-linear? | Yes | Yes | Yes | Maybe | Not clear (cf. Figure S1 F) | Yes |
| Rates (± SD) | $CL_{in\ vitro,\ int}$: 0.214 ± 0.067 mL/h/$10^6$ cells | $CL_{in\ vitro,\ int}$: 0.54 ± 0.10 mL/h/$10^6$ cells (median rate) | $CL_{in\ vitro,\ int}$: 0.044 ± 0.013 mL/h/$10^6$ cells | $CL_{in\ vitro,\ int}$: 0.068 ± 0.016 ml/h/mg protein (Without cofactors) $CL_{in\ vitro,\ int}$: 0.032 ± 0.006 ml/h/mg protein (With cofactors) | | $CL_{in\ vitro,\ int}$: 57.4 mL/h/mg protein Fitted value, not measured |
| Replicates | 3 x 3 | 9 (for each lot 1 experiment) | N/A | 3 | 12 experiments with different material, 3 runs per experiment | Several under different conditions |
| IVIVE BCF (± SD) | 5923 ± 1162 | Not reported | 1528 1206 (Han et al. 2009) | 3276 | 543 ± 7.9 - 2475 ± 95.0 | N/A |
| IVIVE model | Nichols et al. 2013 | | Han et al. 2007 | Han et al. 2007 | Han et al. 2007 | N/A |

| | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 |
|---|---|---|---|---|---|---|
| Comment | Laboratory comparison data<br>Data not selected for comparison (other study with lower stating concentration available) | Data selected for comparison: Newest data and lowest starting concentration | Data not selected for comparison (other study with lower stating concentration available) | Data plot looks slightly scattered (*cf.* Figure 1 of the publication)<br>Data not selected for comparison (other study with lower stating concentration available) | Data not selected for comparison (other study with lower stating concentration available) | $K_M$ value determined: 0.03 µM |

| | Value 7 | Value 8 | Value 9 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Trowell et al. 2018 | Lee et al. 2014 | Lee et al. 2022 |
| Comment | | | |
| Hep. material | | | |
| Donor species | Rainbow trout | Rainbow trout | Rat |
| Hepatic material | Fresh hepatocytes | S9 | S9 |
| Protein or cell concentration | N/A<br>(25 mg/mL) | 6 mg/mL | 1 mg/mL |
| Cell viability | > 85%. | - | - |
| [Characterization of S9 / HEP] | | N/A | Yes |
| Test setup | | | |
| Incubation temperature | N/A | 13.5°C | 37°C |
| Assay duration | 300 min | | 7 min |
| Start conc. | 0.5 µM | 1 µM | 0.025 µM |
| Incubation volume | 0.5 mL | 0.5mL | 4 mL |
| Inactive controls | Yes | Yes, no cofactors | Yes, HI-S9 |
| Inactive controls < 20% loss? | Unknown | N/A | Yes |
| Time points sampled | 6 | N/A | 7 |
| Comment | | | |
| Results | | | |

| | Value 7 | Value 8 | Value 9 |
|---|---|---|---|
| Log-linear? | Yes | Yes | Yes |
| Rates (± SD) | $K_d$: 6.51 (3.55–9.46) $10^{-3}$ $min^{-1}$ (individual. ± 95% CI)<br>$K_d$: 1.44 (0.3–2.58) $10^{-3}$ $min^{-1}$<br>(mixture, ± 95% CI) | $k_r$ = 0.021 ± 0.005 $min^{-1}$ | $k_{met}$= 0.112 ± 0.0145 SE $h^{-1}$ |
| Replicates | N/A | 3 x 3 | 3 |
| **IVIVE BCF (± SD)** | 650–1600 (930) single substance 95% CI and mean<br>2200–10000 (3600) mixture 95% CI and mean | N/A | BMF = 0.027 ± 0.0042 SE kg lipid/kg lipid |
| IVIVE model | Trowell et al. 2018 | N/A | Lee et al. 2022 IVIVE-B model |
| Comment | Data not selected for comparison (other study with lower stating concentration available) | Data not selected for comparison (other study with lower stating concentration available) | Data selected for comparison |

**Table 33: Tier 4 – OECD TG 305 fish in vivo BCF- B[a]P**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Database entry | Literature | Dossier data |
| Source details | OECS QSAR Toolbox | Schlechtriem et al. 2019 | SVHC Support Document, ECHA 2016 |
| Comment | | Not primary source (Jimenez et al. 1987) | |
| Results | | | |
| BCF | 3 – 363 | 631 (recalculated from log data) | 377 - 608 |
| Comment | Different fish species other than rainbow trout | | Test species = *Lepomis macrochirus* |

## 1.12 Chlorpyrifos

**Table 34: Tier 3 – OECD TG 321 HYBIT BCF - chlorpyrifos**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Schlechtriem et al. 2019 |
| Comment | |

| | Value 1 |
|---|---|
| Results | |
| $BCF_k$ | 919 |
| $BCF_{SS}$ | 675 |
| $BCF_{kL}$ | 1938 (5% lipid)<br>1163 (3% lipid) |
| $BCF_{SSL}$ | 1425 (5% lipid)<br>855 (3% lipid) |
| Comment | |
| Validity criteria (TG 321) | |
| Water temp. | N/A |
| Dissolved $O_2$ | N/A |
| TWA stable | N/A |
| Water conc. < solubility | Yes |
| Mortality | N/A |
| [NPOC] | N/A |
| Comment | Study performed prior to TG development<br>Data selected for comparison |

**Table 35: Tier 3 – OECD TG 319 A/B IVIVE BCF - chlorpyrifos**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | This project |
| Source details | Zercher et al. 2024 | Cowan-Ellsberry et al. 2008 | *Cf*. chapter A.5.4 & A.7.1 |
| Comment | Species comparison | | |
| Hep- material | | | |
| Donor species | Rainbow trout | Rainbow trout | Rat (Sprague Dawley) |
| Hepatic material | S9 | S9 | Cryopreserved hepatocytes |
| Protein or cell concentration | 1 mg/mL | 2 mg/mL | |
| Cell viability | | - | |
| [Characterization of S9 / HEP] | | | |
| Test setup | | | |
| Incubation temperature | 13°C | 25°C | |
| Assay duration | 60 min | 60 min | |
| Start conc. | 0.05 µM | 10 µM | |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Incubation volume | 0.5mL | 5 mL | |
| Inactive controls | Yes, room-temperature denatured S9 | | |
| Inactive controls < 20% loss? | N/A | | |
| Time points sampled | 7 | | |
| Comment | DMSO used for spiking (< 1%) | | |
| Results | | | |
| Log-linear? | Yes | | |
| Rates | $CL_{in\ vitro,\ int}$:1.429 mL/h/mg | $CL_m$: 0.325 µM/h/mg protein | |
| Replicates | 3 | | |
| IVIVE BCF | 3570 | 873 - 1071 | |
| IVIVE model | BAT Tool V2.0.2 | Cowan-Ellsberry 2008 | |
| Comment | Data selected for comparison | Data not selected for comparison (newer data with lower starting concentration available) | Data selected for comparison |

**Table 36: Tier 4 – OECD TG 305 fish in vivo BCF - chlorpyrifos**

| | Value 1 | Value 2 | Value 3 | Value 4 |
|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature |
| Source details | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 |
| Comment | Not primary source, Cripe et al. 1986 | Not primary source, Goodman et al. 1985a/b | Not primary source, Tsuda et al. 1997 | Not primary source, Adolfsson-Erici et al. 2012 |
| Results | | | | |
| BCF | 40 – 1995 (reported as log BCF) | 63 – 1000 (reported as log BCF) | 316 – 2512 (reported as log BCF, 14 values) | 3162 (reported as log BCF) |
| Comment | Sheepshead minnow, 27 values | Inland silverside / California grunion | Guppy, Medaka, gold fish, common carp | Rainbow trout |

| | Value 5 | Value 6 | Value 7 |
|---|---|---|---|
| Source | Literature | Literature | Registration data |
| Source details | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 | ECHA Registration Dossier |
| Comment | Not primary source, Jarvinen et al. 1983 | Not primary source, NITE 2005 | EC number 220-864-4 |
| Results | | | |
| BCF | 1585 (reported as log BCF) | 251 – 1995 (reported as log BCF) | 1374 ± 321 |
| Comment | Fathead minnow | Common carp | Rainbow trout |

## 1.13 Simazine

**Table 37: Tier 3 – OECD TG 321 HYBIT BCF - simazine**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Schlechtriem et al. 2019 |
| Comment | $^{14}$C-TRR-based data |
| Results | |
| $BCF_k$ | 5 |
| $BCF_{SS}$ | 3 |
| $BCF_{kL}$ | 15 (5% lipid)<br>9 (3% lipid) |
| $BCF_{SSL}$ | 10 (5% lipid)<br>6 (3% lipid) |
| Comment | |
| Validity criteria (TG 321) | |
| Water temp. | N/A |
| Dissolved $O_2$ | N/A |
| TWA stable | N/A |
| Water conc. < solubility | Yes |
| Mortality | N/A |
| [NPOC] | N/A |
| Comment | Study was performed prior to TG development<br>Data selected for comparison |

**Table 38: Tier 3 – OECD TG 319 A/B IVIVE BCF– simazine**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Black et al. 2021 |
| Comment | Hepatocyte species comparison |
| Hep. material | |
| Donor species | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes |
| Protein or cell concentration | 1 x $10^6$ cells/mL |
| Cell viability | |
| [Characterization of S9 / HEP] | Yes |
| Test setup | |
| Incubation temperature | 12°C |
| Assay duration | 240 min |
| Start conc. | 1 µM |
| Incubation volume | 1 mL |
| Inactive controls | Yes, HI-Hepatocytes |
| Inactive controls < 20% loss? | N/A |
| Time points sampled | 6 |
| Comment | |
| Results | |
| Log-linear? | Unknown |
| Rates | $CL_{in\ vitro,\ int}$: Not significantly different from 0 |
| Replicates | 3 |
| IVIVE BCF | N/A |
| IVIVE model | N/A |
| Comment | Ln instead of $log_{10}$ used<br>Data selected for comparison |

**Table 39: Tier 4 – OECD TG 305 fish in vivo BCF– simazine**

| | Value 1 | Value 2 | Value 3 | Value 4 |
|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature |
| Source details | Schlechtriem et al. 2019 | Schlechtriem et al. 2019 | OECD QSAR Toolbox | OECD QSAR Toolbox |

| | Value 1 | Value 2 | Value 3 | Value 4 |
|---|---|---|---|---|
| Comment | Not primary source, CERI 1992 | Not primary source, Tsuda et al. 1992 | | |
| Results | | | | |
| $BCF_k$ | | | | |
| $BCF_{SS}$ | 3 – 13 (reported as log BCF) | 13 – 25 (reported as log BCF) | 2570 – 8128 (reported as log BCF) | 1 – 214 (reported as log BCF) |
| $BCF_{kL}$ | | | | |
| $BCF_{SSL}$ | | | | |
| Comment | Common carp | Willow shiner | Rainbow trout Substance not found in linked primary source (Oliver & Niimi 1985) | Common carp, *Gnathopogon caerulescens*, guppy |

## 1.14 Terbutryn

**Table 40: Tier 3 – OECD TG 321 HYBIT BCF - terbutryn**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Literature |
| Source details | Kosfeld et al. 2020 | OECD 2024 |
| Comment | | Ring trial summary data |
| Results | | |
| $BCF_k$ | 37 ± 2 | 59/ 49/ 30/ 20/ 30/ 31/ 23 |
| $BCF_{SS}$ | 37 ± 3 | 62/ 66/ 35/ 20/ 32/ 31/ 22 |
| $BCF_{kL}$ | 77 ± 10 (5% lipid)<br>46 ± 6 (3% lipid) | 63/ 46/ 41/ 34/ 36/ 31/ 26 (3% lipid) |
| $BCF_{SSL}$ | 77 ± 12 (5% lipid)<br>46 ± 7 (3% lipid) | 66/ 62/ 47/ 33/ 39/ 31/ 24 (3% lipid) |
| Comment | | 6 experiments conducted by 5 different labs |
| Validity criteria (TG 321) | | |
| Water temp. | | |
| Dissolved $O_2$ | | |
| TWA stable | Yes | |
| Water conc. < solubility | | |
| Mortality | | |
| [NPOC] | | |

| | Value 1 | Value 2 |
|---|---|---|
| Comment | Study performed prior to TG development<br>Data selected for comparison | Data not selected for comparison (median $BCF_{kL}$ 3% lipid = 36, other study represents “worst-case”) |

**Table 41: Tier 3 – OECD TG 319 A/B IVIVE BCF - terbutryn**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Kosfeld et al. 2020 |
| Comment | |
| Hep. Material | |
| Donor species | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes |
| Protein or cell concentration | 2 x $10^6$ cells/mL |
| Cell viability | |
| [Characterization of S9 / HEP] | No |
| Test setup | |
| Incubation temperature | 11°C |
| Assay duration | 45 min |
| Start conc. | 0.1 µM |
| Incubation volume | 1 mL |
| Inactive controls | Yes, HI-hepatocytes |
| Inactive controls < 20% loss? | Yes |
| Time points sampled | 8 |
| Comment | |
| Results | |
| Log-linear? | Yes |
| Rates | |
| Replicates | 3 |
| IVIVE BCF | 73 ($f_U$ = 1)<br>133 ($f_U$ = calc) |
| IVIVE model | Nichols et al. 2013 |
| Comment | Data selected for comparison |

**Table 42: Tier 4 – OECD TG 305 fish in vivo BCF - terbutryn**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Tarja et al. 2003 |
| Comment | |
| Results | |
| $BCF_k$ | |
| $BCF_{SS}$ | 9 (4°C)<br>13 (10°C)<br>44 (17°C) |
| $BCF_{kL}$ | |
| $BCF_{SSL}$ | |
| Comment | Rainbow trout |

## 1.15 Azoxystrobin

**Table 43: Tier 3 – OECD TG 321 HYBIT BCF - Azoxystrobin**

| | Value |
|---|---|
| Source | Literature |
| Source details | Kosfeld et al. 2020 |
| Comment | |
| Results | |
| $BCF_k$ | 4 ± 1.7 |
| $BCF_{SS}$ | 3 ± 0.4 |
| $BCF_{kL}$ | 9 ± 4 (5% lipid)<br>5 ± 2.3 (3% lipid) |
| $BCF_{SSL}$ | 6 ± 1 (5% lipid)<br>4 ± 0.8 (3% lipid) |
| Comment | |
| Validity criteria (TG 321) | |
| Water temp. | |
| Dissolved $O_2$ | |
| TWA stable | Yes |
| Water conc. < solubility | |
| Mortality | |
| [NPOC] | |

| | Value |
|---|---|
| Comment | Data selected for comparison |

**Table 44: Tier 3 – OECD TG 319 A/B IVIVE BCF- Azoxystrobin**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Kosfeld et al. 2020 |
| Comment | |
| Hep. material | |
| Donor species | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes |
| Protein or cell concentration | 2 x $10^6$ cells/mL |
| Cell viability | |
| [Characterization of S9 / HEP] | No |
| Test setup | |
| Incubation temperature | 11°C |
| Assay duration | 45 min |
| Start conc. | 0.04 µM |
| Incubation volume | 1 mL |
| Inactive controls | Yes, HI-hepatocytes |
| Inactive controls < 20% loss? | Yes |
| Time points sampled | 8 |
| Comment | |
| Results | |
| Log-linear? | Yes |
| Rates | |
| Replicates | 3 |
| IVIVE BCF | 12 ($f_U$ = 1)<br>14 ($f_U$ = calc) |
| IVIVE model | Nichols et al. 2013 |
| Comment | Data selected for comparison |

**Table 45: Tier 4 – OECD TG 305 fish in vivo BCF - Azoxystrobin**

| | No data available |
|---|---|
| Source | |

| | No data available |
|---|---|
| Source details | |
| Comment | |

## 1.16 Prochloraz

**Table 46: Tier 3 – OECD TG 321 HYBIT BCF - prochloraz**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Kosfeld et al. 2020 | OECD 2024 | OECD 2024 |
| Comment | | Summary of ring trial data | Summary of ring trial data, TRR-based |
| Results | | | |
| $BCF_k$ | 97 ± 13 | 82/ 128/ 81/ 96/ 97/ 68/ 101/ 91/ 85/ 86<br>Mean: 92 ± 16 | 286 ± 30.9 (semi-static)<br>271 ± 24.4 (flow-through) |
| $BCF_{SS}$ | 94 ± 4 | 96/ 128/ 78/ 97/ 95/ 61/ 109/ 91/ 84/ 91<br>Mean: 93 ± 17.8 | 278 ± 9.3 (semi-static)<br>260 ± 23.1 (flow-through) |
| $BCF_{kL}$ | 308 ± 98 (5% lipid)<br>185 ± 59 (3% lipid) | 79/ 175/ 153/ 90/ 76/ 145/ 160/ 86/ 117/ 112 (3% lipid)<br>Mean: 119 ± 36. 6 | 343 ± 74.4 (semi-static, 3% lipid)<br>88.8 ± 24.1 (flow-through, 3% lipid) |
| $BCF_{SSL}$ | 298 ± 87 (5% lipid)<br>197 ± 52 (3% lipid) | 93/ 174/ 146/ 91/ 75/ 132/ 173/ 86/ 118/ 118 (3% lipid)<br>Mean: 121 ± 35.4 | 333 ± 63.6 (semi-static, 3% lipid)<br>355 ± 85.1 (flow-through, 3% lipid) |
| Comment | | | |
| Validity criteria (TG 321 | | | |
| Water temp. | | | |
| Dissolved $O_2$ | | | |
| TWA stable | | | |
| Water conc. < solubility | | | |
| Mortality | | | |
| [NPOC] | | | |
| Comment | Data selected for comparison | Data not selected for comparison (median $BCF_{kL}$ 3% lipid = 118, | Data not selected for comparison (TRR-based values) |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| | | other study represents "worst-case") | |

**Table 47: Tier 3 – OECD TG 319 A/B IVIVE BCF - prochloraz**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Kosfeld et al. 2020 |
| Comment | |
| Hep. Material | |
| Donor species | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes |
| Protein or cell concentration | 2 x $10^6$ cells/mL |
| Cell viability | |
| [Characterization of S9 / HEP] | No |
| Test setup | |
| Incubation temperature | 11°C |
| Assay duration | 45 min |
| Start conc. | 0.2 µM |
| Incubation volume | 1 mL |
| Inactive controls | Yes, HI-hepatocytes |
| Inactive controls < 20% loss? | Unclear (*cf*. Figure 1) |
| Time points sampled | 8 |
| Comment | |
| Results | |
| Log-linear? | No |
| Rates | |
| Replicates | 3 |
| IVIVE BCF | 366 ($f_U$ = 1)<br>1140 ($f_U$ = calc) |
| IVIVE model | Nichols et al. 2013 |
| Comment | BCF extrapolation: No log linear rate was obtained! |

**Table 48: Tier 4 – OECD TG 305 fish in vivo BCF - prochloraz**

| | Value 1 | Value 2 |
|---|---|---|
| Source | Literature | Admission report |
| Source details | EFSA 2011 | Admission Report for Eleando (German Federal Office of Consumer Protection and Food Safety) |
| Comment | | |
| Results | | |
| BCF | 371 ($^{14}$C-TRR)<br>197 ($^{14}$C-TRR) | 393 |
| Comment | Rainbow trout, Bluegill sunfish | |

## 1.17 Trifloxystrobin

**Table 49: Tier 3 – OECD TG 321 HYBIT BCF - trifloxystrobin**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Kosfeld et al. 2020 |
| Comment | |
| Results | |
| $BCF_k$ | 393 ± 62 |
| $BCF_{SS}$ | 354 ± 17 |
| $BCF_{kL}$ | 947 ± 196 (5% lipid)<br>568 ± 117 (3% lipid) |
| $BCF_{SSL}$ | 852 ± 120 (5% lipid)<br>511 ± 72 (3% lipid) |
| Comment | |
| Validity criteria (TG 321) | |
| Water temp. | |
| Dissolved $O_2$ | |
| TWA stable | |
| Water conc. < solubility | |
| Mortality | |
| [NPOC] | |
| Comment | Data selected for comparison |

**Table 50: Tier 3 – OECD TG 319 A/B IVIVE BCF – trifloxystrobin**

| | **Value 1** |
|---|---|
| Source | Literature |
| Source details | Kosfeld et al. 2020 |
| Comment | |
| Hep. Material | |
| Donor species | Rainbow trout |
| Hepatic material | Cryopreserved hepatocytes |
| Protein or cell concentration | 2 x $10^6$ cells/mL |
| Cell viability | |
| [Characterization of S9 / HEP] | No |
| Test setup | |
| Incubation temperature | 11°C |
| Assay duration | 60 min |
| Start conc. | 0.06 µM |
| Incubation volume | 1 mL |
| Inactive controls | Yes, HI-hepatocytes |
| Inactive controls < 20% loss? | Yes |
| Time points sampled | 8 |
| Comment | |
| Results | |
| Log-linear? | Yes |
| Rates | |
| Replicates | 3 |
| IVIVE BCF | 175 ($f_U$ = 1)<br>725 ($f_U$ = calc) |
| IVIVE model | Nichols et al. 2013 |
| Comment | Data selected for comparison |

**Table 51: Tier 4 – OECD TG 305 fish in vivo BCF – trifloxystrobin**

| | **Value** |
|---|---|
| Source | Literature |
| Source details | Jackson et al. 2009 |

| | Value |
|---|---|
| Comment | Data source: U.S. EPA Environmental Fate Database, http://cfpub.epa.gov/pfate/home.cfm |
| Result | |
| BCF | 372 - 537 (reported as log BCF) |
| Comment | Mean BCF |
| [NPOC] | |
| Comment | |

## 1.18 Diclofenac

**Table 52: Tier 3 – OECD TG 321 HYBIT BCF - diclofenac**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | This project | This project | Literature |
| Source details | *Cf*. chapter A.2.4 | *Cf*. chapter A.2.5 | Kosfeld et al. 2020 |
| Comment | Tested in ionised form | Tested in partly neutral form | Tested in ionised form |
| Results | | | |
| $BCF_k$ | | | 1 |
| $BCF_{SS}$ | | | 3 |
| $BCF_{kL}$ | | | N/A |
| $BCF_{SSL}$ | | | N/A |
| Comment | | | |
| Validity criteria (TG 321) | | | |
| Water temp. | | | |
| Dissolved $O_2$ | | | |
| TWA stable | | | |
| Water conc. < solubility | | | |
| Mortality | | Not valid | |
| [NPOC] | | | |
| Comment | Data selected for comparison | Tested at a nominal pH of 5<br>Data selected for comparison | Data not selected for comparison (no significant difference from the data generated in this project) |

**Table 53: Tier 3 – OECD TG 319 A/B IVIVE BCF - diclofenac**

| | Value 1 | Value 2 | Value 3 | Value 4 |
|---|---|---|---|---|
| Source | Literature | Literature | Literature | Literature |
| Source details | Kosfeld et al. 2020 | Connors et al. 2013 | Pihlaja et al. 2024 | Louisse et al. 2020 |
| Comment | | | | Summary of different studies |
| Hep. Material | | | | |
| Donor species | Rainbow trout | Rainbow trout | Rainbow trout | Rat |
| Hepatic material | Cryopreserved hepatocytes | S9 | S9 | Primary hepatocytes, fresh & cryopreserved |
| Protein or cell concentration | 2 x $10^6$ cells/mL | 1 mg/mL | 1 mg/mL | 0.5 - 2.5 * $10^6$ cells/mL |
| Cell viability | | - | - | N/A |
| [Characterization of S9 / HEP] | No | | | N/A |
| Test setup | | | | |
| Incubation temperature | 11°C | 11°C | 11°C | N/A |
| Assay duration | 150 min | | 180 | N/A |
| Start conc. | 3.54 µM | 1 µM | 1 µM | N/A |
| Incubation volume | 1 mL | | 1 mL | 0.05 - 1 mL |
| Inactive controls | Yes, HI-hepatocytes | Yes, HI-S9 | Yes, HI and room temperature denatured S9 | N/A |
| Inactive controls < 20% loss? | Unclear (*cf*. Figure 1) | No | Unclear (*cf*. Figure 1) | N/A |
| Time points sampled | 8 | 7 | 9 | N/A |
| Comment | | | | |
| Results | | | | |
| Log-linear? | Yes | Yes | Yes | N/A |
| Rates | | $CL_{IN\ VITRO,\ INT}$: 472 mL/h/g liver | $CL_{IN\ VITRO,\ INT}$: 0.118 ± 0.016 mL/h/mg protein (Batch RTL-S9 1802116 $CL_{IN\ VITRO,\ INT}$: 0.147 ± 0.012 mL/h/mg protein (Batch RTL-S9 200629-3 | $CL_{IN\ VITRO,\ INT}$: 86 \| 5.5 \| 5.6 \| 23 \| 26 \| 27.7 \| 59.9 \| 192 µL/min/ $10^6$ cells/mL |

| | Value 1 | Value 2 | Value 3 | Value 4 |
|---|---|---|---|---|
| Replicates | 3 | 2 | | 8 studies were compared |
| IVIVE BCF | 0 ($f_U$ = 1) log $K_{OW}$ = 0.7<br>0 ($f_U$ = calc.) log $K_{OW}$ = 0.7 | N/A | 460/ 9410<br>414/ 8479 | N/A |
| IVIVE model | Nichols et al. 2013 | N/A | Nichols et al. 2013 | N/A |
| Comment | Analytical difficulties, data should be treated with caution<br>Data selected for comparison | Data not selected for comparison (newer data available) | Data selected for comparison | Mean depletion rate used for extrapolation and data comparison |

**Table 54: Tier 3 – OECD TG 319 A/B IVIVE BCF - diclofenac**

| OECD TG 319 A/B - diclofenac | Value 1 |
|---|---|
| Source | Literature |
| Source details | Memmert et al. 2013 |
| Comment | |
| Results | |
| $BCF_k$ | 2 |
| $BCF_{SS}$ | 3 – 5 |
| $BCF_{kL}$ | N/A |
| $BCF_{SSL}$ | 5 – 9 |
| Comment | $^{14}$C-TRR-based values |

## 1.19 Fluoxetine

**Table 55: Tier 3 – OECD TG 321 HYBIT BCF - fluoxetine**

| | Value 1 | Value 2 |
|---|---|---|
| Source | This project | This project |
| Source details | *Cf*. chapter A.2.6 | *Cf*. chapter A.2.7 |
| Comment | Tested in ionised form | Tested in partly neutral form |
| Results | | |
| $BCF_k$ | | |

| | Value 1 | Value 2 |
|---|---|---|
| $BCF_{SS}$ | | |
| $BCF_{kL}$ | | |
| $BCF_{SSL}$ | | |
| Comment | | |
| Validity criteria (TG 321) | | |
| Water temp. | | |
| Dissolved $O_2$ | | |
| TWA stable | | |
| Water conc. < solubility | | |
| Mortality | | |
| [NPOC] | | |
| Comment | Data selected for comparison | Data selected for comparison |

**Table 56: Tier 3 – OECD TG 319 A/B IVIVE BCF - fluoxetine**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Connors et al. 2013 | Connors et al. 2013 | Bourgeois et al. 2022 |
| Comment | Evaluation of chirality on biotransformation | Comparison to humans | Psychotropic mixture testing |
| Hep. Material | | | |
| Donor species | Rainbow trout | Rainbow trout | Rainbow trout |
| Hepatic material | S9 | S9 | Cryopreserved hepatocytes |
| Protein or cell concentration | 1 mg/mL | 1 mg/mL | 1.35 x $10^6$ cells/mL |
| Cell viability | - | - | 86% |
| [Characterization of S9 / HEP] | | Yes | |
| **Test setup** | | | |
| Incubation temperature | 11°C | 11°C | |
| Assay duration | 180 min | 180 min | 150 min |
| Start conc. | 1 µM | 1µM | 5 µg/L (ca. 0.017µM) |
| Incubation volume | 0.2 mL | 0.2 mL | 5 mL |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Inactive controls | Yes, HI-S9 | Yes, HI-S9 | Yes, HI-hepatocytes |
| Inactive controls < 20% loss? | Yes | Yes | Yes |
| Time points sampled | 7 | 7 | 8 |
| Comment | | | |
| **Results** | | | |
| Log-linear? | Yes | Unknown | Yes |
| Rates | Not significantly different from negative control | No metabolism detected | Not significantly different from negative control |
| Replicates | 3 | | |
| IVIVE BCF | N/A | N/A | N/A |
| IVIVE model | N/A | N/A | N/A |
| Comment | None of the tested fluoxetine isomers were biotransformed in RT-S9 Data selected for comparison | Discussion: Smith et al. 2010 showed that trout microsomes metabolize Fluoxetine after induction with carbamazepine. Data selected for comparison | Data selected for comparison |

**Table 57: Tier 4 – OECD TG 305 fish in vivo BCF - fluoxetine**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Nakamura et al. 2008 |
| Comment | |
| Results | |
| $BCF_k$ | |
| $BCF_{SS}$ | 13 (pH 7, body + liver)<br>37 (pH 8, body + liver)<br>330 (pH 9, body + liver) |
| $BCF_{kL}$ | |
| $BCF_{SSL}$ | |
| Comment | Medaka |

## 1.20 17α-Ethinylestradiol

**Table 58: Tier 3 – OECD TG 321 HYBIT BCF - 17α-Ethinylestradiol**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Dussault et al. 2009 |
| Comment | |
| Results | |
| $BCF_k$ | N/A |
| $BCF_{SS}$ | 142 ± 11.6 at 0.02 mg/L<br>71 ± 7.1 at 0.03 mg/L<br>41 ± 2.4 at 0.1 mg/L<br>34 ± 0.7 at 0.29 mg/L<br>47 ± 4.6 at 0.91 mg/L |
| $BCF_{kL}$ | N/A |
| $BCF_{SSL}$ | N/A |
| Comment | Reported as BAFs, values have been derived from water-only exposures |
| Validity criteria (TG 321) | |
| Water temp. | |
| Dissolved $O_2$ | |
| TWA stable | |
| Water conc. < solubility | |
| Mortality | |
| [NPOC] | |
| Comment | Data selected for comparison:<br>Median BCF: 47 |

**Table 59: Tier 3 – OECD TG 319 A/B IVIVE BCF – 17α-Ethinylestradiol**

| | Value 1 |
|---|---|
| Source | Literature/ Database |
| Source details | EURL ECVAM Database (Halder et al. 2018) |
| Comment | Data downloaded 2023-01-11, Unpublished HESI data |
| Hep. Material | |
| Donor species | Rainbow trot |
| Hepatic material | S9 |

| | Value 1 |
|---|---|
| Protein or cell concentration | 2 mg/mL |
| Cell viability | - |
| [Characterization of S9 / HEP] | |
| Test setup | |
| Incubation temperature | 12°C |
| Assay duration | 120 min |
| Start conc. | 0.5 |
| Incubation volume | N/A |
| Inactive controls | N/A |
| Inactive controls < 20% loss? | N/A |
| Time points sampled | N/A |
| Comment | |
| Results | |
| Log-linear? | |
| Rates | $CL_{in\ vitro,\ int}$:<br>6.10E-02 mL/h/mg protein<br>1.54E-01 mL/h/mg protein<br>2.35E-01 mL/h/mg protein<br>1.18E-01 mL/h/mg protein<br>2.50E-02 mL/h/mg protein<br>8.80E-02 mL/h/mg protein<br>6.70E-02 mL/h/mg protein<br>2.10E-02 mL/h/mg protein<br>8.30E-02 mL/h/mg protein |
| Replicates | 9 |
| IVIVE BCF | N/A |
| IVIVE model | N/A |
| Comment | Slope quality rated as “low”<br>Data selected for comparison (no alternatives available) |

**Table 60: Tier 4 – OECD TG 305 fish in vivo BCF – 17α-Ethinylestradiol**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | OECD QSAR Toolbox |
| Comment | |
| Results | |

| | Value 1 |
|---|---|
| $BCF_k$ | 269 - 331 |
| $BCF_{SS}$ | |
| $BCF_{kL}$ | |
| $BCF_{SSL}$ | |
| Comment | |

## 1.21 UV-234

**Table 61: Tier 3 – OECD TG 321 HYBIT BCF – UV-234**

| | Value 1 | Value 2 |
|---|---|---|
| Source | This project | Literature |
| Source details | *Cf*. chapter A.2.3 | Schlechtriem et al. 2022 |
| Comment | | |
| Results | | |
| $BCF_k$ | | 725 |
| $BCF_{SS}$ | | 485 |
| $BCF_{kL}$ | | 1502 (5% lipid)<br>902 (3% lipid, lipid value recalculated from 5% $BCF_{kL}$ and $BCF_k$) |
| $BCF_{SSL}$ | | 1005 (5% lipid)<br>604 (3% lipid, lipid value recalculated from 5% $BCF_{kL}$ and $BCF_k$) |
| Comment | | Biofilm was present. Solvent used for exposure. |
| Validity criteria (TG 321) | | |
| Water temp. | | |
| Dissolved $O_2$ | | |
| TWA stable | | Yes |
| Water conc. < solubility | | |
| Mortality | | |
| [NPOC] | | N/A |
| Comment | Data selected for comparison | Data not selected for comparison (higher solvent usage compared to study in this project) |

**Table 62: Tier 3 – OECD TG 319 A/B IVIVE BCF – UV-234**

| | Value 1 | Value 2 |
|---|---|---|
| Source | This project | This project |
| Source details | *Cf*. chapter A.4.2, A.4.6, A.6.3 | *Cf*. chapter A.5.4 & A.7.3 |
| Comment | | |
| Hep. Material | | |
| Donor species | Rainbow trout | Rat (Sprague Dawley) |
| Hepatic material | Cryopreserved hepatocytes | Cryopreserved hepatocytes |
| Protein or cell concentration | | |
| Cell viability | | |
| [Characterization of S9 / HEP] | | |
| Test setup | | |
| Incubation temperature | | |
| Assay duration | | |
| Start conc. | | |
| Incubation volume | | |
| Inactive controls | | |
| Inactive controls < 20% loss? | | |
| Time points sampled | | |
| Comment | | |
| Results | | |
| Log-linear? | | |
| Rates | | |
| Replicates | | |
| IVIVE BCF | | |
| IVIVE model | | |
| Comment | Data selected for comparison | Data selected for comparison |

**Table 63: Tier 4 – OECD TG 305 fish in vivo BCF – UV-234**

| | Value 1 |
|---|---|
| Source | Registration data |
| Source details | ECHA registration dossier |
| Comment | EC number: 274-570-6 |

| | Value 1 |
|---|---|
| Results | |
| $BCF_k$ | |
| $BCF_{SS}$ | 224 |
| $BCF_{kgL}$ | 1286 |
| $BCF_{SSL}$ | 415 |
| Comment | $^{14}$C-TRR data, rainbow trout |

## 1.22 PFOS

**Table 64: Tier 3 – OECD TG 321 HYBIT BCF - PFOS**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Schlechtriem et al. 2022 | Schlechtriem et al. 2022 | Schlechtriem et al. 2022 |
| Comment | | | |
| Results | | | |
| $BCF_k$ | 302 | 185 | 406 |
| $BCF_{SS}$ | 176 | 111 | 245 |
| $BCF_{kL}$ | N/A | N/A | N/A |
| $BCF_{SSL}$ | N/A | N/A | N/A |
| Comment | Tap water<br>TWA = 49.4 µg/L | Borgmann medium<br>TWA = 50.0 µg/L | Extended uptake phase, tap water<br>TWA = 39.2 µg/L |
| Validity criteria (TG 321) | | | |
| Water temp. | | | |
| Dissolved $O_2$ | | | |
| TWA stable | Yes | Yes | Yes |
| Water conc. < solubility | | | |
| Mortality | | | |
| [NPOC] | | | |
| Comment | Data used for comparison: median BCF = 302 (non-lipid normalized) | | |

**Table 65: Tier 3 – OECD TG 319 A/B IVIVE BCF - PFOS**

| | No data found |
|---|---|
| Source | |

| | No data found |
|---|---|
| Source details | |
| Comment | |

**Table 66: Tier 4 – OECD TG 305 fish in vivo BCF - PFOS**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Literature | Literature |
| Source details | Risk profile on perfluorooctane sulfonate, United Nations Environment Programme | Gust et al. 2025 | Martin et al. 2003 |
| Comment | | | |
| Results | | | |
| $BCF_k$ | 2796 | | 1100 ± 150 (Carcass)<br>4300 ± 570 (Blood)<br>5400 ± 860 (Liver) |
| $BCF_{SS}$ | | 255 – 2136 | |
| $BCF_{kL}$ | | | |
| $BCF_{SSL}$ | | | |
| Comment | Bluegill sunfish | Zebrafish, different exposure concentrations | Rainbow trout |

## 1.23 GenX

**Table 67: Tier 3 – OECD TG 321 HYBIT BCF - GenX**

| | | Value 1 |
|---|---|---|
| | Source | Literature |
| | Source details | Schlechtriem et al. 2022 |
| | Comment | |
| | Results | |
| | $BCF_k$ | 7 |
| | $BCF_{SS}$ | 3 |
| | $BCF_{kL}$ | N/A |
| | $BCF_{SSL}$ | N/A |
| | Comment | |
| | Validity criteria (TG 321 | |
| ) | Water temp. | |

| | | Value 1 |
|---|---|---|
| | Dissolved $O_2$ | |
| | TWA stable | Yes |
| | Water conc. < solubility | |
| | Mortality | |
| | [NPOC] | |
| | Comment | Data used for comparison |

**Table 68: Tier 3 – OECD TG 319 A/B IVIVE BCF - GenX**

| | Value 1 |
|---|---|
| Source | Registration data |
| Source details | ECHA Registration Dossier (EC number: 700-242-3) |
| Comment | |
| Hep. material | |
| Donor species | Rainbow trout |
| Hepatic material | Hepatocytes |
| Protein or cell concentration | N/A |
| Cell viability | N/A |
| [Characterization of S9 / HEP] | N/A |
| **Test setup** | |
| Incubation temperature | N/A |
| Assay duration | N/A |
| Start conc. | N/A |
| Incubation volume | N/A |
| Inactive controls | N/A |
| Inactive controls < 20% loss? | N/A |
| Time points sampled | N/A |
| Comment | |
| **Results** | |
| Log-linear? | N/A |
| Rates | "… suggest the test substance is not metabolized by fish" |
| Replicates | N/A |
| IVIVE BCF | N/A |

| | Value 1 |
|---|---|
| IVIVE model | N/A |
| Comment | Data used for comparison |

**Table 69: Tier 4 – OECD TG 305 fish in vivo BCF - GenX**

| | Value 1 |
|---|---|
| Source | Registration data |
| Source details | ECHA Registration Dossier |
| Comment | EC number: 700-242-3 |
| Results | |
| $BCF_k$ | N/A |
| $BCF_{SS}$ | 8 at 0.01 µg/L<br>7 at 0.1 µg/L<br>2 at 1 µg/L<br>1 at 10 µg/L |
| $BCF_{kL}$ | N/A |
| $BCF_{SSL}$ | 8 at 0.01 µg/L<br>7 at 0.1 µg/L<br>2 at 1 µg/L<br>1 at 10 and 100 µg/L |
| Comment | |

## 1.24 D4 (Octamethylcyclotetrasiloxane)

**Table 70: Tier 3 – OECD TG 321 HYBIT BCF - D4 (Octamethylcyclotetrasiloxane)**

| | No data found |
|---|---|
| Source | |
| Source details | |
| Comment | |

**Table 71: Tier 3 – OECD TG 319 A/B IVIVE BCF - D4 (Octamethylcyclotetrasiloxane)**

| | Value 1 |
|---|---|
| Source | Cantu et al. 2025 |
| Source details | Literature |
| Comment | |
| Hep. Material | |
| Donor species | Rainbow trout |

| | Value 1 |
|---|---|
| Hepatic material | S9 |
| Protein or cell concentration | 1 mg/mL |
| Cell viability | - |
| [Characterization of S9 / HEP] | Yes |
| Test setup | |
| Incubation temperature | 12°C |
| Assay duration | 120 min |
| Start conc. | 0.25µM |
| Incubation volume | 1.5 mL |
| Inactive controls | Yes, HI-S9 |
| Inactive controls < 20% loss? | Yes |
| Time points sampled | 7 |
| Comment | |
| Results | |
| Log-linear? | 1/3 runs yes<br>2/3 runs no |
| Rates | $k_r$: 0.24 ± 0.03 $h^{-1}$<br>Mean ± SE of fish batch 3 |
| Replicates | 3 |
| IVIVE BCF | 11960 |
| IVIVE model | Cantu et al. 2025 |
| Comment | 2/3 $R^2$ are below 0.85, only one slope significantly different from negative control<br>Data used for comparison |

**Table 72: Tier 4 – OECD TG 305 fish in vivo BCF - D4 (Octamethylcyclotetrasiloxane)**

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| Source | Literature | Registration data | Database entry |
| Source details | Brooke et al. 2009 | ECHA Registration dossier | OECD QSAR Toolbox |
| Comment | Not primary source, Fackler et al. 1995 | EC number: 209-136-7 | |
| Results | | | |
| $BCF_k$ | | | |
| $BCF_{SS}$ | 12400 | | 5012 |
| $BCF_{kL}$ | | 14900 | |

| | Value 1 | Value 2 | Value 3 |
|---|---|---|---|
| $BCF_{SSL}$ | | | |
| Comment | $^{14}$C-TRR-based, Fathead minnow | | |

## 1.25 Lauric acid

**Table 73: Tier 3 – OECD TG 321 HYBIT BCF - Lauric acid**

| | Value 1 |
|---|---|
| Source | Literature |
| Source details | Raths et al. 2020 |
| Comment | |
| Results | |
| $BCF_k$ | |
| $BCF_{SS}$ | |
| $BCF_{kL}$ (3% lipid) | 9 ± 3 (5% lipid)<br>6 (3% lipid) |
| $BCF_{SSL}$ | |
| Comment | |
| Validity criteria (TG 321) | |
| Water temp. | |
| Dissolved $O_2$ | |
| TWA stable | |
| Water conc. < solubility | |
| Mortality | |
| [NPOC] | |
| Comment | Data used for comparison |

**Table 74: Tier 3 – OECD TG 319 A/B IVIVE BCF - Lauric acid**

| | No data found |
|---|---|
| Source | |
| Source details | |
| Comment | |

**Table 75: Tier 4 – OECD TG 305 fish in vivo BCF - Lauric acid**

| | **Value 1** |
|---|---|
| Source | Literature |
| Source details | Van Egmont et al 1999 |
| Comment | |
| Results | |
| $BCF_k$ | 255 ± 22 |
| $BCF_{SS}$ | |
| $BCF_{kL}$ | |
| $BCF_{SSL}$ | |
| Comment | Zebrafish |

# 2 BCF QSAR

See Excel file “BCF QSAR supplementary material”

# 3 log $K_{OW}$

The online version of the following publication contains the supplementary material on log $K_{OW}$: Nendza, M., Kosfeld, V., & Schlechtriem, C. (2025). Consolidated octanol/water partition coefficients: combining multiple estimates from different methods to reduce uncertainties in log KOW. Environmental Sciences Europe, 37, Article 44. https://doi.org/10.1186/s12302-025-01072-2

# 4 Log $K_{OA}$

See Excel file “log Koa supplementary material”

# 5 pKa

See Excel file “pKa supplementary material”

# 6 HYBIT (OECD TG 321) – Detailed data

## 6.1 Pyrene

**Table 76: HYBIT - pyrene - tissue concentration**

| Time (h) | Replicate 1 (mg/kg) | Replicate 2 (mg/kg) | Replicate 3 (mg/kg) |
|---|---|---|---|
| 0 | 0.0000 | 0.0000 | 0.0000 |
| 3 | 0.9190 | 1.0067 | 1.0137 |
| 10 | 1.7777 | 1.7680 | 1.8280 |
| 24 | 1.9421 | 1.6411 | 1.6020 |
| 48 | 2.2575 | 2.0962 | 2.0345 |
| 72 | 2.2702 | 1.9788 | 1.9518 |
| 96 | 2.1254 | 2.1068 | 2.1051 |
| 144 | 1.7713 | 1.8830 | 2.1694 |
| 147 | 1.1967 | 1.3385 | 1.3504 |
| 149 | 1.3210 | 1.3968 | 1.2264 |
| 154 | 1.0725 | 0.8951 | 1.0092 |
| 168 | 0.2556 | 0.0739 | 0.2063 |
| 192 | 0.0733 | 0.0509 | 0.0725 |
| 216 | 0.0586 | 0.0450 | 0.0379 |
| 240 | 0.0146 | 0.0325 | 0.0349 |

**Table 77: HYBIT - pyrene - water concentration**

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) |
|---|---|---|
| 0 | 0.00118 | 0.00111 |
| 10 | 0.00098 | 0.00123 |
| 48 | 0.00097 | 0.00089 |
| 72 | 0.00093 | 0.00094 |
| 96 | 0.00102 | 0.00091 |
| 144 | 0.00094 | 0.00094 |
| 172 | 0.00014 | 0.00014 |

**Table 78: HYBIT - pyrene - lipid content determination**

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 31.80 | 1.669* | 5.25* |

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 30.00 | 0.435 | 1.45 |
| 0 | 23.63 | 0.198 | 0.84 |
| 72 | 28.96 | 0.724 | 2.50 |
| 72 | 36.96 | 0.66 | 1.79 |
| 72 | 35.93 | 0.793 | 2.21 |
| 144 | 27.44 | 0.751 | 2.74 |
| 144 | 34.42 | 1.046 | 3.04 |
| 144 | 37.52 | 1.108 | 2.95 |

* = data excluded from calculations

**Table 78: HYBIT - pyrene - monitoring parameters**

| | Temperature (°C) | pH | $O_2$ (mg/L) | $O_2$ (%) | NPOC (mg/L) | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| Mean | 22.5 | 7.68 | 7.97 | 96.12 | 0.5189 | 2.00 |
| Min | 22.0 | 7.39 | 7.79 | 94.60 | 0.9769 | 1.49 |
| Max | 23.2 | 7.95 | 8.15 | 97.20 | 0.7372 | 2.26 |
| SD | 0.3 | 0.15 | 0.10 | 0.78 | 0.2304 | 0.31 |

**Table 80: HYBIT - pyrene - water chemistry analyses**

| Time (h) | Nitrate (mg/L) | Nitrite (mg/L) | Ammonium (mg/L) |
|---|---|---|---|
| 0 | 9 | <0.005 | <0.01 |
| 144 | 8 | <0.005 | <0.01 |
| 240 | 8 | <0.005 | 0.01 |

## 6.2 β-HCH

**Table 81: HYBIT – β-HCH - tissue concentration**

| Time (h) | Replicate 1 (mg/kg) | Replicate 2 (mg/kg) | Replicate 3 (mg/kg) |
|---|---|---|---|
| 0 | 0.0000 | 0.0000 | 0.0000 |
| 3 | 0.7461 | 0.7291 | 0.8841 |
| 6 | 1.0896 | 0.9201 | 0.8455 |
| 10 | 1.1638 | 1.2831 | 1.2958 |
| 24 | 2.4439 | 2.6506 | 2.3127 |
| 48 | 5.2846* | 3.5945 | 3.7773 |

| Time (h) | Replicate 1 (mg/kg) | Replicate 2 (mg/kg) | Replicate 3 (mg/kg) |
|---|---|---|---|
| 72 | 4.4916 | 4.4040 | 3.8091 |
| 96 | 3.6011 | 4.1216 | 3.7463 |
| 99 | 3.4379 | 2.9040 | 3.4536 |
| 102 | 2.7370 | 2.9601 | 2.3212 |
| 106 | 2.8369 | 1.8799 | 1.7663 |
| 120 | 1.0740 | 1.1200 | 0.7475 |
| 144 | 0.3064 | 0.3097 | 0.1491 |
| 168 | 0.1148 | 0.0297 | 0.0496 |
| 192 | 0.0218 | 0.0336 | 0.0493 |

* = data excluded from calculations

**Table 82: HYBIT – β-HCH - water concentration**

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) |
|---|---|---|
| 0 | 0.00690 | 0.00636 |
| 6 | 0.00691 | 0.00678 |
| 24 | 0.00704 | 0.00689 |
| 48 | 0.00779 | 0.00756 |
| 72 | 0.00811 | 0.00808 |
| 96 | 0.00902 | 0.00885 |
| 99 | 0.00053 | 0.00053 |

**Table 83: HYBIT – β-HCH - lipid content determination**

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 42.70 | 0.708 | 1.66 |
| 0 | 51.34 | 0.823 | 1.60 |
| 0 | 41.47 | 0.67 | 1.62 |
| 72 | 35.28 | 0.619 | 1.75 |
| 72 | 34.64 | 0.65 | 1.88 |
| 72 | 32.70 | 0.513 | 1.57 |
| 144 | 45.34 | 0.577 | 1.27 |
| 144 | 41.65 | 0.771 | 1.85 |
| 144 | 34.63 | 0.73 | 2.11 |

**Table 84: HYBIT – β-HCH - monitoring parameters**

| | Temperature (°C) | pH | $O_2$ (mg/L) | $O_2$ (%) | NPOC (mg/L) | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| Mean | 23.6 | 7.78 | 7.93 | 97.02 | 1.066 | 2.04 |
| Min | 23.0 | 7.65 | 7.77 | 94.60 | 0.461 | 1.92 |
| Max | 24.0 | 7.89 | 8.44 | 102.20 | 2.395 | 2.12 |
| SD | 0.33 | 0.08 | 0.22 | 2.25 | 0.912 | 0.08 |

**Table 85: HYBIT – β-HCH - water chemistry analyses**

| water chemistry analyses | Nitrate (mg/L) | Nitrite (mg/L) | Ammonium (mg/L) |
|---|---|---|---|
| 0 | 5 | 0.009 | <0.01 |
| 72 | 5 | <0.005 | 0.06 |
| 168 | 7 | <0.005 | <0.01 |

## 6.3 UV-234

**Table 85: HYBIT – UV-234 - tissue concentration**

| Time (h) | Replicate 1 (mg/kg) | Replicate 2 (mg/kg) | Replicate 3 (mg/kg) |
|---|---|---|---|
| 0 | 0.0000 | 0.0161 | 0.0000 |
| 8 | 0.1338 | 0.1004 | 0.1585 |
| 24 | 0.3381 | 0.3583 | 0.3698 |
| 48 | 0.8224 | 0.9643 | 0.9708 |
| 96 | 1.4233 | 1.4796 | 1.2650 |
| 144 | 1.1868 | 1.2292 | 0.8789 |
| 192 | 1.4766 | 1.6709 | 1.5870 |
| 240 | 2.3606 | 1.6480 | 1.2631 |
| 256 | 0.6784 | 0.7437 | 0.7324 |
| 272 | 0.5794 | 0.4644 | 0.5319 |
| 288 | 6.5857* | 0.4043 | 0.3142 |
| 312 | 0.2429 | 0.3727 | N/A |
| 339 | 0.1797 | 0:1379 | N/A |
| 384 | 0.0938 | 0.1112 | N/A |
| 456 | 0.0244 | 0.0143 | 0.0190 |

* = data excluded from calculations

**Table 87: HYBIT – UV-234 - water concentration**

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) | Replicate 3 (mg/L) |
|---|---|---|---|
| -1 | 0.000836 | 0.00084 | 0.001652 |
| 8 | 0.000944 | 0.001046 | 0.001086 |
| 24 | 0.000826 | 0.000828 | 0.001168 |
| 48 | 0.000932 | 0.000694 | 0.000674 |
| 72 | 0.000868 | 0.000726 | 0.000854 |
| 95 | 0.001138 | 0.000862 | 0.001024 |
| 119 | 0.001196 | 0.001118 | 0.000908 |
| 130 | 0.001502 | 0.001398 | 0.001282 |
| 143 | 0.001132 | 0.001166 | 0.001138 |
| 168 | 0.000802 | 0.000814 | 0.00082 |
| 195 | 0.001272 | 0.001344 | 0.000756 |
| 240 | 0.001438 | 0.000826 | 0.000842 |
| 247 | < LOD | < LOD | < LOD |

**Table 88: HYBIT – UV-234 - lipid content determination**

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 26.79 | 0.34 | 1.27* |
| 0 | 27.75 | 0.32 | 1.15* |
| 0 | 25.78 | 0.33 | 1.28* |
| 72 | 20.87 | 0.58 | 2.78 |
| 72 | 34.31 | 1.16 | 3.38 |
| 72 | 37.60 | 0.76 | 2.02 |
| 144 | 39.87 | 1.32 | 3.31 |
| 144 | 40.63 | 0.77 | 1.90 |
| 144 | 63.55 | 1.04 | 1.64 |

* = data excluded from calculations

**Table 89: HYBIT – UV-234 – monitoring parameters**

| | Temperature (°C) | pH | $O_2$ (mg/L) | $O_2$ (%) | NPOC (mg/L) | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| Mean | 23.1 | 8.17 | 8.36 | 100.2 | 4.4581 | 6.58 |
| Min | 22.3 | 8.01 | 6.66 | 82.9 | 0.6957 | 6.13 |
| Max | 23.5 | 8.63 | 10.76 | 120.8 | 7.4790 | 7.16 |

| | Temperature (°C) | pH | $O_2$ (mg/L) | $O_2$ (%) | NPOC (mg/L) | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| SD | 0.4 | 0.14 | 1.11 | 10.2 | 3.3329 | 0.32 |

**Table 90: HYBIT – UV-234 – water chemistry analyses**

| Time (h) | Nitrate (mg/L) | Nitrite (mg/L) | Ammonium (mg/L) |
|---|---|---|---|
| 0 | 7 | 0.005 | 0.5 |
| 120 | 7 | <0.005 | 0.02 |
| 264 | 8 | <0.005 | <0.01 |
| 456 | 15 | <0.005 | 0.01 |

## 6.4 Diclofenac (ionised)

**Table 91: HYBIT – diclofenac (ionised) - tissue concentration**

| Time (h) | Replicate 1 (mg/kg) | Replicate 2 (mg/kg) | Replicate 3 (mg/kg) |
|---|---|---|---|
| 0 | 0.0000 | 0.0000 | 0.0000 |
| 1 | 0.0173 | 0.0224 | 0.0174 |
| 2 | 0.0224 | 0.0242 | 0.0232 |
| 4 | 0.0391 | 0.0340 | 0.0349 |
| 6 | 0.0529 | 0.0459 | 0.0482 |
| 10 | 0.0572 | 0.0525 | 0.0538 |
| 23 | 0.0700 | 0.0896 | 0.0667 |
| 31 | 0.0849 | 0.0669 | 0.0688 |
| 48 | 0.0744 | 0.0736 | 0.0846 |
| 49 | 0.0564 | 0.0641 | 0.0584 |
| 50 | 0.0400 | 0.0430 | 0.0583 |
| 52 | 0.0404 | 0.0538 | 0.0317 |
| 54 | 0.0297 | 0.0371 | 0.0309 |
| 58 | 0.0270 | 0.0372 | 0.0299 |
| 72 | 0.0181 | 0.0147 | 0.0147 |
| 96 | 0.0068 | 0.0060 | 0.0045 |

**Table 92: HYBIT – diclofenac (ionised) - water concentration**

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) | Replicate 3 (mg/L) |
|---|---|---|---|
| 0 | 0.05627 | 0.05644 | 0.05760 |
| 6 | 0.05243 | 0.05168 | 0.04947 |

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) | Replicate 3 (mg/L) |
|---|---|---|---|
| 24 | 0.05318 | 0.05209 | 0.05305 |
| 48 | 0.05197 | 0.05417 | 0.05404 |

**Table 93: HYBIT – diclofenac (ionised) - lipid content determination**

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 60.90 | 1.357 | 2.23 |
| 0 | 60.19 | 1.161 | 1.93 |
| 0 | 62.78 | 0.936 | 1.49 |
| 48 | 59.30 | 1.230 | 2.07 |
| 48 | 63.15 | 1.392 | 2.20 |
| 48 | 66.40 | 1.402 | 2.11 |
| 96 | 70.31 | 1.429 | 2.03 |
| 96 | 59.68 | 1.272 | 2.13 |
| 96 | 62.09 | 1.488 | 2.40 |

**Table 94: HYBIT – diclofenac (ionised) - monitoring parameters**

| | Temperature (°C) | pH | $O_2$ (mg/L) | $O_2$ (%) | NPOC (mg/L) | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| Mean | 23.6 | 7.88 | 7.70 | 94.92 | 1.765 | 2.06 |
| Min | 23.4 | 7.76 | 7.31 | 89.30 | 1.091 | 2.04 |
| Max | 23.7 | 7.95 | 8.06 | 100.10 | 2.650 | 2.08 |
| SD | 0.1 | 0.07 | 0.29 | 3.61 | 0.661 | 0.02 |

**Table 95: HYBIT – diclofenac (ionised) - water chemistry analyses**

| Time (h) | Nitrate (mg/L) | Nitrite (mg/L) | Ammonium (mg/L) |
|---|---|---|---|
| 0 | 10 | 0.022 | < 0.01 |
| 48 | 10 | 0.059 | < 0.01 |
| 96 | 10 | 0.006 | < 0.01 |

## 6.5 Diclofenac (partly neutral)

**Table 96: HYBIT - Diclofenac (partly neutral) - tissue concentration**

| Time (h) | Replicate 1 (mg/kg) | Replicate 2 (mg/kg) | Replicate 3 (mg/kg) |
|---|---|---|---|
| 0 | 0.000 | 0.000 | 0.000 |
| 4 | 0.725 | 0.539 | 0.594 |
| 8 | 1.105 | 1.273 | 1.771 |

| Time (h) | Replicate 1 (mg/kg) | Replicate 2 (mg/kg) | Replicate 3 (mg/kg) |
|---|---|---|---|
| 24 | 1.715 | 1.122 | 1.512 |
| 48 | 2.815 | 2.546 | 2.360 |
| 72 | 1.853 | 1.818 | 1.905 |
| 96 | 1.910 | 1.576 | 1.659 |
| 120 | 1.583 | 1.237 | 1.238 |
| 124 | 0.681 | 0.897 | N/A |
| 128 | 0.606 | 0.451 | N/A |
| 144 | 0.197 | 0.225 | N/A |
| 168 | 0.047577045 | 0.096710259 | N/A |
| 192 | 0.021050986 | 0.102720947 | N/A |
| 216 | 0.025286065 | 0.016717757 | N/A |
| 240 | 0.00430713 | 0.008729706 | N/A |

**Table 97: HYBIT - Diclofenac (partly neutral) - water concentration**

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) | Replicate 3 (mg/L) |
|---|---|---|---|
| 0 | 0.04558 | 0.05564 | 0.04512 |
| 8 | 0.04543 | 0.04589 | 0.04489 |
| 24 | 0.04473 | 0.04559 | 0.04625 |
| 48 | 0.04202 | 0.04309 | 0.04834 |
| 72 | 0.04368 | 0.04247 | 0.04260 |
| 96 | 0.04446 | 0.04616 | 0.04638 |
| 120 | 0.04711 | 0.05135 | 0.04809 |

**Table 98: HYBIT - Diclofenac (partly neutral) - lipid content determination**

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 83.53 | 0.862 | 1.03 |
| 0 | 73.22 | 0.807 | 1.10 |
| 0 | 79.99 | 1.016 | 1.27 |
| 120 | 78.32 | 1.203 | 1.54 |
| 120 | 74.28 | 1.245 | 1.68 |
| 120 | 73.15 | 1.198 | 1.64 |
| 240 | 43.48 | 0.833 | 1.92 |
| 240 | 40.67 | 0.900 | 2.21 |

**Table 99: HYBIT - Diclofenac (partly neutral) - monitoring parameters**

| | Temperature (°C) | pH* | $O_2$ (mg/L) | $O_2$ (%) | NPOC (mg/L)** | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| Mean | 23.6 | 5.30 | 7.77 | 94.87 | 711.28 | 2.08 |
| Min | 23.5 | 4.88 | 6.58 | 82.00 | 641.50 | 2.06 |
| Max | 23.8 | 5.58 | 8.82 | 102.90 | 799.20 | 2.17 |
| SD | 0.1 | 0.20 | 0.57 | 5.65 | 76.49 | 0.03 |

*Data of pH drop excluded. Full data in Table 100

** Values were above calibration (100 mg/L)

**Table 100: HYBIT - Diclofenac (partly neutral) - pH measurements**

| Time [h] | pH |
|---|---|
| 0 | 5.39 |
| 24 | 4.88 |
| 25 | 3.12 |
| 26 | 3.18 |
| 26.5 | 3.23 |
| 26.75 | 3.41 |
| 27 | 3.2 |
| 27.5 | 3.2 |
| 28 | 4.36 |
| 28.5 | 3.33 |
| 28.75 | 2.98 |
| 29 | 3.13 |
| 30 | 4.74 |
| 31.5 | 4.87 |
| 48 | 5.07 |
| 72 | 5.34 |
| 96 | 5.38 |
| 120 | 5.58 |
| 120 | 5.31 |
| 144 | 5.47 |
| 168 | 5.49 |

| Time [h] | pH |
|---|---|
| 192 | 5.35 |
| 216 | 5.12 |
| 240 | 5.2 |

**Table 101: HYBIT - Diclofenac (partly neutral) - water chemistry analyses**

| Time (h) | Nitrate (mg/L) | Nitrite (mg/L) | Ammonium (mg/L) |
|---|---|---|---|
| 0 | 11 | 0.005 | 0.01 |
| 120 | 10 | 0.008 | 0.04 |
| 240 | 9 | < 0.005 | < 0.01 |

## 6.6 Fluoxetine (ionized)

**Table 102: HYBIT - Fluoxetine (ionized) - tissue concentration**

| Time (h) | Replicate 1 (mg/kg) Fluoxetine | Replicate 2 (mg/kg) Fluoxetine | Replicate 3 (mg/kg) Fluoxetine | Replicate 1 (mg/kg) Norfluoxetine | Replicate 2 (mg/kg) Norfluoxetine | Replicate 3 (mg/kg) Norfluoxetine |
|---|---|---|---|---|---|---|
| 0 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 2 | 0.2760 | 0.2661 | 0.3071 | 0.083 | 0.079 | 0.076 |
| 4 | 0.4571 | 0.4177 | 0.3757 | 0.117 | 0.140 | 0.171 |
| 8 | 0.7605 | 0.7634 | 0.6140 | 0.184 | 0.279 | 0.368 |
| 24 | 1.1472 | 0.9983 | 1.1318 | 0.978 | 0.638 | 0.780 |
| 48 | 1.1783 | 1.0035 | 1.0817 | 0.694 | 0.982 | 1.567 |
| 72 | 0.8014 | 0.9708 | 0.9099 | 1.698 | 1.103 | 1.242 |
| 95 | 1.2819 | 1.0292 | 0.9956 | 1.919 | 1.111 | 1.506 |
| 121 | 0.8951 | 0.8735 | 0.8964 | 1.679 | 1.856 | 2.157 |
| 144 | 0.8257 | 0.8648 | 0.8922 | 1.826 | 1.966 | 2.037 |
| 145 | 0.7128 | 0.9368 | 0.8925 | 1.881 | 1.619 | 2.142 |
| 146 | 0.7515 | 0.6058 | 0.8399 | 2.284 | 1.856 | 2.015 |
| 148 | 0.5555 | 0.4941 | 0.6997 | 2.215 | 2.390 | 2.398 |
| 152 | 0.3201 | 0.5769 | 0.5321 | 1.806 | 1.463 | 1.747 |
| 168 | 0.0704 | 0.1234 | 0.1094 | 2.016 | 0.854 | 1.487 |
| 192 | 0.0000 | 0.0000 | 0.0000 | 0.890 | 0.432 | 0.741 |
| 240 | 0.0000 | 0.0000 | 0.0000 | 0.227 | 0.370 | 0.176 |

**Table 103: HYBIT - Fluoxetine (ionized) - water concentration**

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) | Replicate 3 (mg/L) |
|---|---|---|---|
| 0 | 0.04552 | 0.04043 | 0.04574 |
| 4 | 0.04665 | 0.04802 | 0.04418 |
| 24 | 0.05610 | 0.04466 | 0.05219 |
| 48 | 0.04400 | 0.05171 | 0.04646 |
| 72 | 0.04616 | 0.04728 | 0.04339 |
| 96 | 0.03869 | 0.03655 | 0.03995 |
| 120 | 0.05058 | 0.05466 | 0.05347 |
| 144 | 0.05408 | 0.06527 | 0.04231 |

**Table 104: HYBIT - Fluoxetine (ionized) - lipid content determination**

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 40.62 | 1.05 | 2.58 |
| 0 | 38.25 | 1.04 | 2.72 |
| 0 | 38.86 | 0.88 | 2.26 |
| 144 | 46.27 | 1.46 | 3.16 |
| 144 | 52.85 | 1.76 | 3.33 |
| 144 | 52.40 | 1.70 | 3.24 |
| 240 | 56.27 | 1.69 | 3.00 |
| 240 | 54.14 | 1.92 | 3.55 |

**Table 102: HYBIT - Fluoxetine (ionized) - monitoring parameters**

| | Temperature (°C) | pH | $O_2$ (mg/L) | $O_2$ (%) | NPOC** (mg/L) | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| Mean | 23.5 | 6.02 | 7.77 | 94.9 | 684.38 | 2.04 |
| Min | 23.4 | 5.92 | 6.58 | 82.0 | 577.90 | 1.99 |
| Max | 23.6 | 6.13 | 8.82 | 102.9 | 855.80 | 2.20 |
| SD | 0.1 | 0.06 | 0.57 | 5.7 | 123.04 | 0.03 |

** Values were above calibration (100 mg/L)

**Table 106: HYBIT - Fluoxetine (ionized) - water chemistry analyses**

| Time (h) | Nitrate (mg/L) | Nitrite (mg/L) | Ammonium (mg/L) |
|---|---|---|---|
| 0 | 9 | < 0.005 | < 0.01 |
| 144 | 9 | 0.005 | < 0.01 |
| 240 | 9 | < 0.005 | < 0.01 |

## 6.7 Fluoxetine (partly neutral)

**Table 107: HYBIT - Fluoxetine (partly neutral) - tissue concentration**

| Time (h) | Replicate 1 (mg/kg) Fluoxetine | Replicate 2 (mg/kg) Fluoxetine | Replicate 3 (mg/kg) Fluoxetine | Replicate 1 (mg/kg) Norfluoxetine | Replicate 2 (mg/kg) Norfluoxetine | Replicate 3 (mg/kg) Norfluoxetine |
|---|---|---|---|---|---|---|
| 0 | 0.00 | 0.00 | 0.00 | 0.000 | 0.000 | 0.000 |
| 2 | 2.24 | 2.07 | 2.27 | 0.224 | 0.293 | 0.224 |
| 4 | 2.61 | 2.99 | 2.74 | 0.602 | 0.415 | 0.414 |
| 8 | 3.79 | 3.76 | 3.43 | 0.778 | 2.039 | 0.926 |
| 24 | 7.42 | 6.94 | 6.55 | 4.920 | 4.955 | 5.802 |
| 48 | 13.78 | 7.46 | 12.85 | 6.738 | 6.697 | 12.170 |
| 72 | 9.10 | 7.90 | 13.04 | 6.607 | 9.249 | 10.472 |
| 96 | 11.93 | 18.84 | 10.84 | 10.453 | 8.506 | 11.760 |
| 120 | 12.24 | 10.16 | 15.99 | 14.520 | 12.760 | 9.118 |
| 144 | 27.30 | 20.76 | 9.02 | 13.911 | 10.927 | 15.296 |
| 146 | 26.12 | 12.79 | 16.39 | 11.047 | 15.584 | 16.316 |
| 148 | 18.85 | 22.77 | 9.49 | 9.772 | 10.372 | 16.654 |
| 152 | 9.91 | 14.53 | 18.17 | 10.752 | 14.655 | 19.549 |
| 168 | 6.33 | 9.73 | 1.20 | 13.891 | 10.469 | 19.883 |
| 192 | 4.73 | 5.87 | 1.37 | 11.991 | 9.655 | 5.555 |
| 240 | N/A | 4.78 | 0.28 | 2.919 | 5.311 | 2.731 |

**Table 108: HYBIT - Fluoxetine (partly neutral) - water concentration**

| Time (h) | Replicate 1 (mg/L) | Replicate 2 (mg/L) | Replicate 3 (mg/L) |
|---|---|---|---|
| 0 | 0.04696 | 0.05068 | 0.05290 |
| 4 | 0.04771 | 0.05400 | 0.05094 |
| 24 | 0.05166 | 0.05083 | 0.05290 |
| 48 | 0.05084 | 0.05973 | 0.05639 |
| 72 | 0.01474 | 0.01444 | 0.01620 |
| 96 | 0.04125 | 0.04044 | 0.03778 |
| 120 | 0.04160 | 0.04500 | 0.04500 |
| 144 | 0.04670 | 0.04860 | 0.04430 |

**Table 109: HYBIT - Fluoxetine (partly neutral) - lipid content determination**

| Time (h) | Hyalella sample weight (mg) | Lipid weight (mg) | Lipid content (%) |
|---|---|---|---|
| 0 | 49.34 | 0.685 | 1.388* |
| 0 | 47.07 | 0.604 | 1.283* |
| 0 | 45.45 | 0.583 | 1.283* |
| 144 | 51.00 | 2.174 | 4.263 |
| 144 | 55.07 | 1.333 | 2.421 |
| 144 | 53.05 | 1.347 | 2.539 |
| 240 | 54.45 | 1.360 | 2.498 |
| 240 | 56.50 | 1.530 | 2.708 |
| 240 | 64.07 | 1.608 | 2.510 |

* = data excluded from calculations

**Table 110: HYBIT - Fluoxetine (partly neutral) - monitoring parameters**

| | Temperature (°C) | pH | $O_2$ (mg/L) | $O_2$ (%) | NPOC** (mg/L) | Total flow rate (L/h) |
|---|---|---|---|---|---|---|
| Mean | 23.4 | 8.90 | 7.51 | 91.77 | 452.78 | 2.06 |
| Min | 23.1 | 8.83 | 6.68 | 81.10 | 404.20 | 1.98 |
| Max | 24.1 | 8.99 | 8.01 | 99.70 | 495.70 | 2.16 |
| SD | 0.3 | 0.05 | 0.44 | 5.68 | 39.66 | 0.05 |

** Values were above calibration (100 mg/L)

**Table 111: HYBIT - Fluoxetine (partly neutral) - water chemistry analyses**

| Time (h) | Nitrate (mg/L) | Nitrite (mg/L) | Ammonium (mg/L) |
|---|---|---|---|
| 0 | 9 | < 0.005 | < 0.01 |
| 144 | < 2 | 0.027 | < 0.01 |
| 240 | 8 | < 0.012 | < 0.01 |

# 7 IVIVE raw data

## 7.1 Depletion assay with rainbow trout hepatocytes – pyrene

**Table 112: Depletion assay with rainbow trout hepatocytes - pyrene - concentration data of HI samples**

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 9.25 | 0 | 10.4 | 0 | 8.8 |
| 5 | 9.05 | 2 | 8.9 | 2 | 9.8 |
| 10 | 8.82 | 5 | 8.9 | 5 | 8.9 |
| 20 | 8.88 | 10 | 9.0 | 10 | 8.7 |
| 30 | 9.21 | 20 | 9.0 | 20 | 8.2 |
| 50 | 8.87 | 30 | 8.9 | 30 | 8.3 |

**Table 113: Depletion assay with rainbow trout hepatocytes - pyrene - concentration data of active samples**

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 9.6 | 0 | 9.2 | 0 | 8.6 |
| 5 | 3.1 | 2 | 6.5 | 2 | 5.7 |
| 10 | 1.6 | 5 | 3.6 | 5 | 3.6 |
| 20 | 0.6 | 10 | 1.8 | 10 | 2.0 |
| 30 | 0.3* | 20 | 0.7 | 20 | 0.6 |
| 50 | 0.2* | 30 | 0.4* | 30 | 0.2* |

* Data excluded from calculation

**Table 114: Depletion assay with rainbow trout hepatocytes - pyrene - results of the linear regressions**

| | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| Slope ($h^{-1}$) active | -2.830 | -2.800 | -3.126 |
| $R^2$ | 0.9520 | 0.9605 | 0.9927 |
| Recovery (%) | 27.2 | 31.3 | 36.6 |
| Viability (%) | 84.1 | 85.4 | 81.9 |
| Cell density ($10^6$ cells/mL) | 2.14 | 1.90 | 1.66 |
| Loss in HI at end of incubation (%) | 4.1 | 14.9 | 4.7 |

## 7.2 Depletion assay with rainbow trout hepatocytes – β-HCH

**Table 115: Depletion assay with rainbow trout hepatocytes – β-HCH- concentration data of HI samples**

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 21.4 | 0 | 12.9 | 0 | 21.0 |
| 15 | 13.1 | 15 | 13.6 | 15 | 22.4 |
| 30 | 20.1 | 30 | 15.3 | 30 | 21.8 |
| 60 | 18.1 | 60 | 11.1 | 60 | 22.5 |
| 120 | 19.0 | 120 | 14.1 | 120 | 18.8 |
| 180 | 18.5 | 180 | 15.0 | 180 | 20.2 |
| 240 | 18.2 | 240 | 13.1 | 240 | 18.9 |

**Table 116: Depletion assay with rainbow trout hepatocytes – β-HCH- concentration data of active samples**

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 21.7 | 0 | 13.7 | 0 | 19.6 |
| 15 | 20.2 | 15 | 16.0 | 15 | 22.4 |
| 30 | 22.2 | 30 | 13.8 | 30 | * |
| 60 | 19.7 | 60 | 14.5 | 60 | 21.4 |
| 120 | 21.1 | 120 | 13.7 | 120 | 21.1 |
| 180 | 20.3 | 180 | 14.2 | 180 | 21.7 |
| 240 | 19.4 | 240 | 14.2 | 240 | 20.4 |

* Broken vial

**Table 117: Depletion assay with rainbow trout hepatocytes - Pyrene - Results of the linear regressions**

| | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| Slope ($h^{-1}$) active | -0.0085 | -0.0032 | -0.0007 |
| $R^2$ | 0.3406 | 0.0400 | 0.0030 |
| Recovery (%) | 41.8 | 25.8 | 24.0 |
| Viability (%) | 83.8 | 89.8 | 90.0 |
| Cell density ($10^6$ cells/mL) | 2.09 | 2.44 | 2.36 |
| Loss in HI at end of incubation (%) | 14.8 | -1.0 | 10.0 |

Negative loss = concentration higher than at start of incubation

## 7.3 Depletion assay with rainbow trout hepatocytes – UV-234

**Table 118: Depletion assay with rainbow trout hepatocytes – UV-234 - concentration data of HI samples.**

| Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 15.1 | 0 | 18.9 | 0 | 16.8 |
| 15 | 15.2 | 15 | 22.8 | 15 | 20.1 |
| 30 | 16.0 | 30 | 24.3 | 30 | 20.4 |
| 60 | 16.9 | 60 | 25.2 | 60 | 20.9 |
| 120 | 17.4 | 120 | 24.8 | 120 | 20.0 |
| 180 | 18.1 | 180 | 24.8 | 180 | 21.0 |
| 240 | 16.7 | 240 | 23.4 | 240 | 18.9 |

**Table 119: Depletion assay with rainbow trout hepatocytes – UV-234 - concentration data of active samples. 2 samples were drawn at each sampling time.**

| Run 1 | | | Run 2 | | | Run 3 | | |
|---|---|---|---|---|---|---|---|---|
| (min) | 1 (µg/L) | 2 (µg/L) | (min) | 1 (µg/L) | 2 (µg/L) | (min) | 1 (µg/L) | 2 (µg/L) |
| 0 | 16.5 | 15.1 | 0 | 21.3 | 19.4 | 0 | 14.7 | 17.6 |
| 15 | 16.3 | 16.3 | 15 | 25.6 | 22.4 | 15 | 15.8 | 20.2 |
| 30 | 16.1 | 15.5 | 30 | 25.9 | 24.3 | 30 | 17.3 | 20.6 |
| 60 | 15.6 | 16.4 | 60 | 26.3 | 23.7 | 60 | 19.6 | 21.0 |
| 120 | 16.9 | 17.5 | 120 | 28.1 | 26.8 | 120 | 18.5 | 20.7 |
| 180 | 15.1 | 16.2 | 180 | 27.9 | 22.4 | 180 | 16.7 | 19.7 |
| 240 | 14.8 | 15.1 | 240 | 22.2 | 18.9 | 240 | 15.6 | 19.8 |

**Table 120: Depletion assay with rainbow trout hepatocytes – UV-234 - Results of the linear regressions**

| | Run 1 (mean) | Run 2 (mean) | Run 3 (mean) |
|---|---|---|---|
| Slope ($h^{-1}$) active | -0.0047 | -0.0021 | 0.00204 |
| $R^2$ | 0.1501 | 0.0042 | 0.0091 |
| Recovery (%) | 25.9 | 27.3 | 24.2 |
| Viability (%) | 81.5 | 80.6 | 82.4 |
| Cell density ($10^6$ cells/mL) | 2.00 | 1.99 | 1.67 |
| Loss in HI at end of incubation (%) | -8.0 | -25.0 | -14.1 |

Negative loss = concentration higher than at start of incubation

## 7.4 Depletion assay with rat hepatocytes – Pyrene

**Table 121: Depletion assay with rat hepatocytes - pyrene - concentration data of HI samples**

| Run 2 | | Run 3 | | Run 4 | |
|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 9.9 | 0 | 11.0 | 0 | 9.03 |
| 5 | 10.9 | 5 | 10.3 | 5 | 8.87 |
| 10 | 10.8 | 10 | 10.6 | 10 | 8.97 |
| 20 | 10.6 | 20 | 10.5 | 20 | 9.08 |
| 30 | 11.8 | 30 | 10.2 | 30 | 9.35 |
| 45 | 9.6 | 45 | 9.8 | 45 | 9.36 |

**Table 122: Depletion assay with rat hepatocytes - pyrene - concentration data of active samples**

| Run 2 | | Run 3 | | Run 4 | |
|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 10.1 | 0 | 9.9 | 0 | 8.76 |
| 5 | 8.4 | 5 | 10.1 | 5 | 7.92 |
| 10 | 8.2 | 10 | 9.3 | 10 | 8.05 |
| 20 | 7.2 | 20 | 7.7 | 20 | 7.68 |
| 30 | 7.0 | 30 | 7.0 | 30 | 5.38 |
| 45 | 7.9* | 45 | 6.4 | 45 | 4.98 |

* Data excluded from calculation

**Table 123: Depletion assay with rat hepatocytes - pyrene - Results of the linear regressions**

| | Run 2 | Run 3 | Run 4 |
|---|---|---|---|
| Slope ($h^{-1}$) active | -0.2894 | -0.289 | -0.3439 |
| $R^2$ | 0.8536 | 0.9456 | 0.8957 |
| Viability (%) | 84.1 | 81.7 | 80.7 |
| Cell density ($10^6$ cells/mL) | 1.31 | 1.07 | 1.51 |
| Loss in HI at end of incubation (%) | 3.3 | 11.0 | -3.65 |

Negative loss = concentration higher than at start of incubation

## 7.5 Depletion assay with rat hepatocytes – Chlorpyrifos

**Table 124: Depletion assay with rat hepatocytes - chlorpyrifos - concentration data of HI samples.**

| Run 1 + 2 | |
|---|---|
| (min) | (µg/L) |
| 0 | 14.4 |
| 5 | 15.9 |
| 15 | 17.4 |
| 30 | 20.5 |
| 60 | 12.6 |
| 90 | 14.5 |
| 120 | 13.8 |

**Table 125: Depletion assay with rat hepatocytes - chlorpyrifos - concentration data of active samples.**

| Run 1 | | Run 2 | |
|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) |
| 0 | 11.7 | 0 | 14.1 |
| 5 | 6.0 | 5 | 8.5 |
| 15 | 3.9 | 15 | 5.4 |
| 30 | 2.1 | 30 | 2.4 |
| 60 | 0.5 | 60 | 0.5 |
| 90 | 0.4* | 90 | 0.5* |
| 120 | | 120 | 0.4* |

* Data excluded from calculation

**Table 126: Depletion assay with rat hepatocytes - Chlorpyrifos - Results of the linear regressions**

| | Run 1 | Run 2 |
|---|---|---|
| Slope ($h^{-1}$) active | -1.318 | -1.4022 |
| $R^2$ | 0.9835 | 0.9956 |
| Viability (%) | 78.0 | 77.2 |
| Cell density ($10^6$ cells/mL) | 0.62 | 0.60 |
| Loss in HI at end of incubation (%) | 3.8 | |

## 7.6 Depletion assay with rat hepatocytes – UV-234

**Table 127: Depletion assay with rat hepatocytes – UV-234 - concentration data of HI samples.**

| Run 1 | | Run 2 | | Run 3 | | Run 4 | |
|---|---|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 17.0 | 0 | 7.4 | 0 | 7.4 | 0 | 21.8 |
| 15 | 15.1 | 5 | 8.2 | 5 | 8.2 | 15 | 17.7 |
| 30 | 15.7 | 10 | 9.2 | 10 | 9.2 | 30 | 16.3 |
| 60 | 15.7 | 20 | 13.6 | 20 | 13.6 | 60 | 17.6 |
| 120 | 17.4 | 30 | 8.7 | 30 | 8.7 | 90 | 17.0 |
| 180 | 16.9 | 45 | 8.7 | 45 | 8.7 | 120 | 16.5 |
| 240 | 16.4 | 60 | 8.4 | 60 | 8.4 | 180 | 17.0 |

**Table 128: Depletion assay with rat hepatocytes – UV-234 - concentration data of active samples. The reported concentration is not corrected for the dilution effect that is caused by the stopping solution**

| Run 1 | | Run 2 | | Run 3 | | Run 4 | |
|---|---|---|---|---|---|---|---|
| (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) | (min) | (µg/L) |
| 0 | 15.2 | 0 | 6.2 | 0 | 4.9 | 0 | 24.5 |
| 15 | 14.2 | 5 | 6.0 | 5 | 5.6 | 15 | 14.9 |
| 30 | 13.8 | 10 | 6.3 | 10 | 5.8 | 30 | 15.7 |
| 60 | 12.2 | 20 | 6.6 | 20 | 6.1 | 60 | 16.1 |
| 120 | 10.6 | 30 | 7.8 | 30 | 6.3 | 90 | 16.2 |
| 180 | 9.0 | 45 | 8.5 | 45 | 7.5 | 120 | 16.4 |
| 240 | 8.2 | 60 | 6.3 | 60 | 5.9 | 180 | 16.8 |

* Data excluded from calculation

**Table 129: Depletion assay with rat hepatocytes – UV-234 - Results of the linear regressions**

| | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|
| Slope ($h^{-1}$) active | -0.0674 | 0.0753 | 0.09804 | -0.0137 |
| $R^2$ | 0.9863 | 0.2395 | 0.4147 | 0.0831 |
| Viability (%) | 82.4 | 83.3 | 83.7 | 79.6 |
| Cell density ($10^6$ cells/mL) | 1.66 | 2.51 | 3.14 | 1.05 |
| Loss in HI at end of incubation (%) | 3.5 | -13.4 | -13.4 | 22.5 |

Negative loss = concentration higher than at start of incubation

# 8 Analytical details

## 8.1 General information

### 8.1.1 Chlorpyrifos

**Table 130: Internal standard: Chlorpyrifos-(diethyl-d10)**

| | LOD | LOQ | Calibration range | PRS level* |
|---|---|---|---|---|
| Unit | µg/L | µg/L | µg/L | µg/L |
| Rat | 0.1 | 0.3 | 0.1-10 | 0.5 / 5.0 |

* Instead of classic PRS samples, matrix-matched quality control (QC) samples were measured. Those are identical to the calibration samples, but originate from a second weighing. All samples, calibration samples and QC samples have the same composition in terms of solvent, water and matrix content.

**Table 131: Chromatography conditions**

| | |
|---|---|
| System name | Xevo-TQD |
| Stationary phase | ACQUITY UPLCr BEH C18 1.7µm |
| Mobile phase | A: $H_2O$UHQ:ACN 95:5 + 0.1 %FA<br>B: ACN + 0.1 % FA |
| Injection volume | 10 µL |
| Column temperature | 40 °C |
| Flow rate | 0.3 mL/min |
| Gradient | |

| | Time [min] | Flow rate [mL/min] | % A | % B |
|---|---|---|---|---|
| 1 | Initial | 0.3 | 25.0 | 75.0 |
| 2 | 3.0 | 0.3 | 25.0 | 75.0 |

**Table 132: MS parameter**

| | |
|---|---|
| Type | MRM |
| Span (Da) | 0.2 |
| Ion mode | ES+ |
| Capillary (kV) | 3.20 |
| Cone (V) | 30 |
| Source temperature (°C) | 150 |
| Desolv. Temperature (°C) | 600 |
| Cone gas flow (L/h) | 100 |

**Table 133: Mass transitions**

| | Parent ion (m/z) | Daughter ion (m/z) | Dwell time (s) | Collision energy (eV) |
|---|---|---|---|---|
| Chlorpyrifos (qual) | 351.95 | 153.15 | 0.063 | 13 |
| Chlorpyrifos (quan) | 351.95 | 199.72 | 0.063 | 15 |
| Chlorpyrifos IS (qual) | 361.99 | 130.86 | 0.063 | 18 |
| Chlorpyrifos IS (qual) | 361.99 | 163.10 | 0.063 | 18 |
| Chlorpyrifos IS (quan) | 361.99 | 201.12 | 0.063 | 18 |

quan. = quantifier
qual = qualifier

**Figure 1: Exemplary calibration curve tissue**

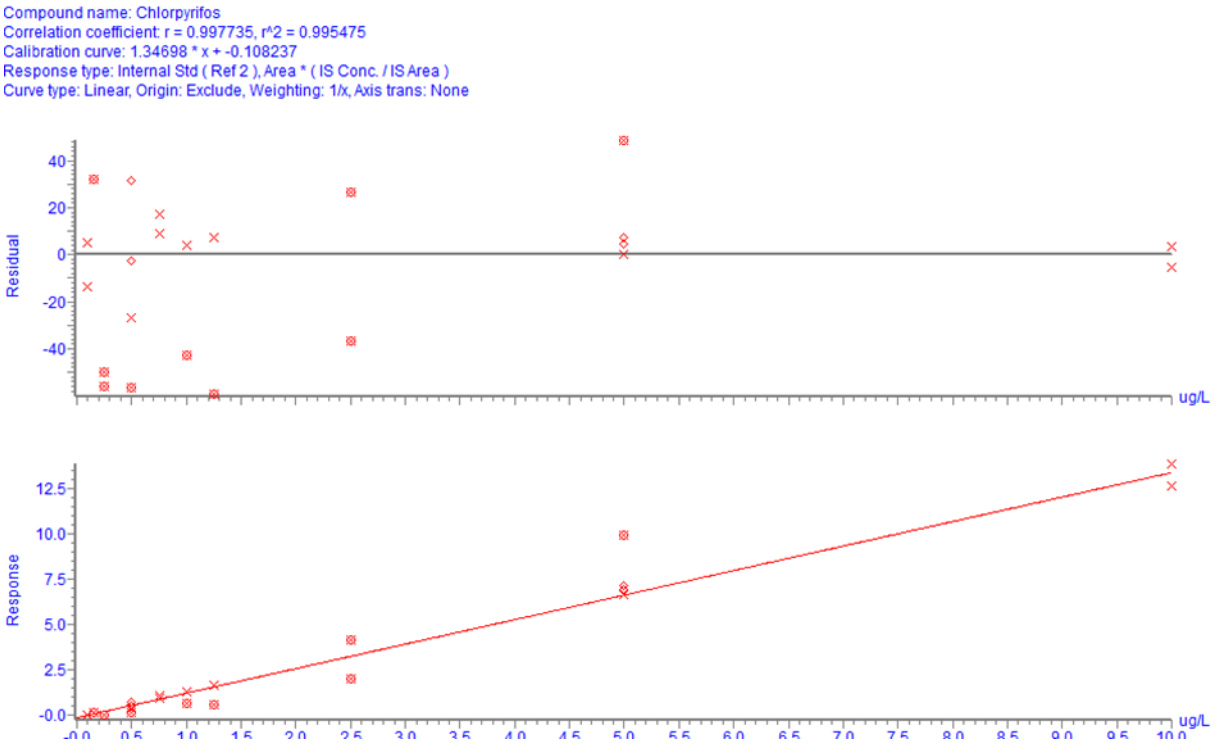
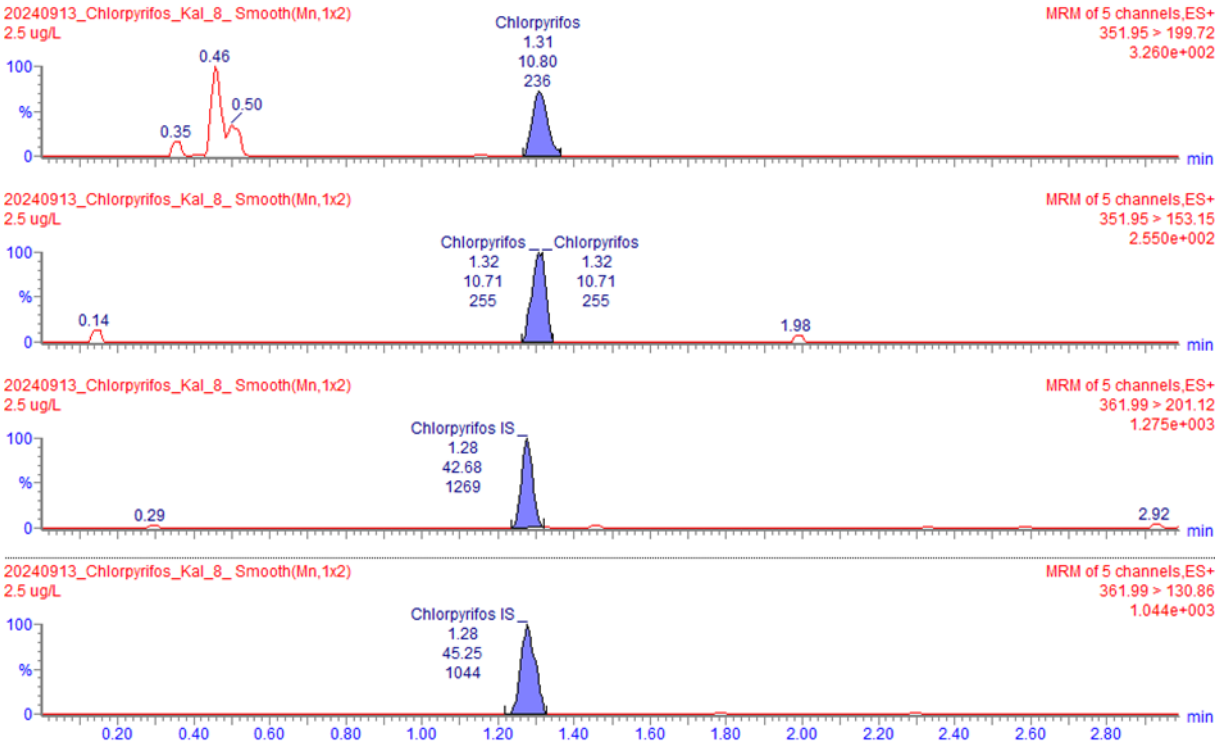


**Figure 2: Exemplary chromatogram**




### 8.1.2 Diclofenac

Storage conditions:

In order to evaluate the stability of the samples until analysis, the storage stability was examined.

The storage stability (recovery of ±20 %) of the water samples over a period of 15 days was checked and confirmed with a recovery of 110 %. The water samples were stored for a maximum of 10 days (pH 5) and 2 days (pH 7.8) until analysis. Storage stability over a period of 12 days of tissue samples has been reviewed and was confirmed with a recovery of 108 %. All tissue samples were analysed within 5 days (pH 5) and 7 days (pH 7.8) after extraction.

**Table 134: Internal standard: Diclofenac d4**

| | LOD | LOQ | Calibration range | PRS level* |
|---|---|---|---|---|
| Unit | µg/L | µg/L | µg/L | µg/L |
| Medium | 0.1 | 0.3 | 0.1-10 | 0.75 / 7.5 |
| Hyalella azteca | 0.1 | 0.3 | 0.1-10 | 0.75 / 7.5 |

* Instead of classic PRS samples, matrix-matched quality control (QC) samples were measured. Those are identical to the calibration samples, but originate from a second weighing. All samples, calibration samples and QC samples have the same composition in terms of solvent, water and matrix content.

**Table 135: Chromatography conditions**

| | |
|---|---|
| System name | Xevo-TQSmicro |

| | |
|---|---|
| Stationary phase | ACQUITY UPLCr BEH C18 1.7µm |
| Mobile phase | A: H2OUHQ:ACN 95:5 + 0.1 %FA<br>B: ACN + 0.1 % FA |
| Injection volume | 10 µL |
| Column temperature | 40 °C |
| Flow rate | 0.3 µL/min |
| Gradient | |

| | Time [min] | Flow rate [mL/min] | % A | % B |
|---|---|---|---|---|
| 1 | Initial | 0.3 | 65.0 | 35.0 |
| 2 | 0.1 | 0.3 | 65.0 | 35.0 |
| 3 | 3.0 | 0.3 | 0.0 | 100.0 |
| 4 | 4.0 | 0.3 | 0.0 | 100.0 |
| 5 | 4.1 | 0.3 | 65.0 | 35.0 |
| 6 | 6.0 | 0.3 | 65.0 | 35.0 |

**Table 136: MS parameter**

| | |
|---|---|
| Type | MRM |
| Span (Da) | 0.5 |
| Ion mode | ES+ |
| Capillary (kV) | 3.5 |
| Cone (V) | 40 |
| Source temperature (°C) | 150 |
| Desolv. Temperature (°C) | 600 |
| Cone gas flow (L/h) | 100 |

**Table 137: Mass transitions**

| | Parent ion (m/z) | Daughter ion (m/z) | Dwell time (s) | Collision energy (eV) |
|---|---|---|---|---|
| Diclofenac (quan) | 295.76 | 215.03 | 0.082 | 13 |
| Diclofenac (qual) | 295.76 | 250.04 | 0.082 | 13 |
| Diclofenac-d4 (IS) (quan) | 299.76 | 254.02 | 0.082 | 13 |
| Diclofenac-d4 (IS) (qual) | 299.76 | 218.93 | 0.082 | 13 |

quan. = quantifier
qual = qualifier

**Figure 3: Exemplary calibration curve medium**

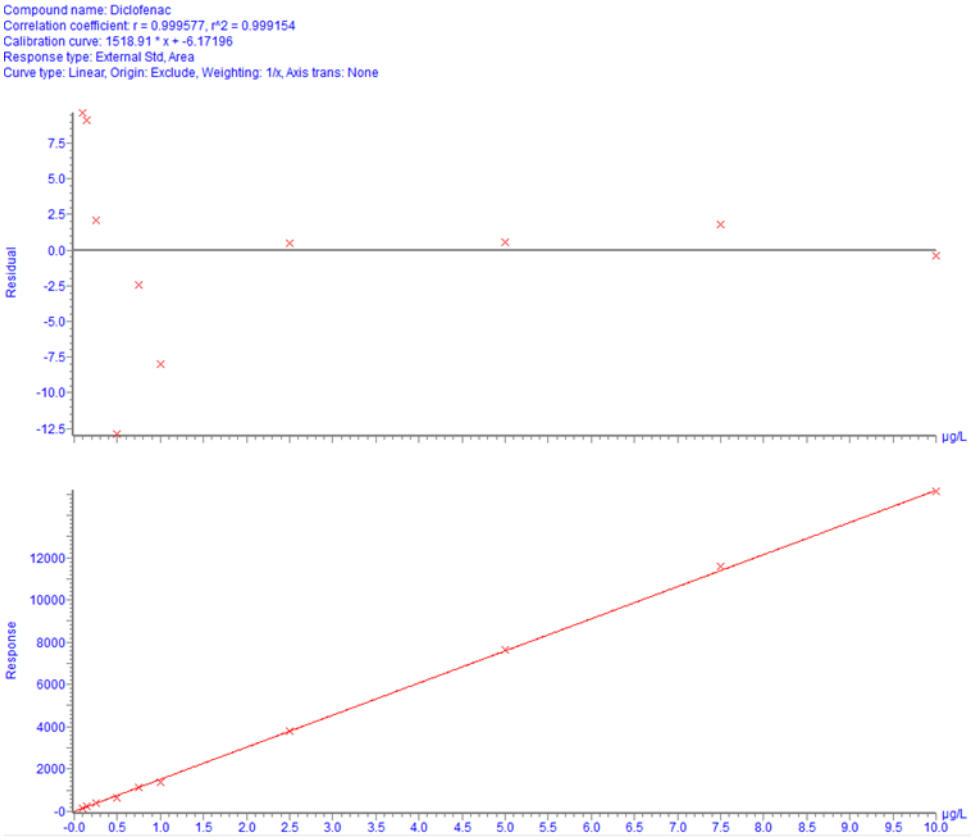



**Figure 4: Exemplary calibration curve tissue**

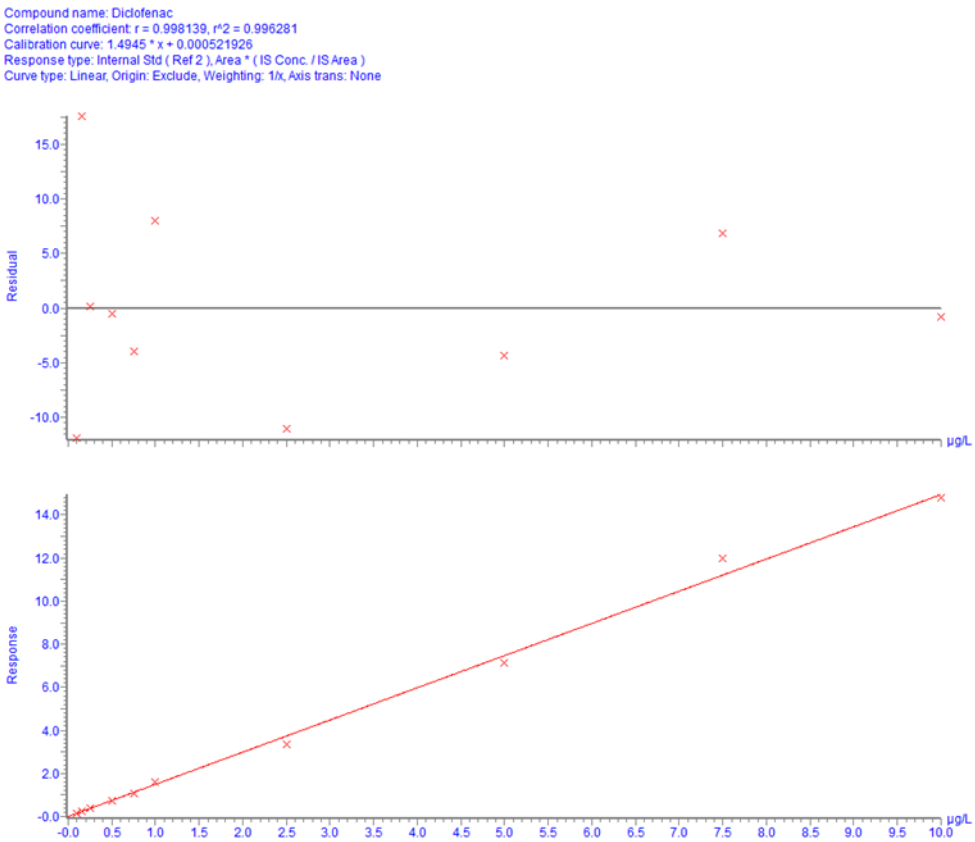


**Figure 5: Exemplary chromatogram - medium**

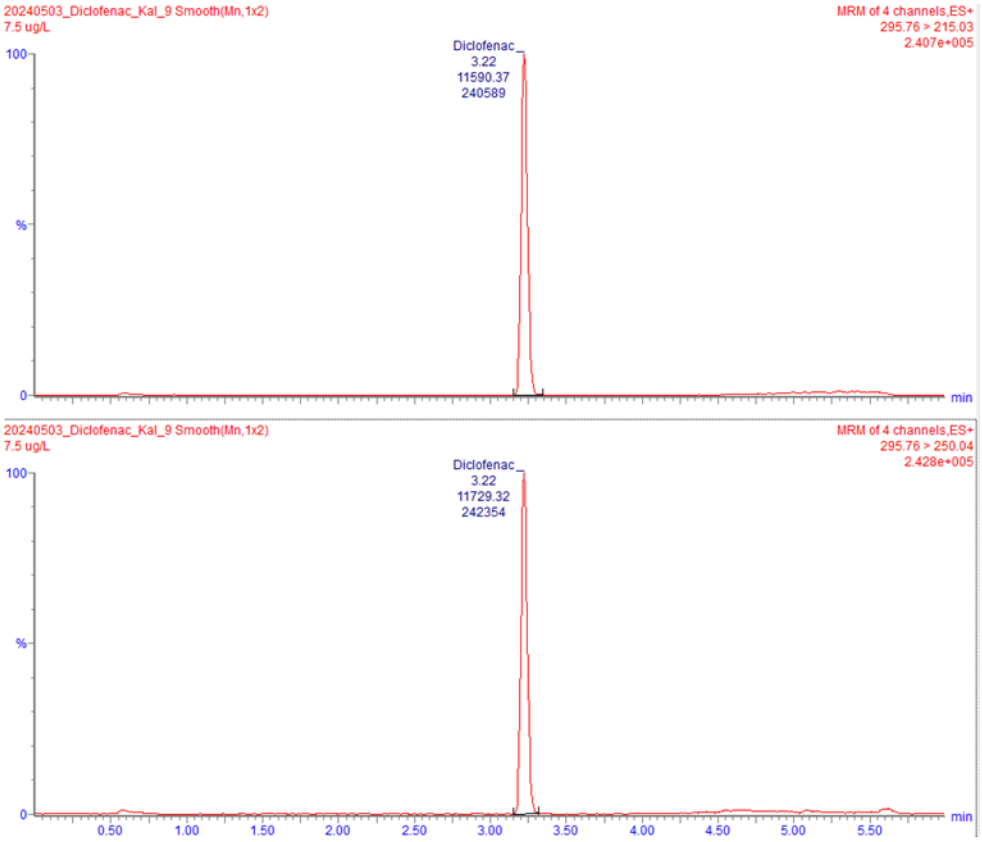
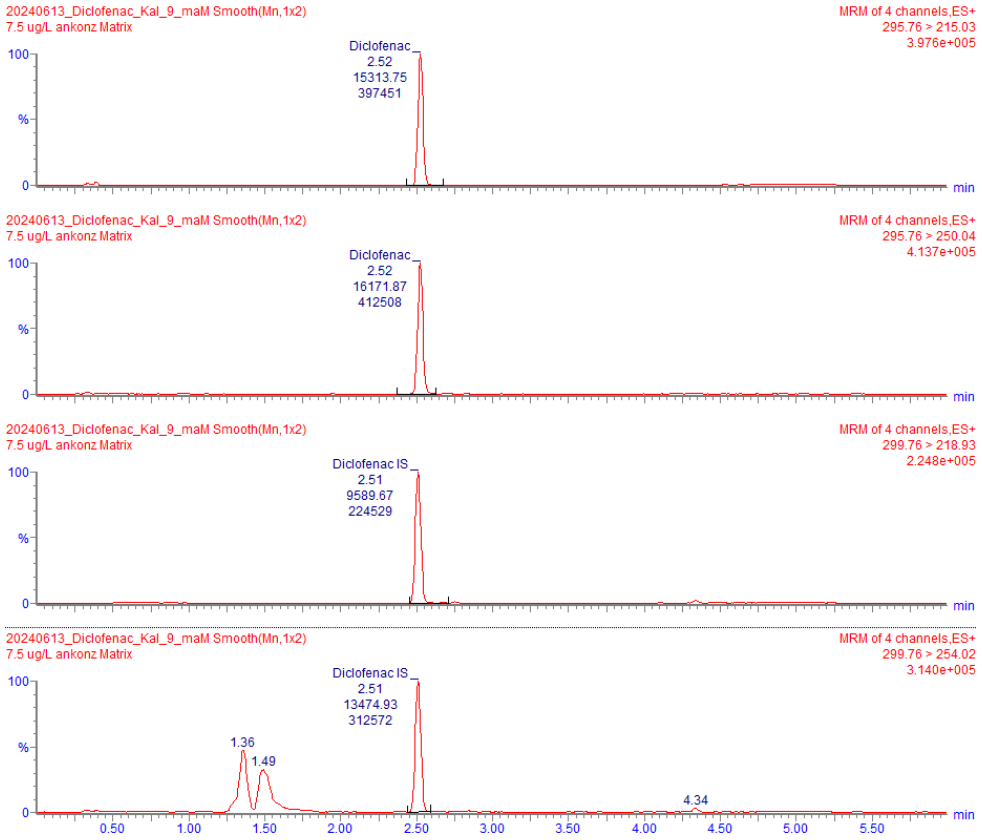



**Figure 6: Exemplary chromatogram - tissue**



### 8.1.3 D4 (Octamethylcyclotetrasiloxane)

No validation according to SANTE/2020/12830 was carried out for D4. A separate calibration was measured for each series of measurements. The validity of each measurement series was verified individually by analyzing quality control standards. In addition, separate limits of detection and quantification were determined for each measurement series (according to the calibration curve method of German norm DIN32645). In order to identify possible impurities/contaminations from the chemicals used and/or the measuring device hexane blanks were analyzed along each batch of samples.

If possible, the samples were measured directly after extraction. Otherwise, the samples were stored at ≤ -18 °C until measurement. The maximum storage time between extraction and measurement was 5 days (except for the samples of the in vitro stability testing).

**Table 138: Internal standard: Tetrakis(trimethylsiloxy)silane (M4Q), final conc. in extracts: 100 µg/L**

| | LOD | LOQ | Calibration range | Quality control standards | | |
|---|---|---|---|---|---|---|
| | | | | QC 1 | Rec.* | QC2 |
| Unit | µg/L | µg/L | µg/L | µg/L | µg/L | µg/L |
| Hexane | 8.35 – 54.7 | 30.9 – 199 | 10 – 1000 (until measurement series of 31.07.24) | 100 | 250 | 500 |
| | 7.56 – 16.7 | 28.3 – 61.9 | 10 – 500 (as of measurement series of 06.08.24) | | | |

* Recalibration standard

No matrix-matched calibration was performed. Calibration standards were prepared by diluting a commercial standard (Neochema GmbH, Bodenheim, Germany) with n-hexane and addition of the internal standard (Neochema GmbH, Bodenheim, Germany).

**Table 139: Chromatography conditions**

| | |
|---|---|
| System name | GC Agilent 7890B |
| Column | 30 m ZB-WAX polyethylene glycol (PEG), 30 m x 0.25 µm x 0.25 mm), with 110 m guardian column |
| Inlet liner | Restek 23308, Double Taper Inlet Liner, 4.0 mm x 6.5 x 78.5 |
| Injection mode | Splitless |
| Injection temperature | 210 °C |
| Injection volume | 1 µL |
| Carrier gas | Helium |
| Carrier gas flow rate | 1.5 mL/min |

| | |
|---|---|
| Transfer line | 1.05 m sulfinert stainless steel capillary |
| Transfer line temperature | 250 °C |

**Table 140: GC oven program**

Subtitle *[if not necessary, please remove]*

| Ramp | Rate °C/min | Oven temperature °C | Hold time min | Run time min |
|---|---|---|---|---|
| Start | | 40 | 1 | 1 |
| 1 | 2.5 | 45 | 0 | 3 |
| 2 | 30 | 80 | 0 | 4.17 |
| 3 | 2.5 | 85 | 0 | 6.17 |
| 4 | 30 | 240 | 8.67 | 20.0 |

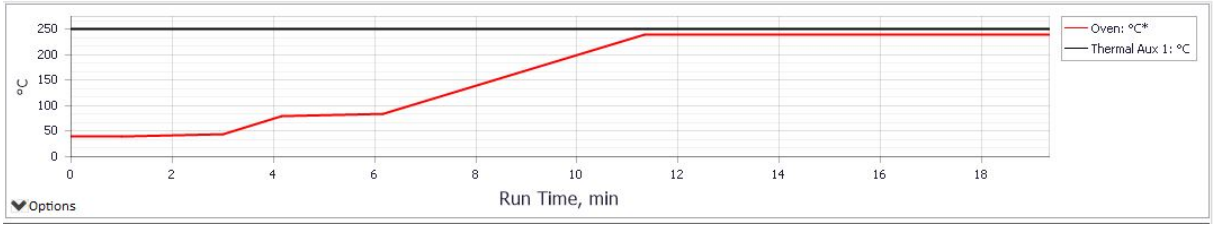
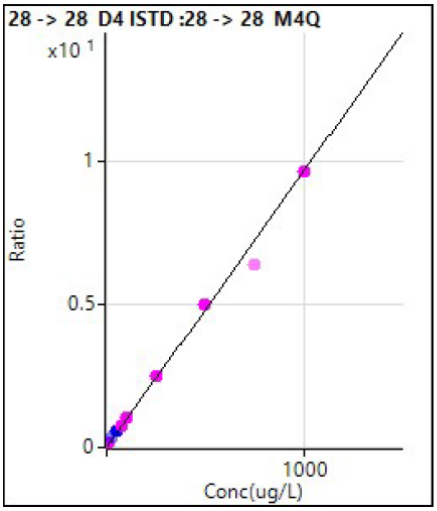



**Table 141: MS parameters**

| | |
|---|---|
| Type | Agilent 8900 ICP-MS/MS |
| Plasma stabilization time | Approx.30 min |
| Plasma gas flow | 15.0 L/min |
| Plasma power | 1550 W |
| Dilution gas flow | 0.70 L/min |
| Gas mode | $H_2$ “on mass” mode |
| Reaction cell gas | Hydrogen |
| Reaction cell gas flow | 4.20 mL/min |
| Isotope and mass shift | $^{28}Si^+ \rightarrow {}^{28}Si^+$ |
| Integration time | 0.6 s |

### 8.1.3.1 Exemplary calibration curves

**Figure 7: Exemplary calibration curve of measurement series from July 31, 2024, calibration 10.0 µg/L – 1000 µg/L.**

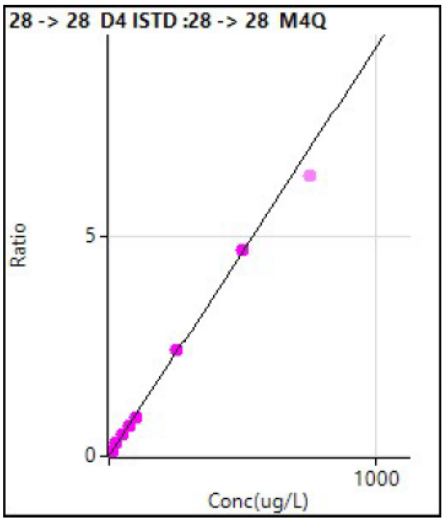



| | Rjct | Conc. | Calc Conc. | Count | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.360 | 121561 | 0.05671 | P | 0.0 | |
| 2 | ☐ | 0.000 | -0.005 | 108492 | 0.05318 | P | 0.0 | |
| 3 | ☐ | 0.000 | -0.355 | 110142 | 0.04979 | P | 0.0 | |
| 4 | ☐ | 10.000 | 10.486 | 351016 | 0.15479 | M | 0.0 | 4.9 |
| 5 | ☑ | 25.000 | | 701247 | 0.33910 | P | 0.0 | |
| 6 | ☐ | 50.000 | 54.163 | 1197699 | 0.57782 | P | 0.0 | 8.3 |
| 7 | ☐ | 75.000 | 72.956 | 1658990 | 0.75984 | M | 0.0 | -2.7 |
| 8 | ☐ | 100.000 | 100.617 | 2647630 | 1.02774 | M | 0.0 | 0.6 |
| 9 | ☐ | 250.000 | 251.686 | 5287946 | 2.49092 | M | 0.0 | 0.7 |
| 10 | ☐ | 500.000 | 512.856 | 10195490 | 5.02046 | M | 0.0 | 2.6 |
| 11 | ☑ | 750.000 | | 13686623 | 6.40145 | M | 0.0 | |
| 12 | ☐ | 1000.000 | 993.029 | 19773029 | 9.67115 | M | 0.0 | -0.7 |

y = 0.0097 * x + 0.0532
R = 0.9999
BEC = 5.495 ug/L

Source: own illustration, Fraunhofer IME

**Figure 8: Exemplary calibration curve of measurement series from August 30, 2024, calibration 10.0 µg/L – 500 µg/L.**




| | Rjct | Conc. | Calc Conc. | Count | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☑ | 0.000 | | 144069 | 0.05604 | M | 0.0 | |
| 2 | ☐ | 0.000 | 0.248 | 111752 | 0.04414 | M | 0.0 | |
| 3 | ☐ | 0.000 | -0.248 | 103447 | 0.03950 | M | 0.0 | |
| 4 | ☐ | 10.000 | 9.025 | 389863 | 0.12626 | M | 0.0 | -9.8 |
| 5 | ☐ | 25.000 | 27.159 | 899151 | 0.29594 | M | 0.0 | 8.6 |
| 6 | ☐ | 50.000 | 48.160 | 1600516 | 0.49244 | M | 0.0 | -3.7 |
| 7 | ☐ | 75.000 | 67.883 | 2166550 | 0.67698 | M | 0.0 | -9.5 |
| 8 | ☐ | 100.000 | 91.946 | 2863051 | 0.90212 | M | 0.0 | -8.1 |
| 9 | ☐ | 250.000 | 256.051 | 7083074 | 2.43759 | M | 0.0 | 2.4 |
| 10 | ☐ | 500.000 | 499.748 | 13612940 | 4.71777 | M | 0.0 | -0.1 |
| 11 | ☑ | 750.000 | | 19699545 | 6.40551 | M | 0.0 | |

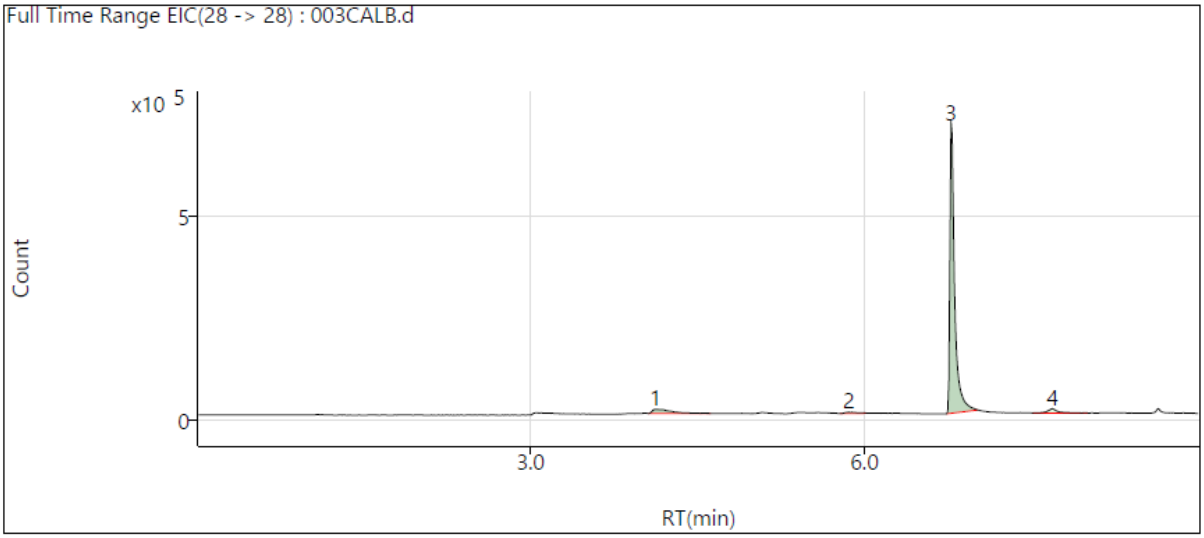
y = 0.0094 * x + 0.0418
R = 0.9997
BEC = 4.47 ug/L

Source: own illustration, Fraunhofer IME

### 8.1.3.2 Exemplary chromatograms

**Figure 9: Exemplary chromatogram of a calibration (hexane) blank with 100 µg/L internal standard from July 31, 2024**

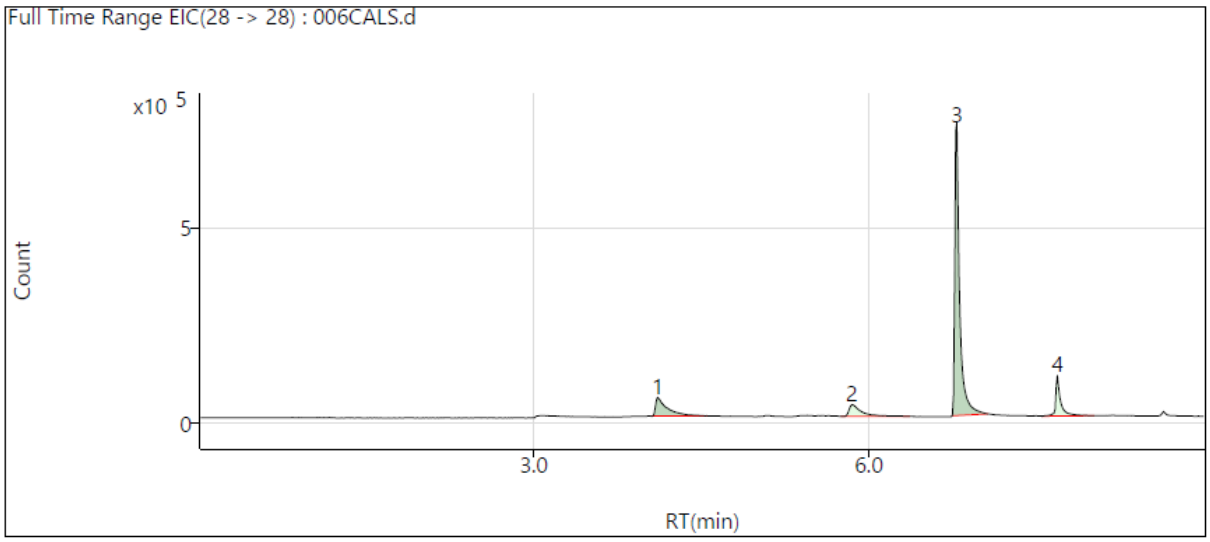
D4: Peak (1) retention time 4.1 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 10: Exemplary chromatogram of a calibration standard 10 µg/L with 100 µg/L internal standard from July 31, 2024.**

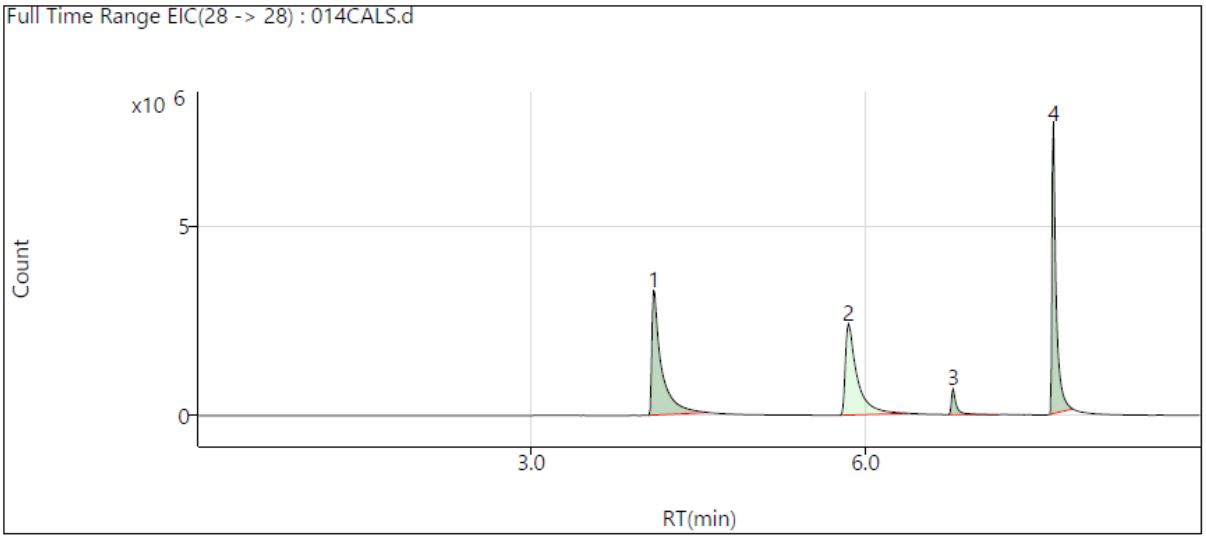
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 11: Exemplary chromatogram of a calibration standard 1000 µg/L with 100 µg/L internal standard from July 31, 2024.**

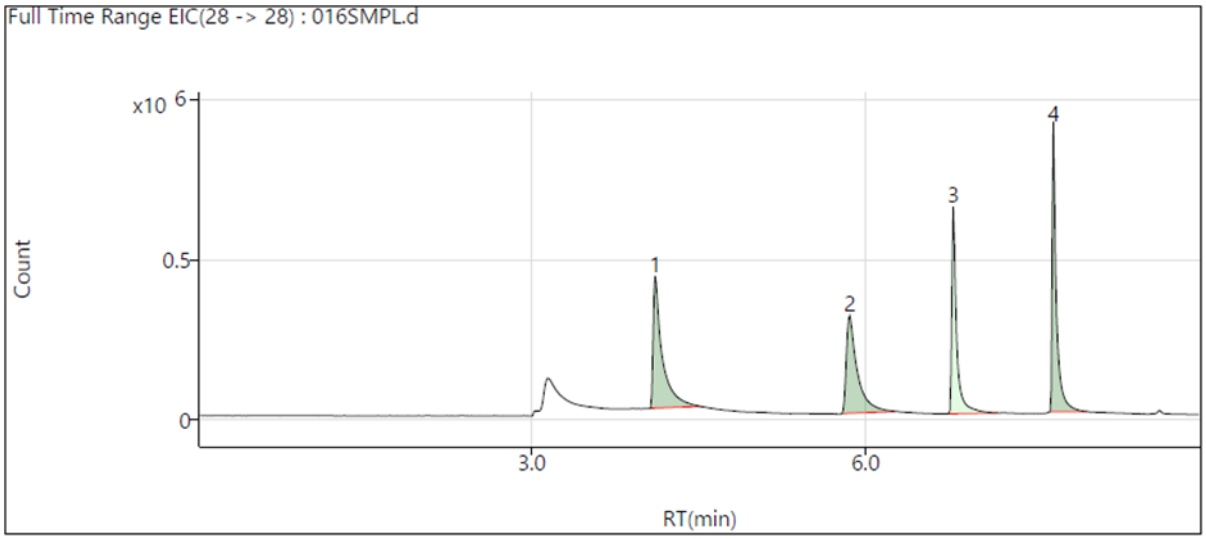
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 12: Exemplary chromatogram of a quality control standard 100 µg/L (QC1) with 100 µg/L internal standard from July 31, 2024.**

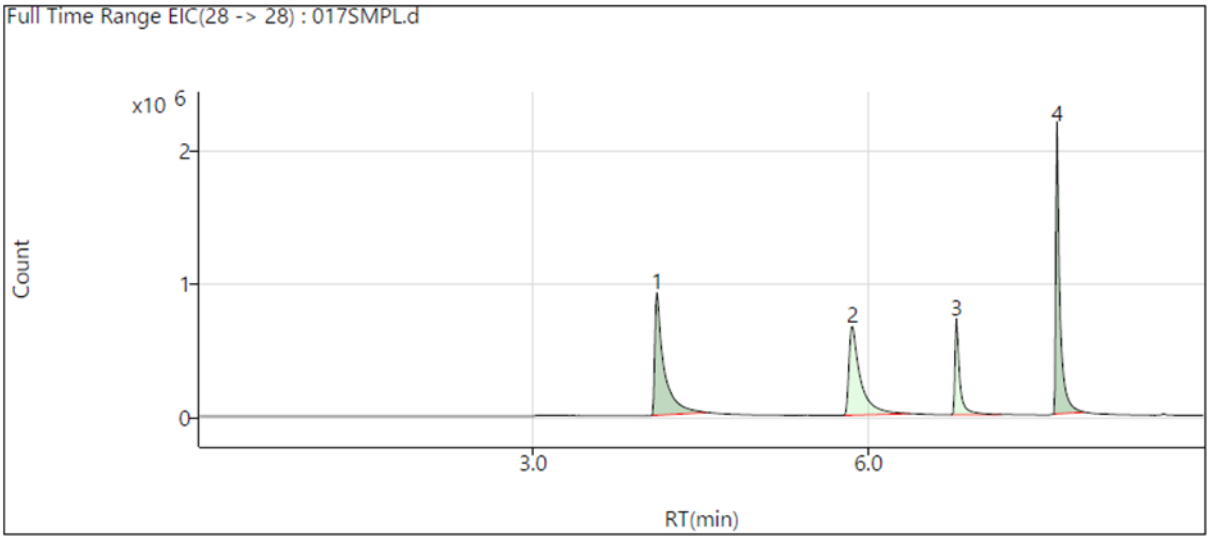
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 13: Exemplary chromatogram of a recalibration standard 250 µg/L (Rec.) with 100 µg/L internal standard from July 31, 2024.**

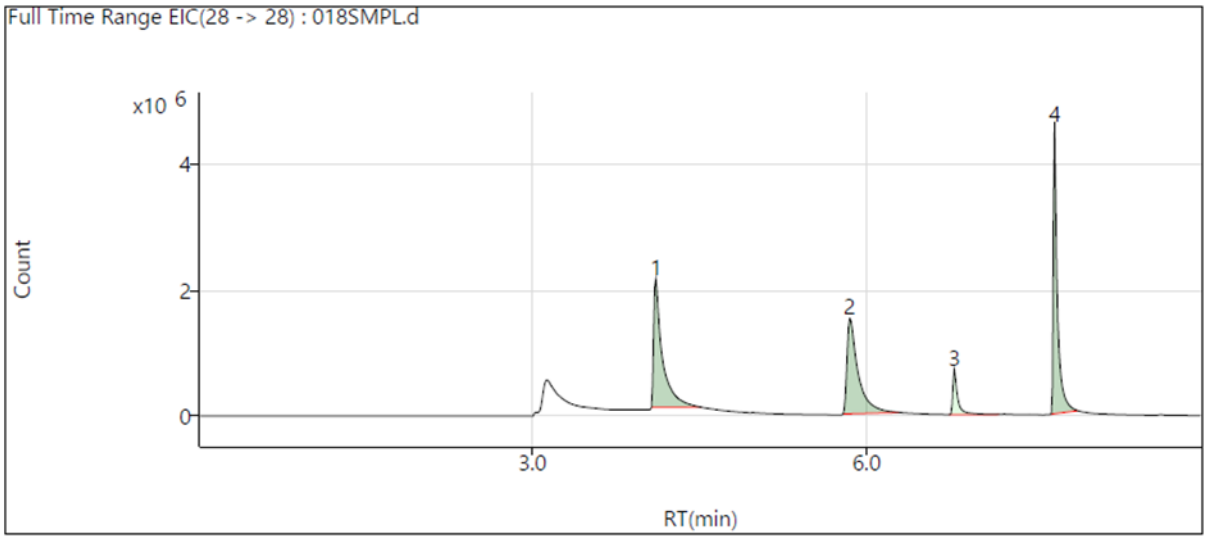
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 14: Exemplary chromatogram of a quality control standard 500 µg/L (QC3) with 100 µg/L internal standard from July 31, 2024.**

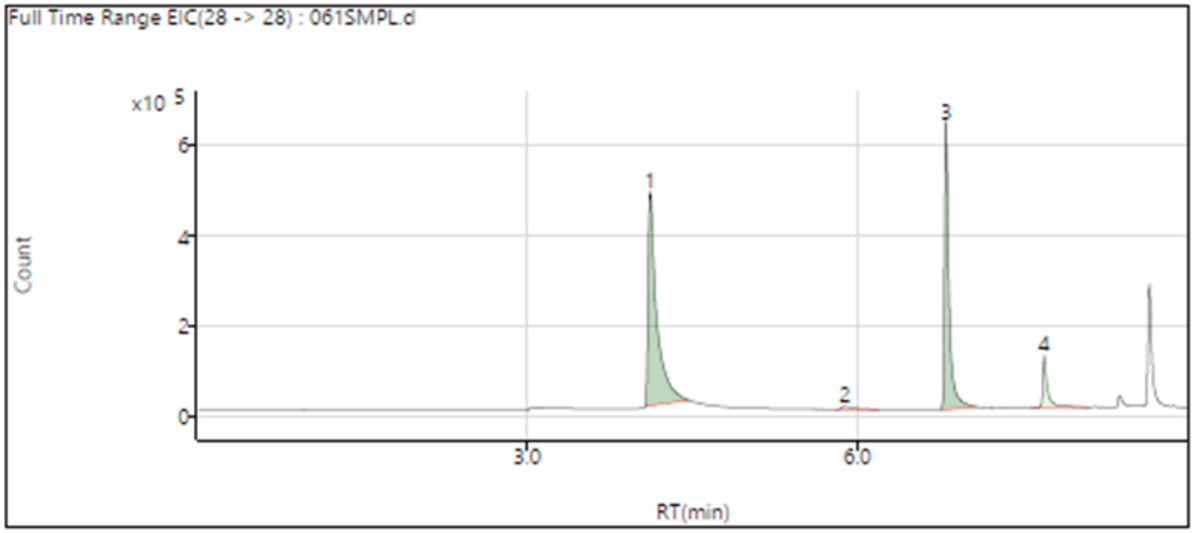
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 15: Chromatogram of in vitro test sample 4.1 (500 µl Williams + 250 µl ACN+ 100 µl n-Hexan (IS)+ 50 µl n-Hexan (IS), sampling time: 0 h, expected conc. 200 µg/L, measured conc. 140 µg/L) from July 31, 2024.**

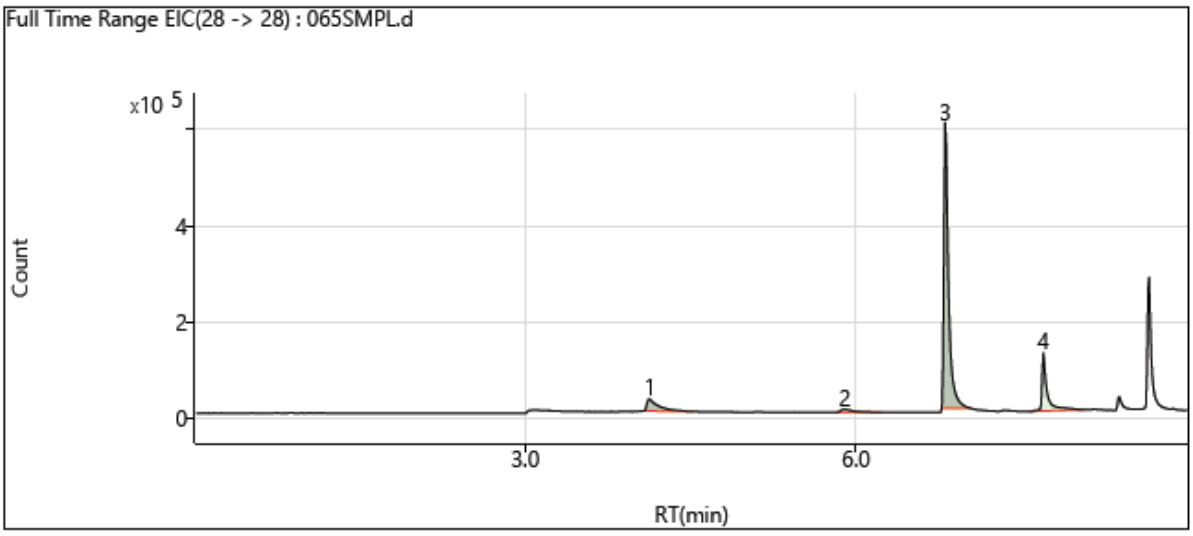
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 16: Chromatogram of in vitro test sample 4.5 (500 µl Williams + 250 µl ACN+ 100 µl n-Hexan (IS)+ 50 µl n-Hexan (IS), sampling time: 4 h, expected conc. 200 µg/L, measured conc. < LOD) from July 31, 2024.**

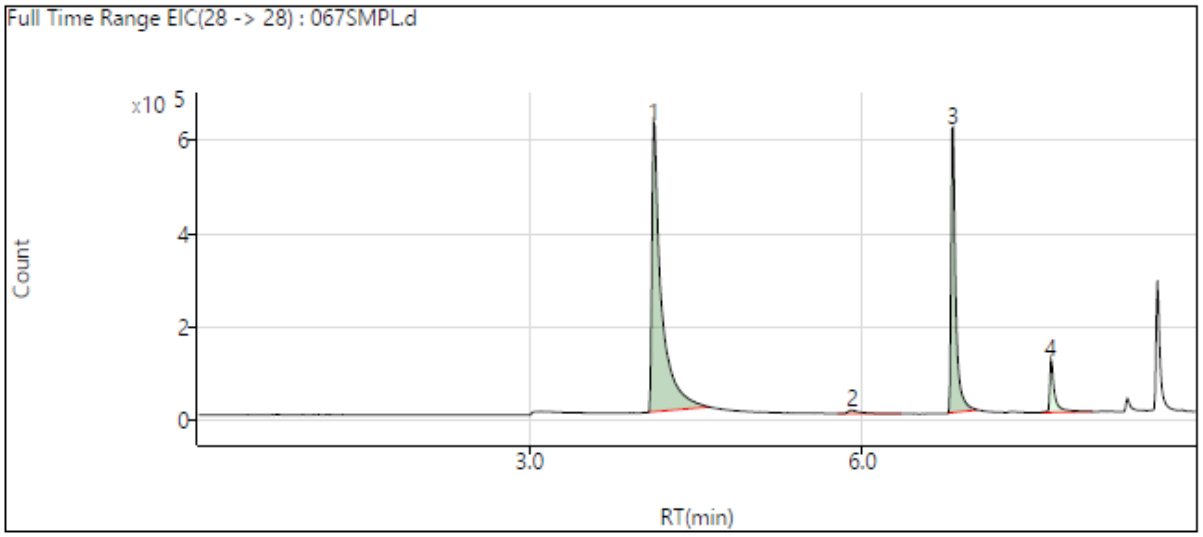
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 17: Chromatogram of in vitro test sample 5.1 (500 µl HI + 250 µl ACN+ 100 µl n-Hexan (IS)+ 50 µl n-Hexan (IS), sampling time: 0 h, expected conc. 200 µg/L, measured conc. 208 µg/L) from July 31, 2024.**

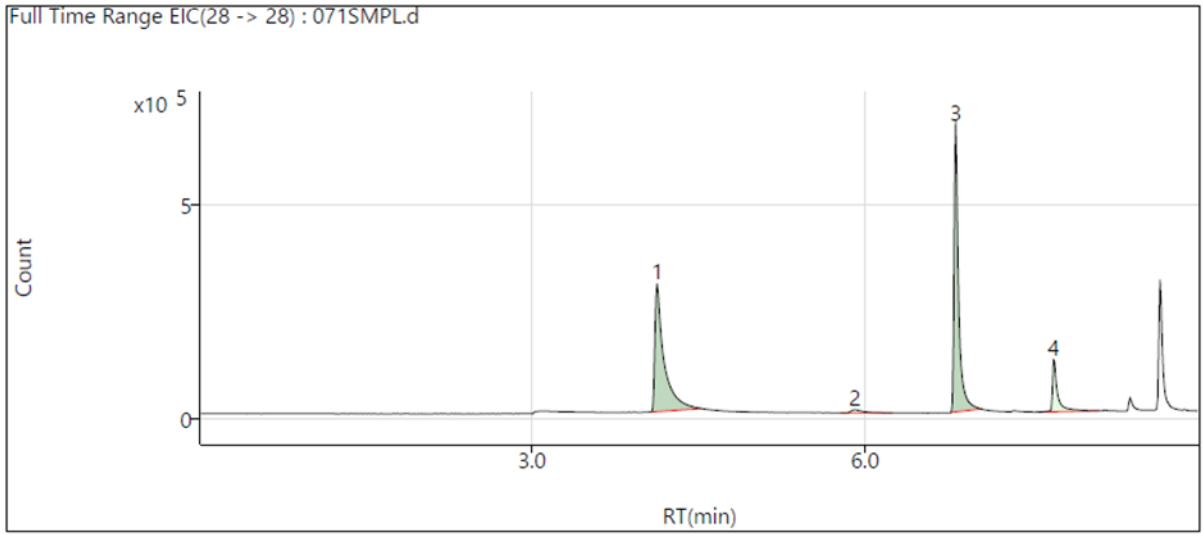
*D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).*




Source: own illustration, Fraunhofer IME

**Figure 18: Chromatogram of in vitro test sample 5.5 (500 µl HI + 250 µl ACN+ 100 µl n-Hexan (IS)+ 50 µl n-Hexan (IS), sampling time: 4 h, expected conc. 200 µg/L, measured conc. 87.8 µg/L) from July 31, 2024.**

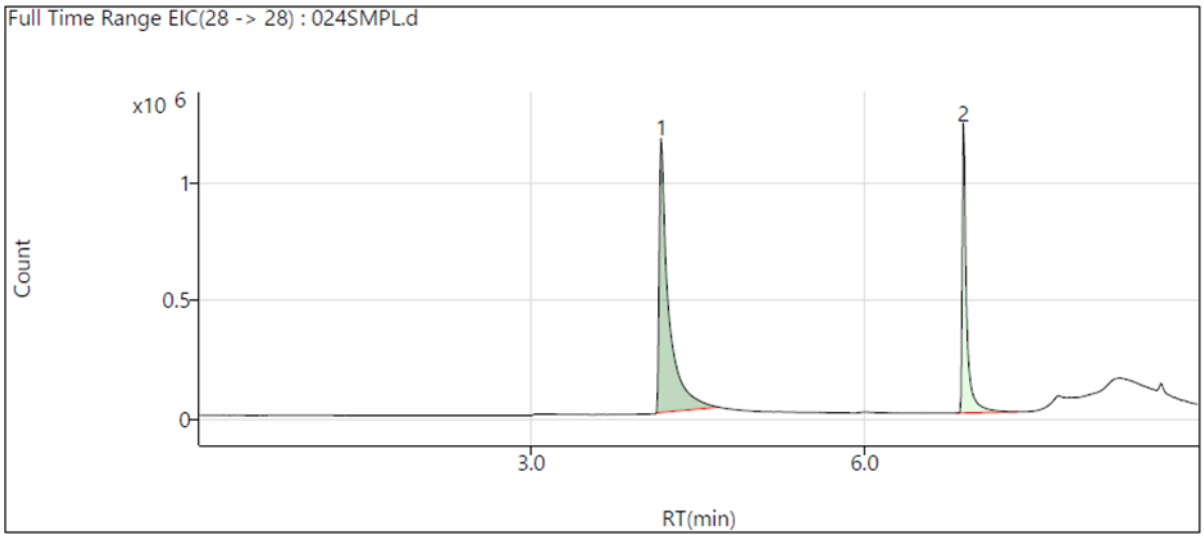
D4: Peak (1) retention time 4.2 min, M4Q (IS): Peak (3), retention time 6.6 min (Peak (2) and Peak (4): D5 (Decamethylcyclopentasiloxane) and D6 (Dodecamethylcyclohexasiloxane), not evaluated for this study).




Source: own illustration, Fraunhofer IME

**Figure 19: Chromatogram of a HYBIT pre-exposure test sample (Test vessel 1, day 35, measured concentration on test sample level: 141 µg/L) from August 30, 2024.**

D4: Peak (1) retention time 4.4 min, M4Q (IS): Peak (3), retention time 7.0 min.




Source: own illustration, Fraunhofer IME

### 8.1.4 Fluoxetine

Storage conditions:

The storage stability of water samples was not investigated as analyses was performed in parallel to the running test with a maximum of 72 h storage at ≤-18 °C.

Storage stability (±20 %) over a period of 11 days of tissue samples has been reviewed and was confirmed with a recovery of 114 % for fluoxetine. The storage stability of the metabolite had a recovery of 75.6 % only. As all tissue samples were analysed with a time delay but latest after 58 days after extraction, concentrations must be reviewed conservatively.

**Table 142: Internal standard: Fluoxetine d6**

| | LOD | LOQ | Calibration range | PRS level* |
|---|---|---|---|---|
| Unit | µg/L | µg/L | µg/L | µg/L |
| medium | 0.1 | 0.3 | 0.1-10 | 0.75 / 7.5 |
| Hyalella azteca | 0.1 | 0.3 | 0.1-10 | 0.75 / 7.5 |

* Instead of classic PRS samples, matrix-matched quality control (QC) samples were measured. Those are identical to the calibration samples, but originate from a second weighing. All samples, calibration samples and QC samples have the same composition in terms of solvent, water and matrix content.

**Table 143: Chromatography conditions**

| | |
|---|---|
| System name | Xevo-TQSmicro |
| Stationary phase | ACQUITY UPLC BEH C18 1,7 µm |
| Mobile phase | A: H2OUHQ:ACN 95:5 + 0.1 % FA<br>B: ACN + 0.1 % FA |
| Injection volume | 10 µL |
| Column temperature | 40 °C |

| | |
|---|---|
| Flow rate | 0.3 µL/min |
| Gradient | |

| | Time [min] | Flow rate [ml/min] | %A | %B |
|---|---|---|---|---|
| 1 | Initial | 0.3 | 75.0 | 25.0 |
| 2 | 0.1 | 0.3 | 75.0 | 25.0 |
| 3 | 3.0 | 0.3 | 15.0 | 85.0 |
| 4 | 3.1 | 0.3 | 0.0 | 100.0 |
| 5 | 4.0 | 0.3 | 0.0 | 100.0 |
| 6 | 4.1 | 0.3 | 95.0 | 5.0 |
| 7 | 5.0 | 0.3 | 95.0 | 5.0 |
| 8 | 5.1 | 0.3 | 75.0 | 25.0 |
| 9 | 6.0 | 0.3 | 75.0 | 25.0 |

**Table 144: MS parameter**

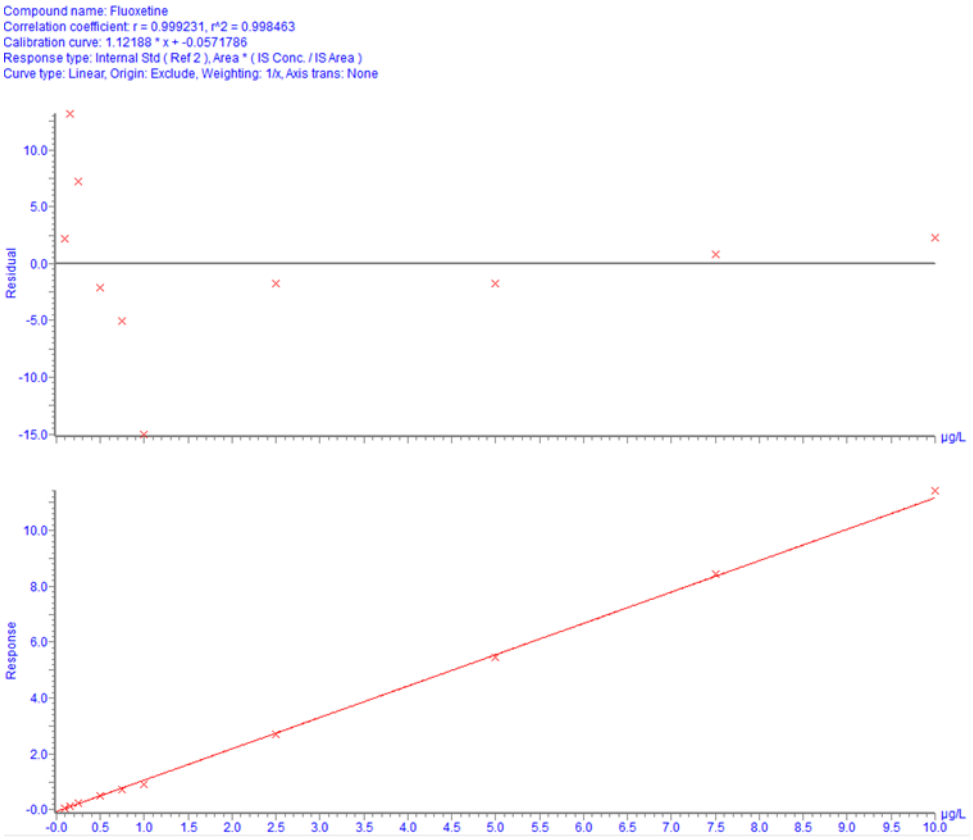
| | |
|---|---|
| Type | MRM |
| Span (Da) | 0.5 |
| Ion mode | ES+ |
| Capillary (kV) | 0.5 |
| Cone (V) | 10 |
| Source temperature (°C) | 150 |
| Desolv. Temperature (°C) | 600 |
| Cone gas flow (L/h) | 100 |

**Table 145: Mass transitions**

| | Parent ion (m/z) | Daughter ion (m/z) | Dwell time (s) | Collision energy (eV) |
|---|---|---|---|---|
| Fluoxetine | 310.06 | 147.94 | 0.109 | 15 |
| Norfluoxetine (Metabolit) | 296.04 | 133.83 | 0.109 | 5 |
| Fluoxetine d6 (IS) | 316.10 | 153.84 | 0.109 | 15 |

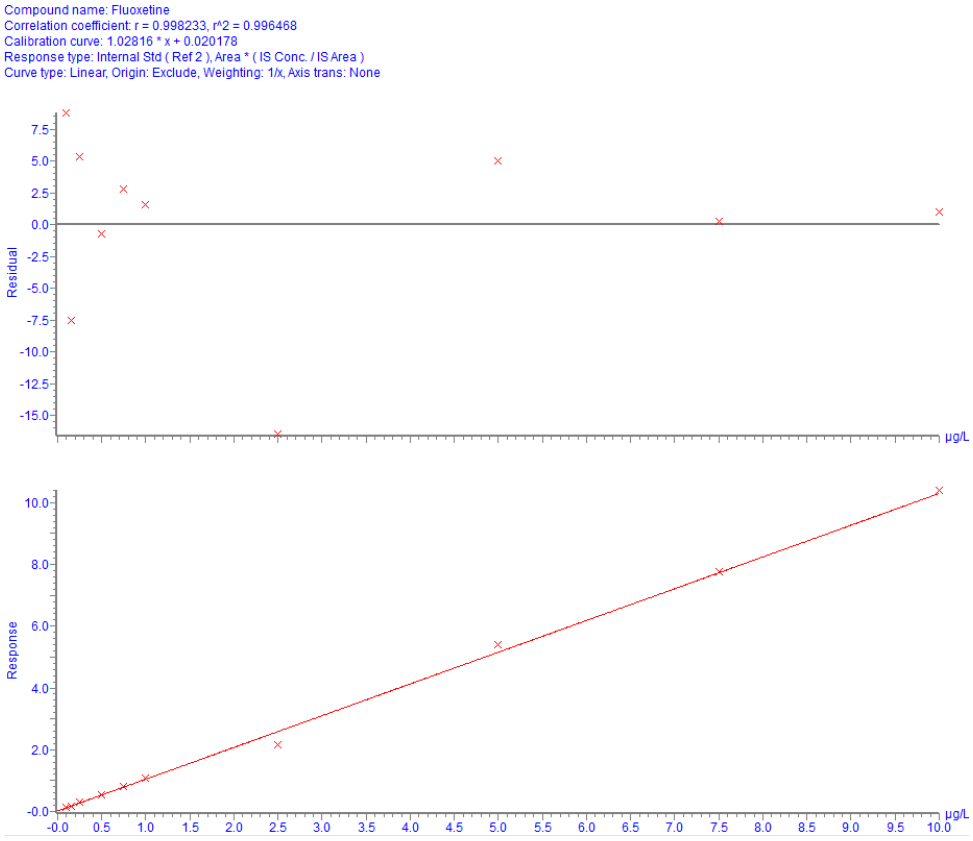
quan. = quantifier
qual = qualifier

**Figure 20: Exemplary calibration curve medium**



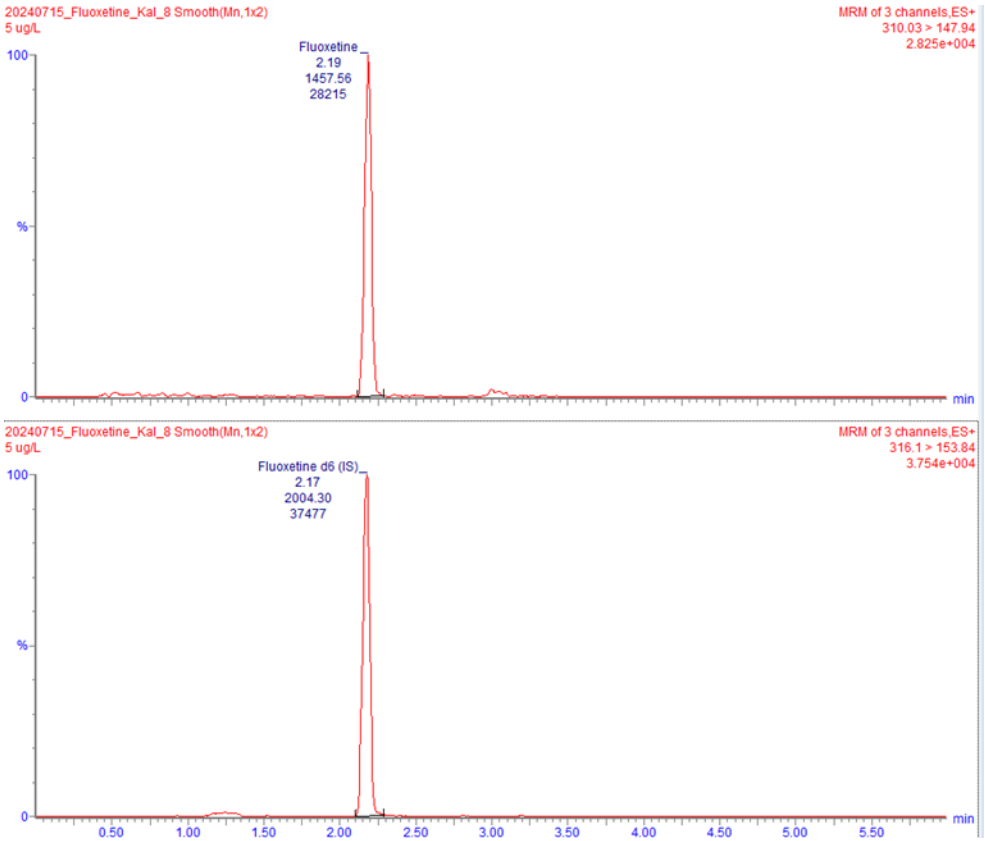

Source: own illustration, Fraunhofer IME

**Figure 21: Exemplary calibration curve tissue**



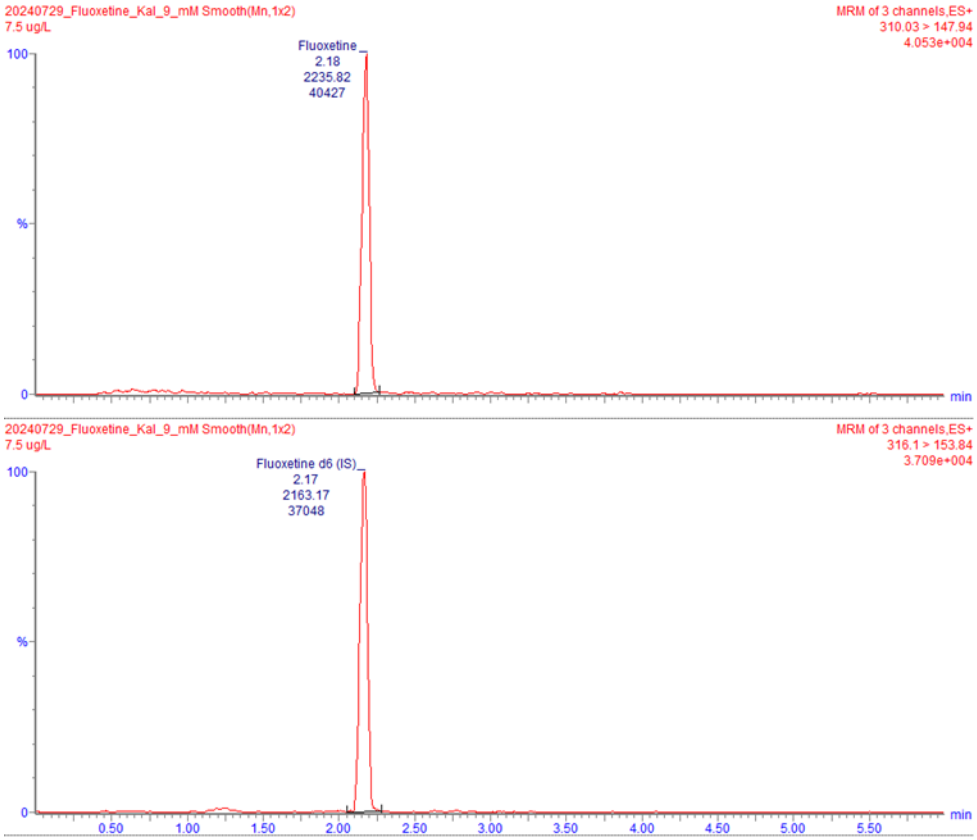
Source: own illustration, Fraunhofer IME

**Figure 22: Exemplary chromatogram - medium**




Source: own illustration, Fraunhofer IME

**Figure 23: Exemplary chromatogram - tissue**




Source: own illustration, Fraunhofer IME

### 8.1.5 Pyrene & β-HCH

**Table 146: Internal standard**

Pyrene: d10-pyrene (CAS-Number: 1718-52-1)

β-HCH: HCB (CAS-Number: 118-74-1)

| | LOD | LOQ | Calibration range | PRS level |
|---|---|---|---|---|
| Water | 1 µg/L | | 1 – 100 µg/L | 2 µg/L<br>60 µg/L |
| *H. azteca* tissue | 5 ng (abs.) | | 5 – 1000 ng (abs.) | 10 ng (abs.)<br>60 ng (abs.) |
| Hepatocyte extracts | 0.5 µg/L cell matrix | | 0.5 – 120 ng (abs.) | 1 µg/L cell matrix<br>60 µg/L cell matrix |

**Table 147: Stock solutions**

Water sample analyses

| In acetone | | Pyrene, ß-HCH | | | | Pyrene-d10, HCB (IS-standards) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Volume (mL) | Stock II [µL] | Conc. (ng/mL) | 50 µL per Vial (ng abs.) | Conc. [µg/L water] | Stock II [µL] | Conc. (ng/mL) | 50 µL per Vial (ng abs.) | Conc. [µg/L water] |

| In acetone | | Pyrene, ß-HCH | | | | Pyrene-d10, HCB (IS-standards) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cal 1 | 10 | 10 | 100 | **5** | **1** | 250 | 2500 | **125** | **25** |
| Cal 2 | 10 | 20 | 200 | **10** | **2** | 250 | 2500 | **125** | **25** |
| Cal 3 | 10 | 40 | 400 | **20** | **4** | 250 | 2500 | **125** | **25** |
| Cal 4 | 10 | 80 | 800 | **40** | **8** | 250 | 2500 | **125** | **25** |
| Cal 5 | 10 | 150 | 1500 | **75** | **15** | 250 | 2500 | **125** | **25** |
| Cal 6 | 10 | 300 | 3000 | **150** | **30** | 250 | 2500 | **125** | **25** |
| Cal 7 | 10 | 600 | 6000 | **300** | **60** | 250 | 2500 | **125** | **25** |
| Cal 8 | 10 | 1000 | 10000 | **500** | **100** | 250 | 2500 | **125** | **25** |

**Table 148: Stock solutions**

H. azteca tissue sample analyses

| In acetone | Pyrene, ß-HCH | | | | Pyrene-d10, HCB (IS-standards) | | | |
|---|---|---|---|---|---|---|---|---|
| | Stock II (µL) | Volume (mL) | Conc. (µg/mL) | 25 µL per Vial (ng abs.) | Stock IS I (µL) | Volume Acetone (mL) | Conc. (µg/mL) | 25 µL per Vial (ng abs.) |
| Cal 0 | 0 | 10 | 0,0 | **0** | 200 | 10 | 2 | **50** |
| Cal 1 | 20 | 10 | 0,2 | **5** | 200 | 10 | 2 | **50** |
| Cal 2 | 40 | 10 | 0,4 | **10** | 200 | 10 | 2 | **50** |
| Cal 3 | 80 | 10 | 0,8 | **20** | 200 | 10 | 2 | **50** |
| Cal 4 | 150 | 10 | 1,5 | **37** | 200 | 10 | 2 | **50** |
| Cal 5 | 300 | 10 | 3 | **75** | 200 | 10 | 2 | **50** |
| Cal 6 | 600 | 10 | 6 | **150** | 200 | 10 | 2 | **50** |
| Cal 7 | 1200 | 10 | 12 | **300** | 200 | 10 | 2 | **50** |
| Cal 8 | 1200 | 5 | 24 | **600** | 100 | 5 | 2 | **50** |
| Cal 9 | 2000 | 5 | 40 | **1000** | 100 | 5 | 2 | **50** |

**Table 149: Chromatography conditions**

| Parameter | Description |
|---|---|
| System name | GC 5590, MSD 5977B |
| Stationary phase | Rxi-5Sil MS (30m x 250µm ID x 0,1µm FD) |
| Injection volume (µL) | 1 |
| Injection mode | Splitless |
| Flow rate | 1 mL/min |

| Parameter | Description |
|---|---|
| Inlet temperature (°C) | 290 |
| Oven | <table><tr><td></td><td>Rate (°C/ min)</td><td>Value (°C)</td><td>Hold time (min)</td><td>Run time (min)</td></tr><tr><td>Initial</td><td></td><td>40</td><td>1</td><td>1</td></tr><tr><td>Ramp 1</td><td>10</td><td>100</td><td>0</td><td>7</td></tr><tr><td>Ramp 2</td><td>30</td><td>300</td><td>6.333</td><td>20</td></tr></table> |
| MS Transferline (°C) | 270 |
| Solvent delay (min) | 3 |

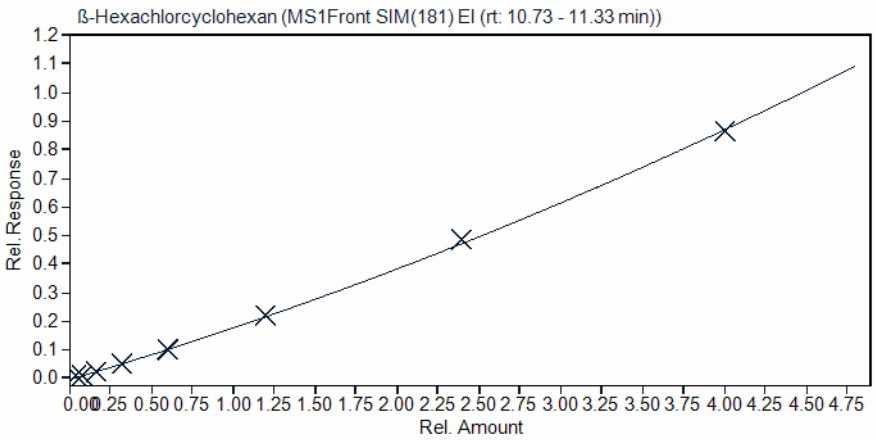
**Table 150: MS parameter**

| Parameter | Description |
|---|---|
| MS mode | SIM |
| Ion mode | EI |
| Source temperature | 230 °C |
| Quad temperature | 150 °C |

**Table 151: Mass transitions**

| | Retention time (min) | Quantifier mass (m/z) | Qualifier mass (m/z) |
|---|---|---|---|
| HCB, internal standard | 10.86 | 284 | 142 |
| β-HCH | 11.02 | 181 | 219 |
| d-10 pyrene, internal standard | 12.34 | 212 | 106 |
| Pyrene | 12.35 | 202 | none |

**Figure 24: Exemplary calibration curve medium, ß-HCH in water**



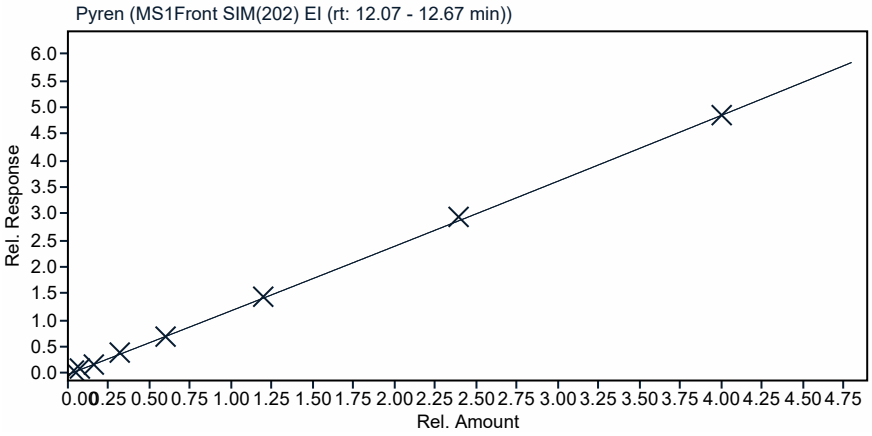

| | |
|---|---|
| R: | 0.999749 |
| R^2: | 0.99965 |
| Formula: | $y = ax^2 + bx + c$ |
| a: | 0.01247 |

b: 0.16908

c: -0.0011

Source: own illustration, Fraunhofer IME

**Figure 25: Exemplary calibration curve medium, pyrene in water**




R: 0.999944

R^2: 0.99993

Formula: $y = ax^2 + bx + c$

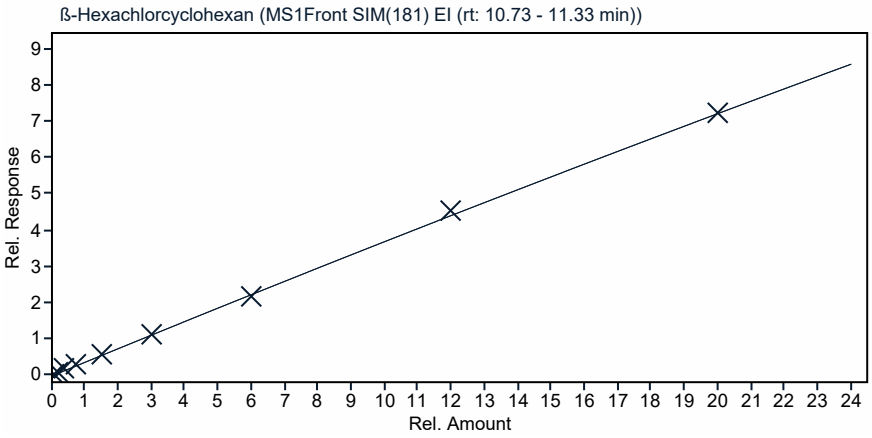
a: 0.00627

b: 1.19396

c: -0.00392

Source: own illustration, Fraunhofer IME

**Figure 26: Exemplary calibration curve tissue, ß-HCH in *H. azteca***




R: 0.999716

R^2: 0.99966

Formula: $y = ax^2 + bx + c$

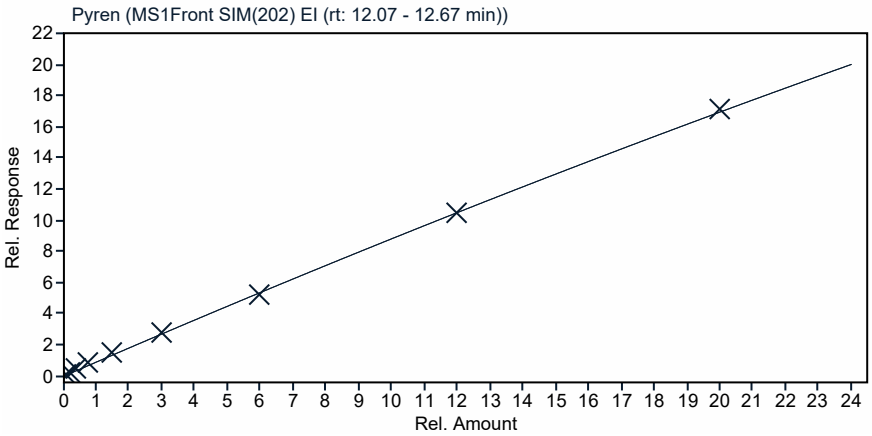
a: -0.00089

b: 0.37925

c: 0.00572

Source: own illustration, Fraunhofer IME

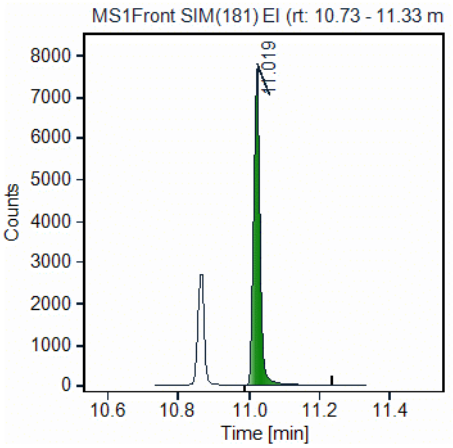
**Figure 27: Exemplary calibration curve medium, pyrene in *H. azteca***




| | |
|---|---|
| R: | 0.999557 |
| R^2: | 0.99979 |
| Formula: | $y = ax^2 + bx + c$ |
| a: | -0.0035 |
| b: | 0.91818 |
| c: | 0.04525 |

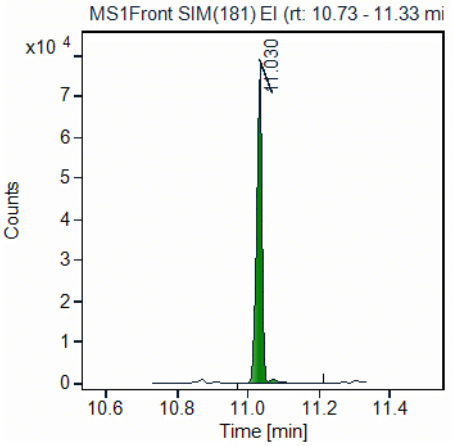
Source: own illustration, Fraunhofer IME

**Figure 28: Exemplary chromatogram: ß-HCH water sample 72h replicate 1**



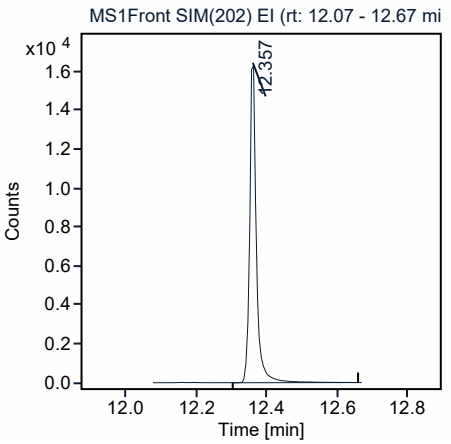

Source: own illustration, Fraunhofer IME

**Figure 29: Exemplary chromatogram: ß-HCH *H. azteca* sample 48h replicate 1**



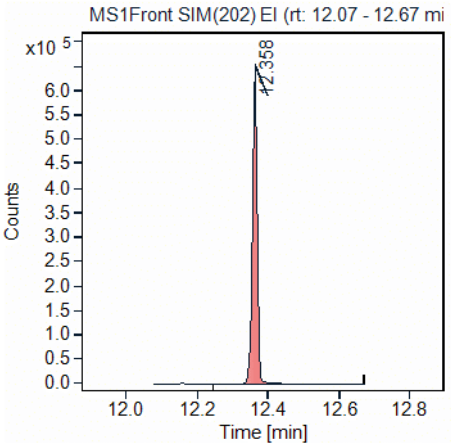
Source: own illustration, Fraunhofer IME

**Figure 30: Exemplary chromatogram: Pyrene water sample 10h replicate 1**




Source: own illustration, Fraunhofer IME

**Figure 31: Exemplary chromatogram: Pyrene *H. Azteca sample 24h replicate 1***




Source: own illustration, Fraunhofer IME

#### 8.1.5.1 Calculation of concentration in measured sample

Water

No calculations were necessary.

H. azteca tissue

The determined content of the target substance was divided by the documented wet weight of the *H. azteca* sample

Hepatocyte extracts

No calculations were necessary.

### 8.1.6 UV-234

**Table 152: internal standards**

2-Benzotriazol-2-yl-4,6-bis(1-methyl-1-phenylethyl) phenol-d4 (UV-234-d4)

| | LOD | LOQ | Calibration range | PRS level |
|---|---|---|---|---|
| Unit | µg/L | µg/L | µg/L | µg/L |
| Medium | 0.05 | 0.15 | 0.05-5 | 0.25 / 2.5* |
| Hyalella | 0.05 | 0.15 | 0.05-5 | See chapter 8.2.5.2 |
| Rainbow trout in vitro | 0.25 | 0.75 | 0.25-25 | 1.0 / 10* |
| Rat in vitro | 0.25 | 0.75 | 0.25-10 | 0.5 / 7.5* |

* Instead of classic PRS samples, matrix-matched quality control (QC) samples were measured. Those are identical to the calibration samples, but originate from a second weighing. All samples, calibration samples and QC samples have the same composition in terms of solvent, water and matrix content.

**Table 153: Chromatography conditions**

| | |
|---|---|
| System name | Xevo-TQD |
| Stationary phase | ACQUITY UPLCr BEH C18 1.7µm |
| Mobile phase | C: H2O:MeOH 95:5 + 0.1 % FA<br>D: MeOH + 0.1 % FA |
| Injection volume | 10 µL |
| Column temperature | 40 °C |
| Flow rate | 0.3 µL/min |
| Gradient | <table><tr><td></td><td>Time [min]</td><td>Flow rate [mL/min]</td><td>% C</td><td>% D</td></tr><tr><td>1</td><td>Initial</td><td>0.3</td><td>5.0</td><td>95.0</td></tr><tr><td>2</td><td>3.0</td><td>0.3</td><td>5.0</td><td>95.0</td></tr></table> |

Sub caption of table – for example source, additional information.

**Table 154: MS parameter**

| | |
|---|---|
| Type | MRM |
| Span (Da) | 0.2 |
| Ion mode | ES+ |
| Capillary (kV) | 2.2 |
| Cone (V) | 50.0 |
| Source temperature (°C) | 150 |
| Desolv. Temperature (°C) | 500 |
| Cone gas flow (L/h) | 150 |

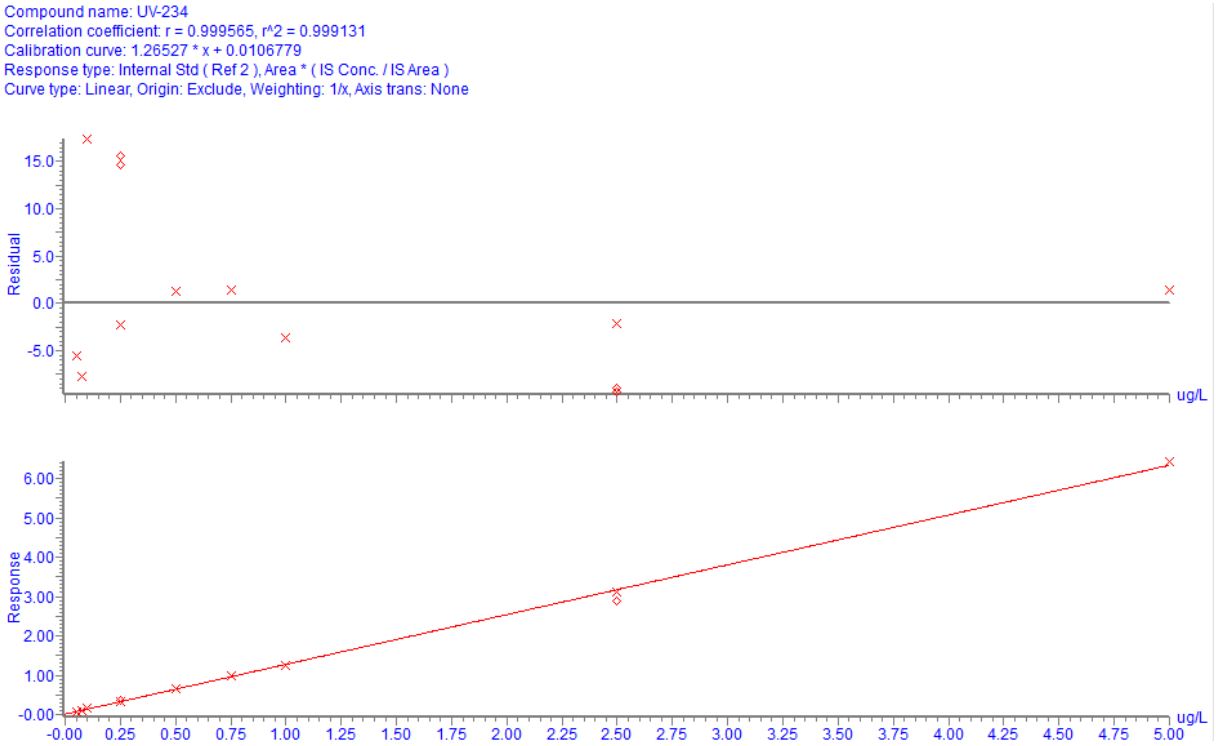
Sub caption of table – for example source, additional information.

**Table 155: Mass transitions**

| | Parent ion (m/z) | Daughter ion (m/z) | Dwell time (s) | Collision energy (eV) |
|---|---|---|---|---|
| UV-234 (quan) | 448.37 | 119.06 | 0.108 | 35 |
| UV-234 (qual) | 448.37 | 370.29 | 0.108 | 20 |
| UV-234 IS (quan) | 452.31 | 374.28 | 0.108 | 20 |

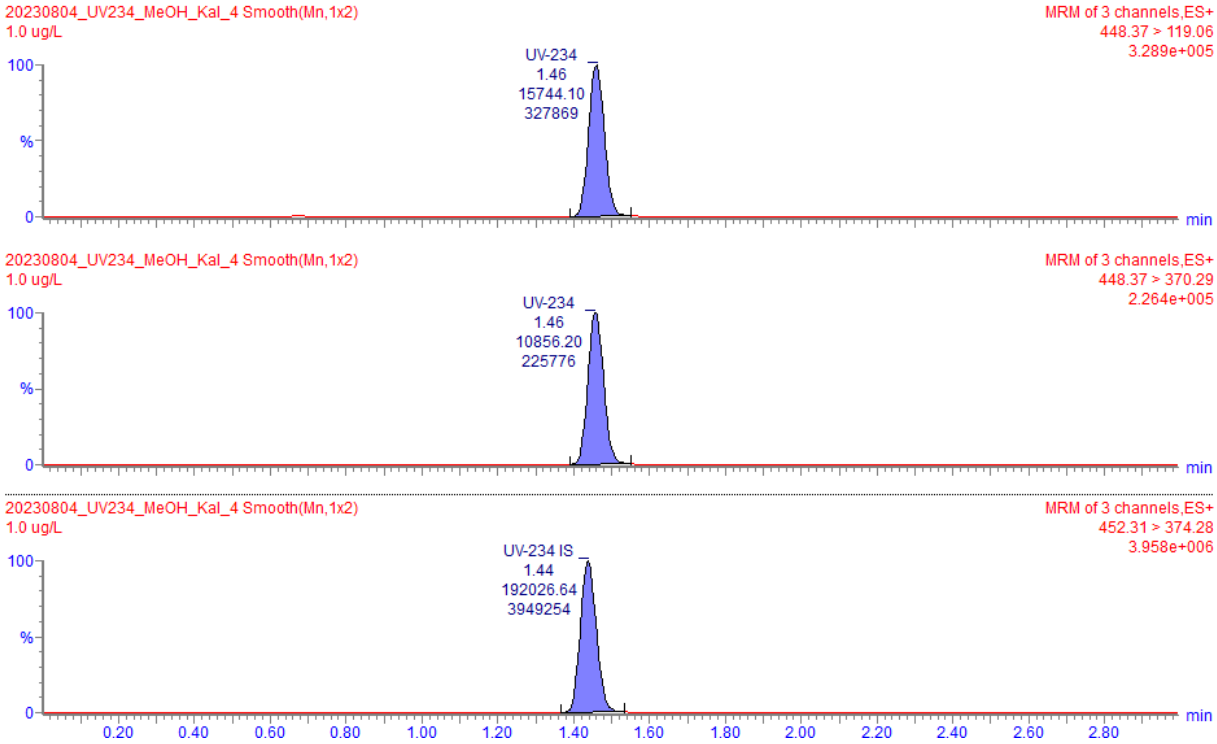
quan. = quantifier
qual = qualifier

**Figure 32: Exemplary calibration curve medium**



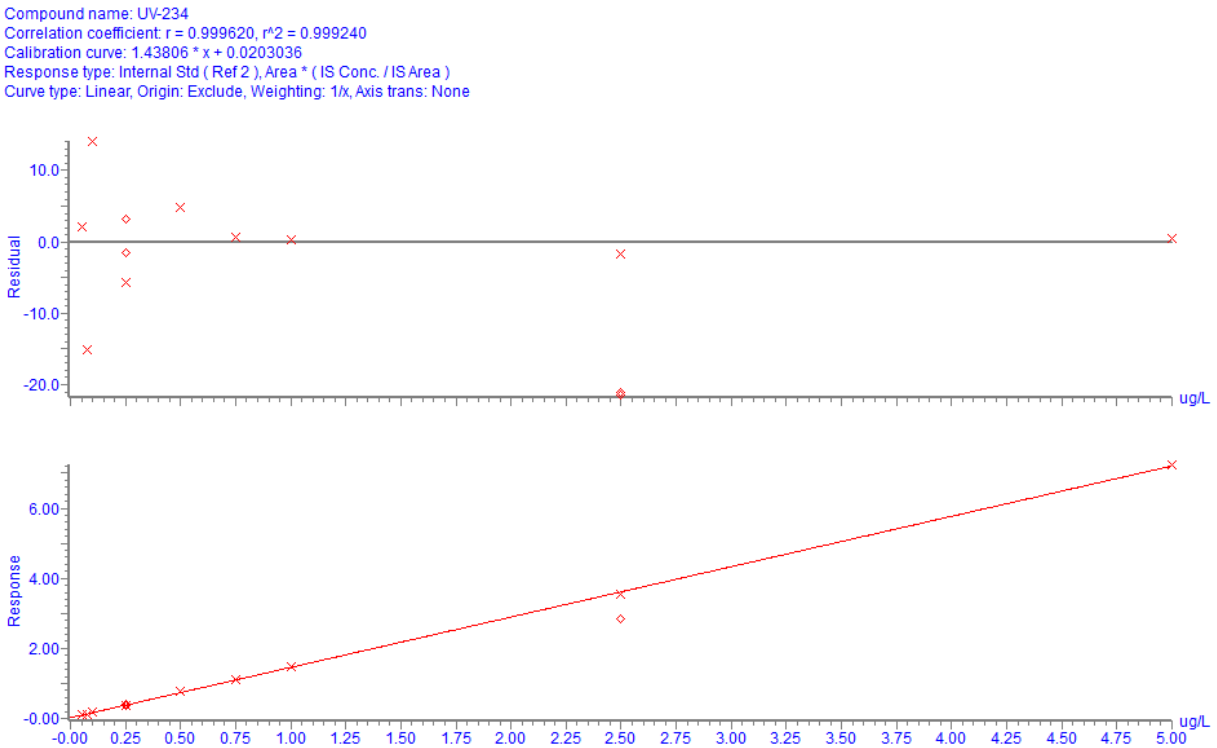

Source: own illustration, Fraunhofer IME

**Figure 33: Exemplary chromatogram medium**



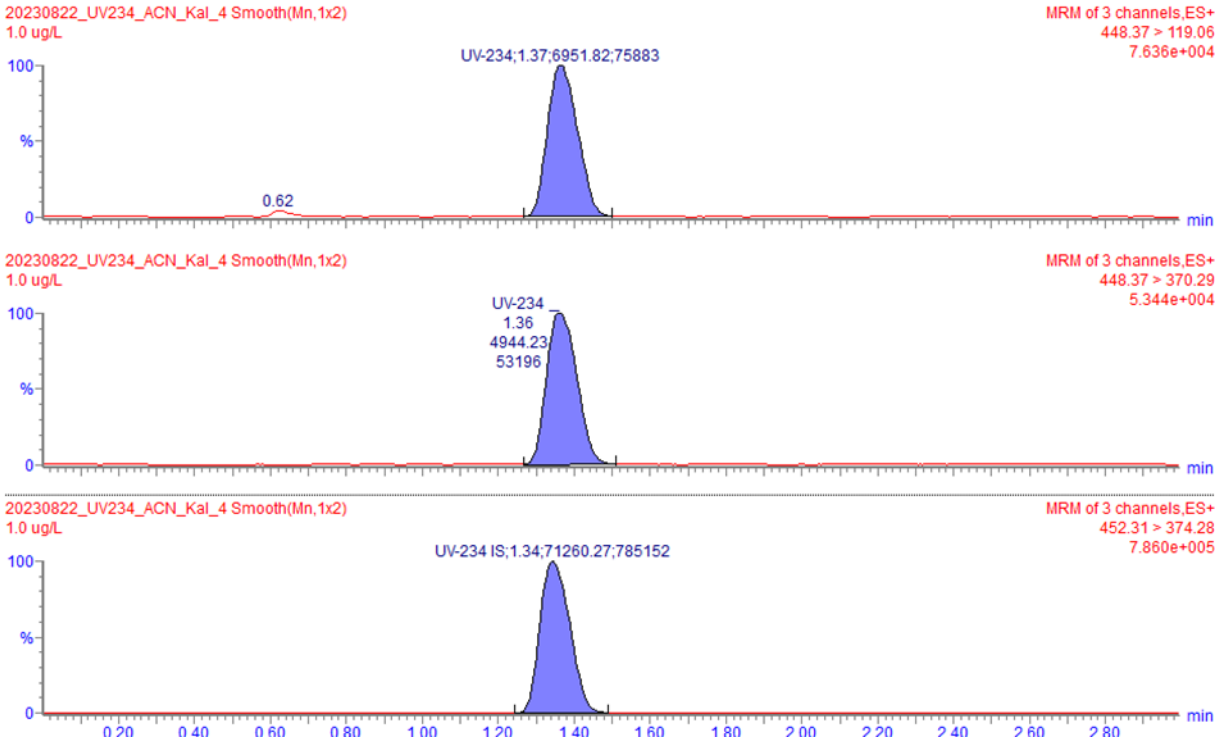

Source: own illustration, Fraunhofer IME

**Figure 34: Exemplary calibration curve *H. azteca* tissue BCF**



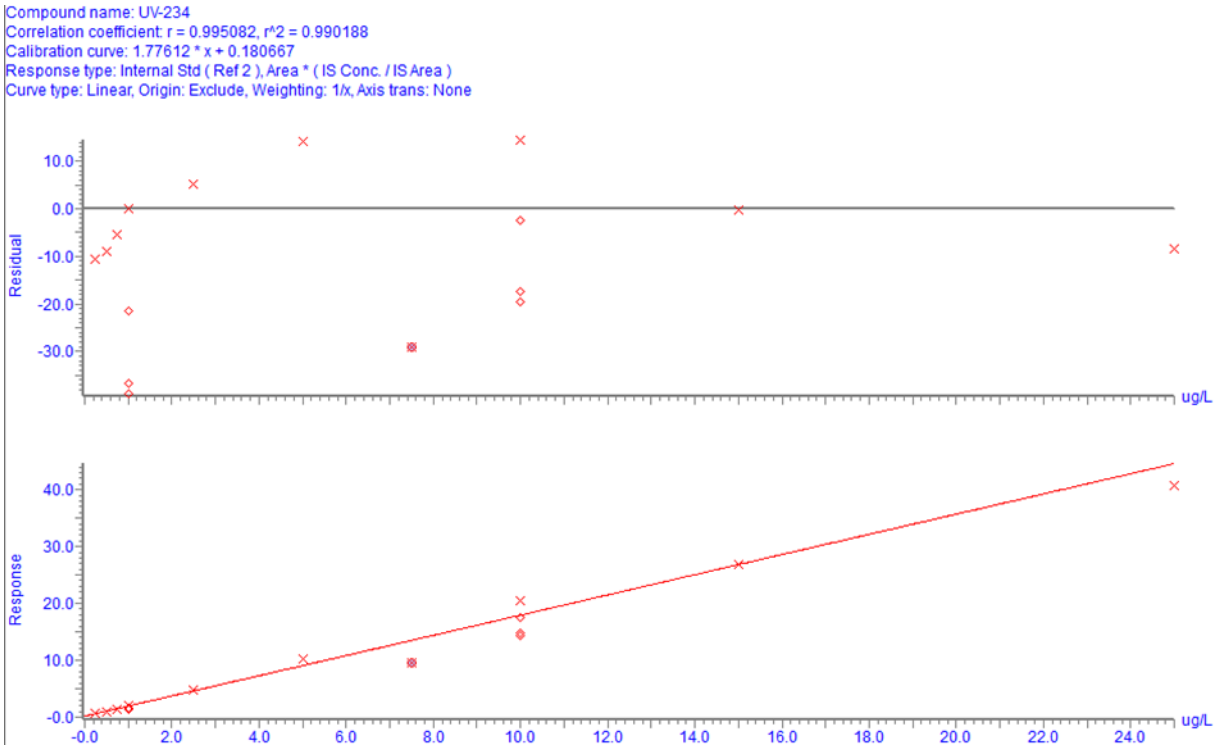

Source: own illustration, Fraunhofer IME

**Figure 35: Exemplary chromatogram *H. azteca* tissue BCF**



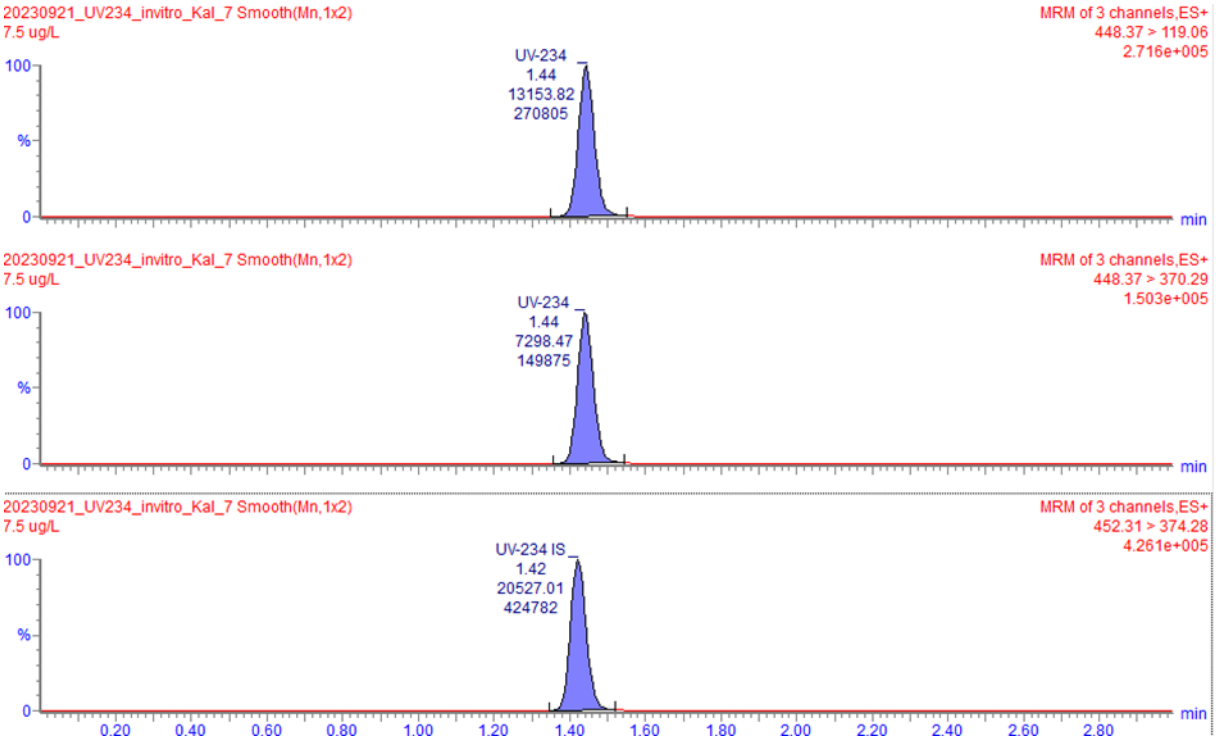

Source: own illustration, Fraunhofer IME

**Figure 36: Exemplary calibration curve tissue trout in vitro**




Source: own illustration, Fraunhofer IME

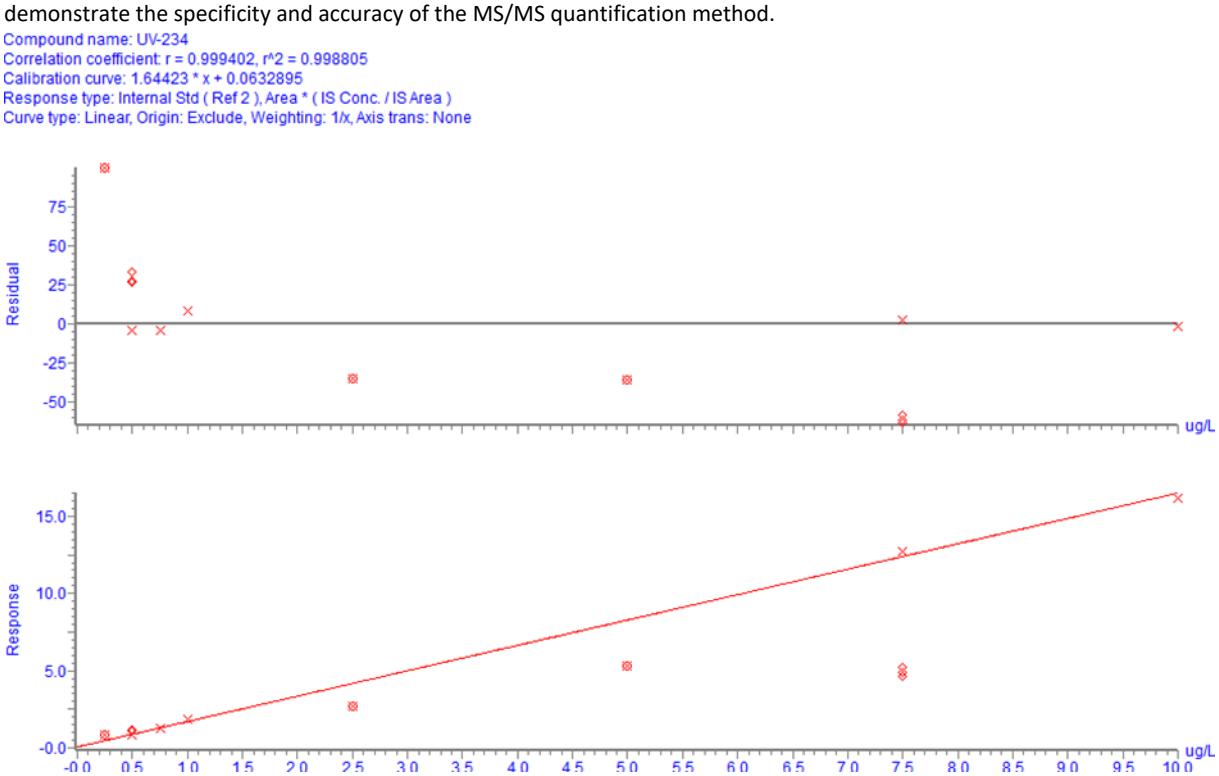
**Figure 37: Exemplary chromatogram trout in vitro**




Source: own illustration, Fraunhofer IME

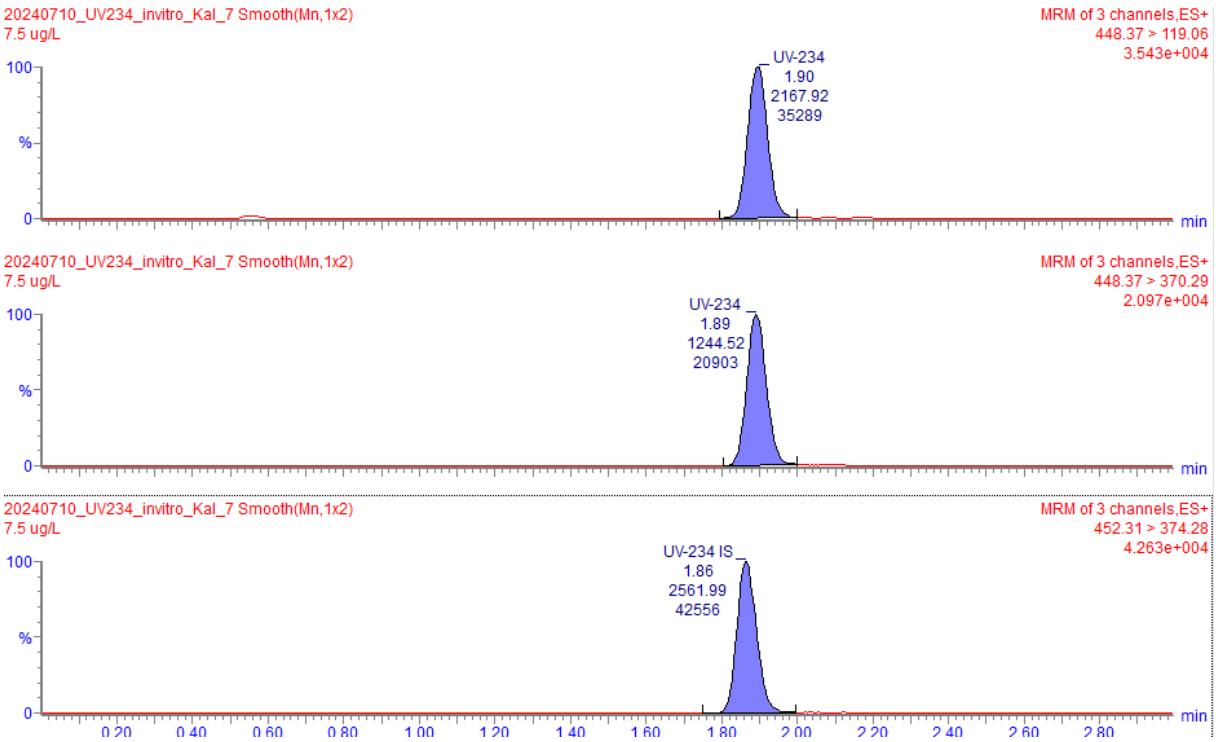
**Figure 38: Exemplary calibration curve tissue Rat in vitro**

Representative MRM chromatograms for UV-234 at 1.0 µg/L. The clear single peaks at a retention time of 1.37 minutes demonstrate the specificity and accuracy of the MS/MS quantification method.




Source: own illustration, Fraunhofer IME

**Figure 39: Exemplary chromatogram Rat in vitro**




Source: own illustration, Fraunhofer IME

## 8.2 Sample preparation

### 8.2.1 Chlorpyrifos

*Cf.* chapter A.4.2

### 8.2.2 Diclofenac

The chemical analysis of the test substances was performed by liquid chromatography (LC) with coupled mass spectrometry (MS). The analyses of medium and *H. azteca* samples were carried out in the multiple reaction monitoring (MRM) mode and quantification of the analytes was performed internally with deuterated standards. Quality control samples with one low ($c_{low}$ = 0.75 µg/L) and one high ($c_{high}$ = 7.5 µg/L) concentration were run alongside each measurement.

#### 8.2.2.1 Water and tissue samples

Calibrations for water and tissue analysis of Diclofenac samples collected during the bioconcentration tests consisted at least of 6, mostly 10, points ranging from 0.1 – 10 µg/L. All medium samples were measured after 1:5 dilution with holding and dilution water (HDW) and ACN (1:1 *v:v)*.

*H. azteca* samples were processed by solid-liquid extraction. Therefore, 1.5 mL ACN and 1.5 g FastPrep™ Beads were added to the biological sample, homogenized for 20 seconds and then treated in an ultrasonic bath for 10 min. The samples were centrifuged for 5 min at 21.1 g and supernatants were transferred into a volumetric flask. The second and third extraction step, were similar to the first one. The combined supernatants were filled up to a total volume of 5 mL. This was followed by centrifugation for 5 min at 4696 g. The extracts obtained were subsequently analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). For the experiment at pH 7.8 samples were concentrated by factor 10 and measured after 1:3.75

dilution. Samples of the experiment at pH 5 were either directly measured after 1:3.75 dilution with HDW and ACN (1:1 *v:v* ) or were concentrated by factor 10. The concentration step was performed for the last four sampling points of the depuration. Concentrated samples were measured after 1:3.75 dilution with HDW and ACN (1:1 *v:v*). The concentration step was performed by reducing the volume of the samples by evaporating the solvent by means of a nitrogen stream.

#### 8.2.2.2 Determination of extraction efficiency

Beforehand, the efficiency rates for this protocol were tested. Blank H. azteca samples were spiked with a specific amount of the test substance in three replicates. After extraction of the samples as described above, the recovery rates were calculated based on the amount of the spiked compound. For the analyte, the extraction protocol was efficient. The recovery rate was determined to be 112 ±7.6 % with acetonitrile.

### 8.2.3 D4 (Octamethylcyclotetrasiloxane)

#### 8.2.3.1 Water samples pre-exposure HYBIT test

10 mL test medium were pipetted into a 15 mL polypropylene vial (Sarstedt, Nümbrecht, Germany). Subsequently, 2 g sodium chloride (ChemSolute, Th. Geyer GmbH, Renningen, Germany), 1 ml n-hexane (Supelco, Darmstadt, Germany) and 100 µL internal standard solution with a concentration of 1 mg/L were added and the sample was shaken on a horizontal shaker for 30 min. After centrifugation for 15 min at 4500 rpm and 4 °C, the hexane phase was removed and a second extraction step was performed (addition of 1 mL n-hexane, shaking for 30 min on a horizontal shaker, centrifugation for 15 min at 4500 rpm and at 4 °C). The n-hexane phases were combined and evaporated to a volume of approx. 1 mL under a gentle nitrogen flow. The samples were then sent directly to the analysis via ICP-GC-MS/MS or stored at ≤ -18 °C until analysis.

The extraction and analysis of D4 in water samples is based on an existing method for the extraction and analysis of D4 in fish fillet samples (Radermacher, 2020).

“Radermacher G, Rüdel H, Wesch C, Böhnhardt A, Koschorreck J (2020), Retrospective analysis of cyclic volatile methylsiloxanes in archived German fish samples covering a period of two decades. Sci Total Environ 706, 136011 https://doi.org/10.1016/j.scitotenv.2019.136011 (open access)”

#### 8.2.3.2 *H. azteca* samples

No method was developed for extraction of *H. azteca* samples, since the study was aborted due to the impossibility of the application of D4.

#### 8.2.3.3 Hepatocyte extracts

Hepatocyte extractions were extracted using n-hexane. Different volume ratios of n-hexane to hepatocyte solution were tested. Testing was not continued to due insufficient recoveries of the test substance.

### 8.2.4 Pyrene & β-HCH

#### 8.2.4.1 Water samples

- 5 mL of water are placed in an 8 mL screw vial. Then, 50 µL of the IS standard solution (in acetone) and 500 µL n-hexane are added for extraction.

- The sample is shaken for 30 minutes, 250 µL of the organic extract are pipetted into a micro vial, and subsequently analysed by GC-MS

#### 8.2.4.2 PRS- samples (water)

- 5 mL of water are placed in an 8 mL screw vial. Subsequently, 50 µL of the standard solution (in acetone) and 500 µL n-hexane are added for extraction.
- The sample is shaken for 30 minutes, 250 µL of the organic extract are pipetted into a micro vial, followed by GC-MS analysis.
- Calibration range of the standard solution in solvent: 100 – 10,000 ng/mL
- Calibration range based on the test medium: 1 – 100 µg/L water
- PRS samples included Cal-level 2 (2 µg/L water) and Cal-level 7 (60 µg/L water)

#### 8.2.4.3 *H. azteca* samples

- The *H. azteca* sample is transferred to a 1.5 mL measuring vial and 25 µL acetonic IS standard (Cal 0) are added.
- The *H. azteca* are carefully ground with a suitable metal rod until the sample is homogeneous. Then, 975 µL n-hexane are added, the vial is sealed and shaken for 30 minutes (extraction).
- The weight of the *H. azteca* sample (wet weight) is documented and factored into the measurement result.
- For PRS samples, Cal-level 2 (10 ng abs.) and Cal-level 8 (600 ng abs.) were also measured.

#### 8.2.4.4 PRS samples (*H. azteca*)

- Approximately 50 mg of *H. azteca* are placed in a 1.5 mL glass vial and 25 µL of acetonic calibration standard are added.
- The *H. azteca* are carefully ground with a suitable metal rod until the sample is homogeneous. Then 975 µL of n-hexane are added, the vial is sealed and shaken for 30 minutes (extraction).
- Calibration range: 5 – 1000 ng abs.
- As PRS samples, Cal-level 2 (10 ng abs.) and Cal-level 8 (600 ng abs.) were also measured.

#### 8.2.4.5 Hepatocyte extracts

*Cf.* chapters A.4.2 & A.5.4

### 8.2.5 UV-234

The chemical analysis of UV-234 was performed by LC-MS/MS. The analyses of medium and *H. azteca* samples were carried out in multiple reaction monitoring (MRM) mode using a deuterated internal standard.

For *H. Azteca* and water measurements, quality control samples with one low ($c_{low}$ = 0.25 µg/L) and one high ($c_{high}$ = 25 µg/L) concentration were run alongside each measurement.

For *in vitro* measurements, quality control samples with one low ($c_{low,T}$ = 1 µg/L; $c_{low,R}$ = 0.5 µg/L) and one high ($c_{high,T}$ = 10 µg/L; $c_{high,R}$ = 7.5 µg/L) concentration were run alongside each measurement for trout (T) and rat (R) hepatozytes, respectively.

#### 8.2.5.1 Water samples

For sampling of water, a volumetric pipette was equilibrated in the test vessel since start of the application. 5 mL of water were drawn from the vessel with the equilibrated pipette during a movement across the vessel and mixed with 5 mL of methanol.

Before LC-MS/MS measurement, 15 µL of internal standard solution (UV-234-d4, c = 1000 µg/L) were added to 1 mL of stabilised sample. The matrix-matched calibration ranged from 0.1–25 µg/L with 11 calibration points.

#### 8.2.5.2 *H. azteca* samples

*H. azteca* samples were processed by solid-liquid extraction. First, 15 µL of internal standard solution (c = 1000 µg/L) were added to the tissue samples. The extraction was performed for 60 s in a bead beating homogenizer using 4 mL ACN and approx. 1.5 g FastPrep™ beads (yttria-stabilised zirconium oxide, 2mm diameter). The extract was treated with ultrasound for 5 min and centrifuged for 5 min afterwards. Supernatants were transferred into a volumetric flask. The extraction was repeated for a second and third time with 3 mL of acetonitrile and the supernatants were combined and filled up to a total volume of 10 mL with acetonitrile. Samples were centrifuged again and analysed by LC-MS/MS with different dilutions to fit the calibration range.

A solvent calibration (range 0.05–5 µg/L) was used for quantification of UV-234 using the internal standard for compensation of matrix effects.

To confirm the suitability of the extraction and analysis method, procedural recovery samples (PRS) with the same dilutions as the test samples were measured alongside the *H. Azteca* samples. For preparation of PRS, *H. Azteca* blank matrix was spiked with defined amounts of the test item and extracted as described for the test samples. The dilution was done with solvent to reach a ratio of 200 µL methanol to 800 µL acetonitrile (including the respective amount of blank matrix) as follows:

**Table 156: Dilution of *H. Azteca* and respective procedural recovery samples**

| Dilution factor | Concentration PRS level 1 / 2 in diluted extract | *H. Azteca* samples analysed with same dilution |
|---|---|---|
| 1 (no dilution) | 0.25 / 2.5 µg/L | days 0, 14 |
| 2 | 0.125 / 1.25 µg/L | days 1, 11, 12, 13 |
| 5 | 0.5 / 2 µg/L | days 2, 3, 8, 9 |
| 10 | 0.25 / 1 µg/L | days 4, 5, 6, 7 |

Recoveries of the PRS samples were between 88.1 and 109.3 % and confirmed the suitability of the quantification method of UV-234 in *H. Azteca*.

#### 8.2.5.3 Hepatocyte extracts

*Cf.* chapters A.4.2, A.4.6, A.5.2

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