

Supporting Information

Effects of benzo[a]pyrene, cortisol and 17 β -estradiol on liver microsomal EROD activity of *Anguilla anguilla*: An *in vitro* approach

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Table S1 - Buffers, solvents and solutions used during Experimental Setup

| <u>Buffers</u> | |
|-------------------------|---|
| BS | 0.5 μ M etoxyresorufin in Tris-HCl 0.1 M pH 7.4 with KCl 0.15M and 20% glycerol |
| B | Tris-HCl 0.1 M pH 7.4 with KCl 0.15M and 20% glycerol |
| BSC | BS with cortisol (final concentration in cuvette = 5.997 ng/ml) |
| BSE₂ | BS with E ₂ (final concentration in cuvette = 5.997 ng/ml) |
| <u>Solvents</u> | |
| DMSO | Dimethyl sulfoxide |
| <u>Solutions</u> | |
| B[a]P | Benzo[a]pyrene (0.1, 0.3, 0.9 and 2.7 μ M) in DMSO |

Table S2 - Experimental liver microsomal EROD activity assay procedures to assess effects of cortisol (C), 17 β -estradiol (E₂) and benzo[a]pyrene (B[a]P), alone or in combination. The numbers (1, 2, 3 and 4) represent the sequence of compounds added to the cuvette [Tris-HCl 0.1 M pH 7.4 with KCl 0.15 M 20% glycerol (B) with 0.5 μ M ethoxyresorufin (BS); BS with cortisol 5.997 ng/ml (BSC); BS with E₂ 5.997 ng/ml (BSE₂), dimethyl sulfoxide (DMSO), benzo[a]pyrene (B[a]P)].

| Experimental assay | 5 μ l microsomes | 1090 μ l BS | 1090 μ l BSC | 1090 μ l BSE ₂ | 5 μ l B | 5 μ l DMSO | 5 μ l B[a]P (in DMSO) | 10 μ l NADPH (10nM) |
|--|----------------------|-----------------|------------------|-------------------------------|-------------|----------------|---------------------------|-------------------------|
| Effects of 5.997 ng/ml of C | 1 | | 2 | | | 3 | | 4 |
| Effects of 5.997 ng/ml of E ₂ | 1 | | | 2 | | 3 | | 4 |
| Effects of B[a]P (0.1, 0.3, 0.9 and 2.7 μ M) | 1 | 2 | | | | | 3 | 4 |
| Effects of B[a]P (0.1, 0.3, 0.9 and 2.7 μ M) after pre-exposure to 5.997 ng/ml of C | 1 | | 2 | | | | 3 | 4 |
| Effects of B[a]P (0.1, 0.3, 0.9 and 2.7 μ M) after pre-exposure to 5.997 ng/ml of E ₂ | 1 | | | 2 | | | 3 | 4 |

