**Supplementary material**

**Toxic effects of imidacloprid and sulfoxaflor on *Rana nigromaculata* tadpoles: growth, antioxidant indices and thyroid hormone-related endocrine system**

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**Text S1**. **Detailed information about the instruments (LC-MS/MS)**

Imidacloprid and sulfoxaflor were separated by the Shimadzu 20 AD-XR liquid chromatography system (Tokyo, Japan) using a Phenomenex Kinetex F5 (100 × 2.1 mm, 2.6 μm) and a CHIRALPAK IH-3 column (0.46 cm i.d.×15 cm×3 µm) with columns temperature of 35°C and 25°C, respectively. The flow rate of mobile phases was 0.4 mL/min and the injection volume were 1 µL and 2 µL, as for imidacloprid, the mobile phases were water (A) and methanol (B) (80/20, V/V), as for sulfoxaflor, the mobile phases were acetonitrile and 0.05% trifluoroacetic acid water (25/75, V/V). Results were confirmed using an AB Sciex 4500Q trap mass spectrometer device (Foster City, CA, USA). Multiple reaction monitoring (MRM) mode and a positive mode electrospray ionisation source (ESI+) were used to determine concentrations of imidacloprid and sulfoxaflor. The ESI parameters are as following: ion spray voltage, 5500 V; ion source temperature, 550°C; curtain gas was 25 psi, as for imidacloprid ion source gases 1 and 2 pressures were 65 and 55 psi, respectively. The transitions employed for qualification and qualitative analysis were *m/z* 256.10/209.00 and 256.10/170.10, and collision energies were 20.84 and 27.40 eV, respectively. As for sulfoxaflor, ion source gases 1 and 2 pressures were 55 and 55 psi, respectively. The transitions employed for qualification and qualitative analysis were *m/z* 278.10/174.10 and 256.10/105.00, and collision energies were 55.25 and 57.30 eV, respectively.

**Table S1**.LC50 values of sulfoxaflor and imidacloprid against R. nigromaculata tadpole

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound | aLC50  (mg/L) | bConfidence intervals  (mg/L) | cRegression equation | dR2 |
| sulfoxaflor | 427.37 | 392.59-467.94 | Y=8.61x-22.89 | 0.983 |
| imidacloprid | 173.55 | 152.17-199.43 | Y=4.48x-10.07 | 0.982 |

a LC50, the effective concentration that results in 50% mortality of the test population compared with the control.

b 95% confidence intervals surrounding each estimated LC50 are bracketed.

c the concentration–response curves were established as the correlation between the concentration and the mortality of the test population. X is the logarithm of concentration.

d R2 represents the correlation coefficient.

**Table S2**. Calibration equation, R2, limit of quantification (LOQ)) for imidacloprid in solvent and matrices.

|  |  |  |  |
| --- | --- | --- | --- |
| Matrices | Calibration equation | R2 | LOQ  (mg/kg) |
| Methanol | y = 1×107x + 464343 | 0.9952 | - |
| Tadpoles | y = 1×107x + 498709 | 0.9963 | 0.005 |
| Water | y = 6×106x + 39520 | 0.9992 | 0.01 |

**Table S3**. Average recoveries and relative standard deviation (RSD) of imidacloprid under various matrices and spiked levels.

|  |  |  |  |
| --- | --- | --- | --- |
| Matrix | Spiked levels  (mg/kg) | Average recovery  (%, n=6) | RSD  (%) |
|
| Tadpoles | 0.02 | 84.92 | 4.51 |
| 0.2 | 76.18 | 4.57 |
| 2 | 78.92 | 2.58 |
| Water | 0.01 | 100.01 | 3.75 |
| 0.1 | 98.69 | 4.12 |
| 1 | 101.14 | 2.36 |

**Table S4**. Calibration equation, R2, limit of quantification (LOQ) for sulfoxaflor in solvent and matrices.

|  |  |  |  |
| --- | --- | --- | --- |
| Matrices | Calibration equation | R2 | LOQ  (mg/kg) |
| Methanol | y = 2×106x + 128854 | 0.9965 | - |
| Tadpoles | y = 2×106x + 466300 | 0.9939 | 0.3 |
| Water | y =2×106x + 100000 | 0.9922 | 0.01 |

**Table S5**. Average recoveries and relative standard deviation (RSD) of sulfoxaflor under various matrices and spiked levels.

|  |  |  |  |
| --- | --- | --- | --- |
| Matrix | Spiked levels  (mg/kg) | Average recovery  (%, n=6) | RSD  (%) |
|
| Tadpoles | 0.3 | 82.51 | 5.79 |
| 3 | 84.56 | 1.06 |
| 30 | 115.21 | 5.30 |
| Water | 0. 1 | 90.58 | 7.69 |
| 1 | 98.78 | 3.01 |
| 10 | 105.30 | 2.40 |



**Fig S1**. Concentrations of imidacloprid and sulfoxaflor in water (A) (B), Note: The left and right y axis in A and B is relative to the value of concentration in water exposure to 1.74 and 17.36 mg/L, 4.27 and 42.73 mg/L (Error bars represent the means ± SD), respectively.