SUPPLEMENTARY MATERIAL

**Effects of long-term chlorpyrifos exposure on mortality and reproductive tissues of Banded Gourami (*Trichogaster fasciata*)**

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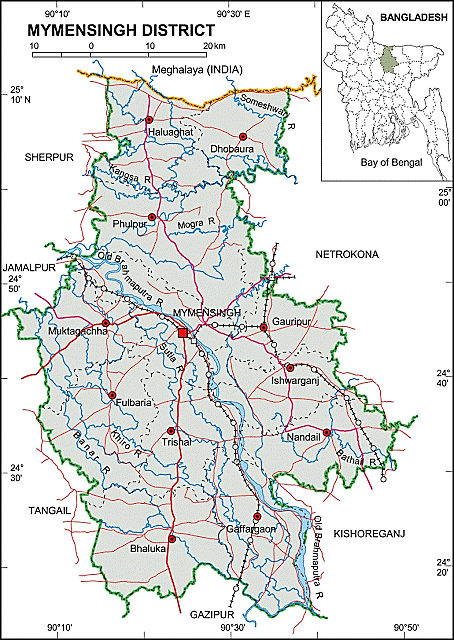


Fig. S1. Mymensingh District map (Gauripur) showing the banded gourami fish collection area.

**Histology of gonads**

Table S1. Dehydration process of gonad tissue before clearing with benzene.

|  |  |  |  |
| --- | --- | --- | --- |
| Step | Reagent | Temperature (ºC) | Programme  (min) |
| 1 | 80% Ethanol | Ambient | 720 |
| 2 | 95% Ethanol | Ambient | 60 |
| 3 | 95% Ethanol | Ambient | 60 |
| 4 | 100% Ethanol | Ambient | 60 |
| 5 | 100% Ethanol | Ambient | 60 |
| 6 | 100% Ethanol | Ambient | 60 |

Table S2. Clearing process of gonad tissue before infiltration with paraffin.

|  |  |  |  |
| --- | --- | --- | --- |
| Step | Reagent | Temperature (ºC) | Programme  (min) |
| 1 | 100% Benzene | Ambient | 60 |
| 2 | 100% Benzene | Ambient | 60 |

Table S3: Infiltration process of gonad tissue with paraffin to enable slicing.

|  |  |  |  |
| --- | --- | --- | --- |
| Step | Reagent | Temperature (ºC) | Programme  (min) |
| 1 | Paraffin | 60 | 40 |
| 2 | Paraffin | 60 | 40 |

Table S4: Staining process of gonad tissuebefore mounting.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Step | Reagent | Temperature  (ºC) | Programme  (min) | Process |
| 1 | Xylene | Ambient | 10 minutes | Clearing |
| 2 | Xylene | Ambient | 10 minutes |
| 3 | Xylene | Ambient | 10 minutes |
| 4 | 100% alcohol | Ambient | 5 minutes | Dehydration |
| 5 | 100% alcohol | Ambient | 5 minutes |
| 6 | 90% alcohol | Ambient | 3 minutes |
| 7 | 80% alcohol | Ambient | 3 minutes |
| 8 | 70% alcohol | Ambient | 3 minutes |
| 9 | 50% Ethyl alcohol | Ambient | 2 minutes | Staining |
| 10 | Distilled water | Ambient | 15 dips |
| 11 | Haematoxylene (Mayer’s) | Ambient | 3 minutes |
| 12 | Wash in tap water | Ambient | 15 minutes |
| 13 | 50% Ethyl alcohol | Ambient | 10-15 dips |
| 14 | 95% Ethyl alcohol | Ambient | 30 seconds |
| 15 | Eosin Y | Ambient | 1 minute |
| 16 | 95% Ethyl alcohol | Ambient | 2 minutes | Rehydration |
| 17 | 100% Ethyl alcohol | Ambient | 1 minute |
| 18 | 100% Ethyl alcohol | Ambient | 3 minutes |
| 19 | 100% Ethyl alcohol | Ambient | 1 minutes |
| 20 | Xylene | Ambient | 20 minutes | Clearing |
| 21 | Xylene | Ambient | 20 minutes |
| 22 | Drying | Ambient | Over night |
| 23 | D.P.X. | Ambient | Over night | Mounting |

Table S5: Acute toxicity (96-h LC50) of chlorpyrifos for Banded Gourami (*Trichogaster fasciata*) according to OECD (mean ± SD; n = 3).

|  |  |  |
| --- | --- | --- |
| Chlorpyrifos concentration (µg/L) | Total number of individuals | Number of dead individuals at 96 h |
| 0 | 7 ± 0 | 0 |
| 150 | 7 ± 0 | 0 |
| 250 | 7 ± 0 | 1 ± 1 |
| 350 | 7 ± 0 | 1.3 ± 0.6 |
| 450 | 7 ± 0 | 1.7 ± 0.6 |
| 550 | 7 ± 0 | 2 ± 1 |
| 650 | 7 ± 0 | 2.7 ± 0.6 |
| LC10 value with 95% confidence limits |  | 258 (158-421) |
| LC50 value with 95% confidence limits |  | 833 (506-1371) |
| LC90 value with 95% confidence limits |  | 2689 (727-9942) |

Table S6: Mortality (%) of male and female Banded Gourami (mean ± SD; n = 3) exposed to different chlorpyrifos concentrations during the experimental period.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chlorpyrifos concentrations  (µg/L) | Male mortality (%) | | | | | Female mortality (%) | | | | |
| Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 |
| 0 | 0 | 3.7±6.4 | 4.2±7.2 | 9.5±8.3 | 11±9.5 | 0 | 3.7±6.4 | 4.2±7.2 | 9.5±8.3 | 16.7±16.6 |
| 15 | 0 | 0 | 16.7±7.2 | 33.4±8.3 | 100±0 | 3.3±5.8 | 3.7±6.4 | 16.7±7.2 | 28.6±0 | 33.3±0 |
| 50 | 3.3±5.8 | 11±11 | 33.3±7.2 | 52.4±8.2 | 100±0 | 6.7±5.8 | 22.2±0 | 13.3±14.4 | 42.8±14.3 | 61±9.5 |
| 150 | 20±0 | 48±6.5 | 100±0 | 100±0 | 100±0 | 10±10 | 33.3±22.2 | 62.5±12.5 | 100±0 | 100±0 |
| 500 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 |

Table S7: No observed effect concentrations (NOECs) in µg/L of male and female mortality, GSI and histopathological alterations of ovary and testes during the experimental period.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Days of exposure | Mortality | | GSI | | Ovary alterations | | | | | | | | Testes alterations | | | | | |
| Male | Female | Male | Female | CC | CR | AF | DPNO | AD | DOL | IFS | NE | ISST | BST | DICL | DSC | TO | EL |
| 15 | 50 | 50 | <15 | 50 | 50 | 50 | 50 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | 50 | ­­50 | 50 | 50 | ≥ 150 | ≥ 150 |
| 30 | 50 | 50 | <15 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 150 | 50 | 50 | ≥ 150 | ≥ 150 |
| 45 | <15 | 15 | ≥ 50 | ≥ 150 | 50 | 50 | 15 | 15 | 15 | ≥ 150 | ≥ 150 | ≥ 150 | ≥ 50 | ≥ 50 | ≥ 50 | ≥ 50 | 15 | 15 |
| 60 | <15 | <15 | ≥ 50 | ≥ 50 | ≥ 50 | ≥ 50 | 15 | ≥ 50 | ≥ 50 | <15 | ≥ 50 | 15 | ≥ 50 | 15 | <15 | ≥ 50 | ≥ 50 | 15 |
| 75 | <15 | <15 | NC | ≥ 50 | ≥ 50 | ≥ 50 | 15 | ≥ 50 | 15 | 15 | ≥ 50 | 15 | NC | NC | NC | NC | NC | NC |

NC= Not calculated due to 100% mortality

Table S8: Chronic NOECs for mortality of different fishes exposed to chlorpyrifos.

|  |  |  |  |
| --- | --- | --- | --- |
| Species name | Days of exposure | NOEC values (µg/L) | Reference |
| *Clarias batrachus* | 30 | 0.21 | ECOTOX Database (<http://cfpub.epa.gov/ecotox/quick_query.htm)> |
| *Pimephales promelas* | 32 | \*1.88 (n = 2) |
| *Cyprinus carpio* | 40 | \*2.33 (n = 75) |
| *Tilapia zillii* | 90 | \*2.67 (n = 5) |
| *Oreochromis niloticus* | 28 | \*8.6 (n = 5) |
| *Oncorhynchus tshawytscha* | 60 | \*3.7 (n = 2) |
| *Fundulus heteroclitus* | 28 | \*7.65 (n = 5) |

\*when more than 1 value was available for a species, the geometric mean was calculated