Supplemental Information

Chronic embryo-larval exposure of fathead minnows to the pharmaceutical drug metformin: survival, growth, and microbiome

Figure S1. Standard curves for metformin (top) and guanylurea (bottom).
Table S1a. Dilution water chemistry. Measurements were performed monthly (three times for each parameter over 3 months).

| Analyte                              | Units  | Mean  | s.d.  |
|--------------------------------------|--------|-------|-------|
| Alkalinity, Total (CaCO3)            | mg/L   | 90.7  | 4.6   |
| Ammonia as N                         | mg/L   | 0.0080| 0.0042|
| Calcium                              | mg/L   | 35.5  | 1.7   |
| Carbon, Dissolved Inorganic          | mg/L   | 23.0  | 1.1   |
| Carbon, Dissolved Organic            | mg/L   | 0.8   | 0.1   |
| Carbon, Particulate Organic          | mg/L   | 0.028 | 0.013 |
| Chloride                             | mg/L   | 28.4  | 2.4   |
| Fluoride                             | mg/L   | 0.67  | 0.04  |
| Hardness                             | mg/L   | 125   | 5     |
| Magnesium                            | mg/L   | 8.82  | 0.30  |
| Nitrate/Nitrite as N                 | mg/L   | 0.448 | 0.082 |
| Nitrogen, Particulate Organic        | mg/L   | 0.003 | 0.002 |
| pH                                   | pH     | 7.20  | 0.05  |
| Phosphorus, Total                    | mg/L   | 0.0044| 0.0054|
| Potassium                            | mg/L   | 1.71  | 0.06  |
| Silica                               | mg/L   | 1.01  | 0.17  |
| Sodium                               | mg/L   | 15.4  | 1.1   |
| Specific conductance (25°C)          | µS/cm  | 323   | 19    |
| Sulfate                              | mg/L   | 24.9  | 0.9   |
Table S1b. Individual beaker water chemistry. Measurements were performed three times for each parameter. Abbreviations lrvl week = larval week

| Beaker | Measured metformin (µg/L) | Stage | Temperature (°C) | Conductivity (µS/cm) | pH | Dissolved Oxygen (mg/L) | Free NH₃ (mg/L) |
|--------|--------------------------|-------|------------------|----------------------|----|------------------------|----------------|
| 1      | Controls                 | egg   | 24.9             | 375.5                | 8.47| 8.02                   | 0.0000         |
| 2      | Controls                 | egg   | 24.6             | 379.5                | 8.49| 8.08                   | 0.0000         |
| 6      | Controls                 | egg   | 24.7             | 377.4                | 8.46| 8.05                   | 0.0000         |
| 10     | Controls                 | egg   | 24.6             | 378.4                | 8.46| 8.04                   | 0.0000         |
| 11     | Controls                 | egg   | 24.7             | 380.6                | 8.48| 8.03                   | 0.0000         |
| 13     | Controls                 | egg   | 24.7             | 379.8                | 8.48| 8.07                   | 0.0000         |
| 16     | Controls                 | egg   | 24.9             | 378.7                | 8.48| 8.01                   | 0.0000         |
| 18     | Controls                 | egg   | 25.0             | 386.9                | 8.47| 8.03                   | 0.0000         |
| 21     | Controls                 | egg   | 24.8             | 378.0                | 8.45| 8.06                   | 0.0000         |
| 22     | Controls                 | egg   | 24.9             | 379.7                | 8.46| 8.04                   | 0.0000         |
| 25     | Controls                 | egg   | 25.0             | 380.7                | 8.48| 8.01                   | 0.0000         |
| 29     | Controls                 | egg   | 24.8             | 375.1                | 8.46| 8.03                   | 0.0000         |
| 31     | Controls                 | egg   | 25.0             | 359.7                | 8.42| 8.00                   | 0.0000         |
| 32     | Controls                 | egg   | 24.8             | 362.1                | 8.43| 8.03                   | 0.0000         |
| 34     | Controls                 | egg   | 24.5             | 362.5                | 8.43| 8.09                   | 0.0000         |
| 37     | Controls                 | egg   | 24.6             | 360.5                | 8.38| 8.07                   | 0.0000         |
| 42     | Controls                 | egg   | 24.7             | 360.2                | 8.44| 8.06                   | 0.0000         |
| 43     | Controls                 | egg   | 24.6             | 362.7                | 8.44| 8.04                   | 0.0000         |
| 1      | Controls                 | 1st lrvl wk | 25.5             | 363.2                | 8.40| 7.90                   | 0.0000         |
| 2      | Controls                 | 1st lrvl wk | 25.5             | 358.6                | 8.40| 7.89                   | 0.0000         |
| 6      | Controls                 | 1st lrvl wk | 25.4             | 360.1                | 8.41| 7.97                   | 0.0000         |
| 10     | Controls                 | 1st lrvl wk | 25.0             | 356.8                | 8.41| 7.94                   | 0.0000         |
| 11     | Controls                 | 1st lrvl wk | 24.9             | 356.7                | 8.40| 7.97                   | 0.0000         |
| 13     | Controls                 | 1st lrvl wk | 24.9             | 361.2                | 8.41| 8.17                   | 0.0000         |
| 16     | Controls                 | 1st lrvl wk | 25.2             | 358.5                | 8.41| 7.95                   | 0.0000         |
| 18     | Controls                 | 1st lrvl wk | 25.3             | 359.0                | 8.40| 7.92                   | 0.0000         |
| 21     | Controls                 | 1st lrvl wk | 25.2             | 356.4                | 8.40| 7.93                   | 0.0000         |
| 22     | Controls                 | 1st lrvl wk | 25.1             | 356.5                | 8.38| 7.91                   | 0.0000         |
| Beaker | Measured metformin (µg/L) | Stage          | Temperature (°C) | Conductivity (µS/cm) | pH  | Dissolved Oxygen (mg/L) | Free NH₃ (mg/L) |
|--------|--------------------------|----------------|-----------------|----------------------|-----|------------------------|----------------|
| 25     | Controls                 | 1st lrvl wk   | 24.8            | 361.3                | 8.39| 7.96                   | 0.0000         |
| 29     | Controls                 | 1st lrvl wk   | 24.9            | 358.8                | 8.40| 7.95                   | 0.0000         |
| 31     | Controls                 | 1st lrvl wk   | 25.2            | 357.4                | 8.40| 7.93                   | 0.0000         |
| 32     | Controls                 | 1st lrvl wk   | 25.2            | 355.7                | 8.38| 7.95                   | 0.0000         |
| 34     | Controls                 | 1st lrvl wk   | 25.0            | 353.8                | 8.39| 7.99                   | 0.0000         |
| 37     | Controls                 | 1st lrvl wk   | 25.0            | 354.5                | 8.40| 7.99                   | 0.0000         |
| 42     | Controls                 | 1st lrvl wk   | 24.9            | 354.7                | 8.39| 8.03                   | 0.0000         |
| 43     | Controls                 | 1st lrvl wk   | 24.9            | 349.2                | 8.40| 7.98                   | 0.0000         |
| 1      | Controls                 | 2nd lrvl wk   | 24.9            | 360.9                | 8.35| 8.05                   | 0.0000         |
| 2      | Controls                 | 2nd lrvl wk   | 24.9            | 359.8                | 8.33| 8.04                   | 0.0000         |
| 6      | Controls                 | 2nd lrvl wk   | 24.7            | 362.5                | 8.33| 8.03                   | 0.0000         |
| 10     | Controls                 | 2nd lrvl wk   | 24.8            | 356.6                | 8.33| 7.99                   | 0.0000         |
| 11     | Controls                 | 2nd lrvl wk   | 24.8            | 360.4                | 8.32| 7.97                   | 0.0000         |
| 13     | Controls                 | 2nd lrvl wk   | 24.8            | 361.8                | 8.35| 8.01                   | 0.0000         |
| 16     | Controls                 | 2nd lrvl wk   | 25.0            | 358.0                | 8.31| 7.93                   | 0.0000         |
| 18     | Controls                 | 2nd lrvl wk   | 24.9            | 363.7                | 8.29| 7.93                   | 0.0000         |
| 21     | Controls                 | 2nd lrvl wk   | 24.8            | 357.6                | 8.33| 7.94                   | 0.0000         |
| 22     | Controls                 | 2nd lrvl wk   | 24.7            | 357.0                | 8.31| 7.97                   | 0.0000         |
| 25     | Controls                 | 2nd lrvl wk   | 24.7            | 362.2                | 8.29| 7.98                   | 0.0000         |
| 29     | Controls                 | 2nd lrvl wk   | 24.9            | 357.4                | 8.34| 7.93                   | 0.0000         |
| 31     | Controls                 | 2nd lrvl wk   | 24.8            | 357.5                | 8.31| 7.95                   | 0.0000         |
| 32     | Controls                 | 2nd lrvl wk   | 24.9            | 354.3                | 8.33| 7.97                   | 0.0000         |
| 34     | Controls                 | 2nd lrvl wk   | 24.7            | 357.1                | 8.33| 8.02                   | 0.0000         |
| 37     | Controls                 | 2nd lrvl wk   | 24.8            | 357.1                | 8.33| 8.01                   | 0.0000         |
| 42     | Controls                 | 2nd lrvl wk   | 24.8            | 358.1                | 8.33| 8.03                   | 0.0000         |
| 43     | Controls                 | 2nd lrvl wk   | 24.7            | 358.3                | 8.35| 8.03                   | 0.0000         |
| Controls mean |               | 24.9           | 363.2           | 8.39                | 8.00| 0.0000               |
| Controls s.d.  |                | 0.2            | 9.1             | 0.06               | 0.06| 0.0000               |
| Beaker | Measured metformin (µg/L) | Stage       | Temperature (°C) | Conductivity (µS/cm) | pH   | Dissolved Oxygen (mg/L) | Free NH₃ (mg/L) |
|--------|---------------------------|-------------|------------------|----------------------|------|------------------------|----------------|
| 3      | 3.4 µg/L                  | egg         | 24.7             | 379.6                | 8.48 | 8.09                   | 0.0000         |
| 7      | 3.4 µg/L                  | egg         | 24.1             | 384.0                | 8.48 | 8.08                   | 0.0000         |
| 9      | 3.4 µg/L                  | egg         | 24.5             | 380.6                | 8.48 | 8.02                   | 0.0000         |
| 23     | 3.4 µg/L                  | egg         | 24.4             | 378.8                | 8.47 | 8.07                   | 0.0000         |
| 24     | 3.4 µg/L                  | egg         | 24.8             | 382.2                | 8.47 | 8.00                   | 0.0000         |
| 27     | 3.4 µg/L                  | egg         | 24.5             | 383.3                | 8.49 | 8.05                   | 0.0000         |
| 35     | 3.4 µg/L                  | egg         | 24.4             | 362.3                | 8.46 | 8.06                   | 0.0000         |
| 39     | 3.4 µg/L                  | egg         | 24.7             | 362.1                | 8.46 | 8.03                   | 0.0000         |
| 44     | 3.4 µg/L                  | egg         | 24.3             | 362.9                | 8.43 | 8.08                   | 0.0000         |
| 3      | 3.4 µg/L                  | 1st lrvl wk | 24.9             | 358.9                | 8.40 | 8.01                   | 0.0000         |
| 7      | 3.4 µg/L                  | 1st lrvl wk | 24.8             | 362.5                | 8.40 | 7.98                   | 0.0000         |
| 9      | 3.4 µg/L                  | 1st lrvl wk | 24.8             | 358.0                | 8.42 | 7.98                   | 0.0000         |
| 23     | 3.4 µg/L                  | 1st lrvl wk | 24.8             | 358.7                | 8.41 | 7.96                   | 0.0000         |
| 24     | 3.4 µg/L                  | 1st lrvl wk | 24.8             | 358.8                | 8.41 | 7.94                   | 0.0000         |
| 27     | 3.4 µg/L                  | 1st lrvl wk | 24.7             | 358.0                | 8.41 | 7.98                   | 0.0000         |
| 35     | 3.4 µg/L                  | 1st lrvl wk | 24.7             | 354.9                | 8.42 | 8.00                   | 0.0000         |
| 39     | 3.4 µg/L                  | 1st lrvl wk | 24.8             | 357.3                | 8.41 | 8.00                   | 0.0000         |
| 44     | 3.4 µg/L                  | 1st lrvl wk | 24.7             | 357.5                | 8.42 | 8.03                   | 0.0000         |
| 3      | 3.4 µg/L                  | 2nd lrvl wk | 24.5             | 358.6                | 8.34 | 8.03                   | 0.0000         |
| 7      | 3.4 µg/L                  | 2nd lrvl wk | 24.4             | 363.9                | 8.33 | 8.04                   | 0.0000         |
| 9      | 3.4 µg/L                  | 2nd lrvl wk | 24.8             | 357.9                | 8.32 | 8.01                   | 0.0000         |
| 23     | 3.4 µg/L                  | 2nd lrvl wk | 24.6             | 356.4                | 8.33 | 7.99                   | 0.0000         |
| 24     | 3.4 µg/L                  | 2nd lrvl wk | 24.6             | 363.0                | 8.32 | 7.99                   | 0.0000         |
| 27     | 3.4 µg/L                  | 2nd lrvl wk | 24.6             | 359.6                | 8.32 | 8.02                   | 0.0000         |
| 35     | 3.4 µg/L                  | 2nd lrvl wk | 24.6             | 355.4                | 8.32 | 7.99                   | 0.0000         |
| 39     | 3.4 µg/L                  | 2nd lrvl wk | 24.7             | 360.4                | 8.32 | 8.02                   | 0.0000         |
| 44     | 3.4 µg/L                  | 2nd lrvl wk | 24.7             | 357.9                | 8.35 | 8.03                   | 0.0000         |
| 3.4 µg/L | mean                |             | 24.6             | 364.2                | 8.40 | 8.02                   | 0.0000         |
|        | s.d.                     |             | 0.2              | 9.7                  | 0.06 | 0.04                   | 0.0000         |
| Beaker | Measured metformin (µg/L) | Stage | Temperature (°C) | Conductivity (µS/cm) | pH   | Dissolved Oxygen (mg/L) | Free NH₃ (mg/L) |
|--------|---------------------------|-------|------------------|----------------------|------|------------------------|----------------|
| 4      | 33.6 µg/L                 | egg   | 24.1             | 380.4                | 8.47 | 8.09                   | 0.0000         |
| 8      | 33.6 µg/L                 | egg   | 24.0             | 381.4                | 8.47 | 8.09                   | 0.0000         |
| 15     | 33.6 µg/L                 | egg   | 24.1             | 380.8                | 8.48 | 8.06                   | 0.0000         |
| 19     | 33.6 µg/L                 | egg   | 24.5             | 386.3                | 8.49 | 8.04                   | 0.0000         |
| 26     | 33.6 µg/L                 | egg   | 24.4             | 390.4                | 8.49 | 8.04                   | 0.0000         |
| 30     | 33.6 µg/L                 | egg   | 24.4             | 374.7                | 8.47 | 8.04                   | 0.0000         |
| 33     | 33.6 µg/L                 | egg   | 24.4             | 359.9                | 8.44 | 8.03                   | 0.0000         |
| 36     | 33.6 µg/L                 | egg   | 24.4             | 361.9                | 8.44 | 8.06                   | 0.0000         |
| 40     | 33.6 µg/L                 | egg   | 24.5             | 361.1                | 8.45 | 8.06                   | 0.0000         |
| 4      | 33.6 µg/L 1st lrvl wk     |       | 24.8             | 361.9                | 8.40 | 7.98                   | 0.0000         |
| 8      | 33.6 µg/L 1st lrvl wk     |       | 24.7             | 362.7                | 8.39 | 8.00                   | 0.0000         |
| 15     | 33.6 µg/L 1st lrvl wk     |       | 24.5             | 360.4                | 8.42 | 7.99                   | 0.0000         |
| 19     | 33.6 µg/L 1st lrvl wk     |       | 24.9             | 358.9                | 8.42 | 7.94                   | 0.0000         |
| 26     | 33.6 µg/L 1st lrvl wk     |       | 24.7             | 362.4                | 8.42 | 7.98                   | 0.0000         |
| 30     | 33.6 µg/L 1st lrvl wk     |       | 24.6             | 359.1                | 8.42 | 8.00                   | 0.0000         |
| 33     | 33.6 µg/L 1st lrvl wk     |       | 24.8             | 358.9                | 8.41 | 7.98                   | 0.0000         |
| 36     | 33.6 µg/L 1st lrvl wk     |       | 24.9             | 354.0                | 8.41 | 7.95                   | 0.0000         |
| 40     | 33.6 µg/L 1st lrvl wk     |       | 24.9             | 354.6                | 8.42 | 7.97                   | 0.0000         |
| 4      | 33.6 µg/L 2nd lrvl wk     |       | 24.5             | 363.1                | 8.32 | 8.03                   | 0.0000         |
| 8      | 33.6 µg/L 2nd lrvl wk     |       | 24.3             | 361.2                | 8.31 | 8.04                   | 0.0000         |
| 15     | 33.6 µg/L 2nd lrvl wk     |       | 24.4             | 360.9                | 8.33 | 8.02                   | 0.0000         |
| 19     | 33.6 µg/L 2nd lrvl wk     |       | 24.3             | 360.5                | 8.35 | 8.05                   | 0.0000         |
| 26     | 33.6 µg/L 2nd lrvl wk     |       | 24.6             | 364.0                | 8.33 | 8.03                   | 0.0000         |
| 30     | 33.6 µg/L 2nd lrvl wk     |       | 24.4             | 360.2                | 8.34 | 8.01                   | 0.0000         |
| 33     | 33.6 µg/L 2nd lrvl wk     |       | 24.4             | 361.4                | 8.35 | 8.05                   | 0.0000         |
| 36     | 33.6 µg/L 2nd lrvl wk     |       | 24.4             | 359.4                | 8.34 | 8.09                   | 0.0000         |
| 40     | 33.6 µg/L 2nd lrvl wk     |       | 24.6             | 358.8                | 8.33 | 8.03                   | 0.0000         |
| 33.6 µg/L | mean                  |       | 24.5             | 365.2                | 8.40 | 8.02                   | 0.0000         |
|        | s.d.                     |       | 0.2              | 9.9                  | 0.06 | 0.04                   | 0.0000         |
| Beaker | Measured metformin (µg/L) | Stage       | Temperature (°C) | Conductivity (µS/cm) | pH | Dissolved Oxygen (mg/L) | Free NH₃ (mg/L) |
|--------|---------------------------|-------------|------------------|----------------------|----|------------------------|----------------|
| 5      | 269 µg/L                  | egg         | 24.3             | 381.0                | 8.46 | 8.07                  | 0.0000         |
| 12     | 269 µg/L                  | egg         | 24.2             | 382.1                | 8.48 | 8.04                  | 0.0000         |
| 14     | 269 µg/L                  | egg         | 24.1             | 379.3                | 8.48 | 8.04                  | 0.0000         |
| 17     | 269 µg/L                  | egg         | 24.6             | 379.4                | 8.48 | 8.00                  | 0.0000         |
| 20     | 269 µg/L                  | egg         | 24.5             | 377.6                | 8.46 | 7.99                  | 0.0000         |
| 28     | 269 µg/L                  | egg         | 24.3             | 377.9                | 8.46 | 8.05                  | 0.0000         |
| 38     | 269 µg/L                  | egg         | 24.2             | 360.2                | 8.44 | 8.06                  | 0.0000         |
| 41     | 269 µg/L                  | egg         | 24.1             | 363.6                | 8.44 | 8.06                  | 0.0000         |
| 45     | 269 µg/L                  | egg         | 24.1             | 360.3                | 8.44 | 8.07                  | 0.0000         |
| 5      | 269 µg/L                  | 1st lrvl wk | 24.9             | 360.8                | 8.42 | 7.95                  | 0.0000         |
| 12     | 269 µg/L                  | 1st lrvl wk | 24.8             | 358.9                | 8.43 | 7.98                  | 0.0000         |
| 14     | 269 µg/L                  | 1st lrvl wk | 24.6             | 357.2                | 8.42 | 7.96                  | 0.0000         |
| 17     | 269 µg/L                  | 1st lrvl wk | 25.0             | 362.0                | 8.43 | 7.89                  | 0.0000         |
| 20     | 269 µg/L                  | 1st lrvl wk | 25.0             | 356.0                | 8.42 | 7.94                  | 0.0000         |
| 28     | 269 µg/L                  | 1st lrvl wk | 24.7             | 358.2                | 8.43 | 7.96                  | 0.0000         |
| 38     | 269 µg/L                  | 1st lrvl wk | 24.7             | 353.8                | 8.40 | 7.97                  | 0.0000         |
| 41     | 269 µg/L                  | 1st lrvl wk | 24.4             | 359.5                | 8.39 | 7.99                  | 0.0000         |
| 45     | 269 µg/L                  | 1st lrvl wk | 24.4             | 354.7                | 8.41 | 8.01                  | 0.0000         |
| 5      | 269 µg/L                  | 2nd lrvl wk | 24.6             | 358.8                | 8.34 | 8.05                  | 0.0000         |
| 12     | 269 µg/L                  | 2nd lrvl wk | 24.7             | 360.4                | 8.34 | 8.01                  | 0.0000         |
| 14     | 269 µg/L                  | 2nd lrvl wk | 24.5             | 358.8                | 8.34 | 8.03                  | 0.0000         |
| 17     | 269 µg/L                  | 2nd lrvl wk | 24.4             | 362.7                | 8.33 | 8.03                  | 0.0000         |
| 20     | 269 µg/L                  | 2nd lrvl wk | 24.2             | 358.8                | 8.32 | 8.06                  | 0.0000         |
| 28     | 269 µg/L                  | 2nd lrvl wk | 24.4             | 360.4                | 8.34 | 8.01                  | 0.0000         |
| 38     | 269 µg/L                  | 2nd lrvl wk | 24.3             | 360.0                | 8.33 | 8.05                  | 0.0000         |
| 41     | 269 µg/L                  | 2nd lrvl wk | 24.4             | 359.2                | 8.31 | 8.03                  | 0.0000         |
| 45     | 269 µg/L                  | 2nd lrvl wk | 24.4             | 356.5                | 8.34 | 8.03                  | 0.0000         |
|        | 269 µg/L                  | mean        | 24.5             | 363.6                | 8.40 | 8.01                  | 0.0000         |
|        | s.d.                      |             | 0.3              | 9.0                  | 0.06 | 0.05                  | 0.0000         |
|        | All controls and metformin| mean        | 24.7             | 363.9                | 8.40 | 8.01                  | 0.0000         |
|        | s.d.                      |             | 0.3              | 9.3                  | 0.06 | 0.05                  | 0.0000         |
Table S2a. Liquid chromatography gradient conditions for metformin and guanylurea analysis in exposure beakers during the 21-dfathead minnow embryo-larval test.

| TIME (min) | FLOW (ml/min) | %Water | %Methanol |
|------------|---------------|--------|-----------|
| Metformin  |               |        |           |
| Initial    | 0.15          | 90     | 10        |
| 5          | 0.15          | 90     | 10        |
| 12         | 0.15          | 0      | 100       |
| 15         | 0.15          | 0      | 100       |
| 20         | 0.15          | 90     | 10        |
| 35         | 0.15          | 90     | 10        |
| Guanylurea |               |        |           |
| Initial    | 0.15          | 90     | 10        |
| 1.2        | 0.15          | 0      | 100       |
| 5          | 0.15          | 0      | 100       |
| 7          | 0.15          | 90     | 10        |
| 20         | 0.15          | 90     | 10        |
Table S2b. Precursor to product transitions monitored for native and labelled metformin and guanylurea. Two ions were chosen so that a ratio between the two could be used as a further confirmation of compound identity.

| Electrospray Conditions |  |
|-------------------------|--|
| Collision Gas Flow (ml/min) | 0.15 |
| Desolvation Temperature | 300 °C |
| Gas Flow: Desolvation (L/Hr) | 800 |
| Cone (L/Hr) | 150 |
| Nebuliser (Bar) | 7 |
| Source Voltages: Capillary (KV) | 0.94 |
| Cone (V) | 0 |
| Source Offset (V) | 50 |
| Source Temperature | 150 °C |

| MS/MS Transitions |  |
|-----------------|--|
| Compound | Precursor (m/z) | Product (m/z) | Dwell Time (sec) | Cone (V) | Collision Energy (V) |
| Metformin | 130.1 | 60.2 | 0.25 | 15 | 18 |
| Metformin | 130.1 | 71.2 | 0.25 | 15 | 20 |
| D6 Metformin | 136.3 | 77.2 | 0.25 | 16 | 19 |
| D6 Metformin | 136.3 | 119.3 | 0.25 | 16 | 14 |
| Guanylurea | 102.87 | 43.1 | 0.25 | 47 | 14 |
| Guanylurea | 102.87 | 60.5 | 0.25 | 38 | 14 |
| Guanylurea | 102.87 | 86.5 | 0.25 | 53 | 14 |
| $^{15}$N$_4$ Guanylurea | 107.53 | 63.4 | 0.25 | 40 | 11 |
| $^{15}$N$_4$ Guanylurea | 107.53 | 89.4 | 0.25 | 40 | 11 |
Table S3. All metformin measurements in beaker exposure water for fathead minnow embryo-larval 21-d exposure to metformin.

| week | Nominal metformin (µg/L) | PRE - Three replicate measurements (µg/L) | PRE - Mean metformin concentration (µg/L) | POST - Three replicate measurements (µg/L) | POST - Mean metformin concentration (µg/L) |
|------|--------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| Controls | | 0.0093 | | 0.0093 |
| 1 Controls | | 3.1 | 3.01 | 3.28 | 3.65 | 3.74 |
| 1 Controls | | 3.1 | 3.31 | | 3.64 | |
| 1 Controls | | 3.1 | 3.53 | | 3.92 | |
| 1 Controls | | 31 | 32.29 | 32.31 | 33.80 | 36.17 |
| 1 Controls | | 31 | 33.20 | | 36.41 | |
| 1 Controls | | 31 | 31.45 | | 38.30 | |
| 1 Controls | | 312 | 298.77 | 280.48 | 260.55 | 276.13 |
| 1 Controls | | 312 | 265.22 | | 276.00 | |
| 1 Controls | | 312 | 277.46 | | 291.85 | |
| 2 Controls | | 0.00 | 0.01 | 0.06 | 0.03 | |
| 2 Controls | | 0.00 | | 0.00 | | |
| 2 Controls | | 0.03 | | 0.06 | | |
| 2 Controls | | 0.00 | 0.02 | 0.06 | 0.03 | |
| 2 Controls | | 0.04 | | 0.04 | | |
| 2 Controls | | 0.04 | | 0.03 | | |
| 2 Controls | | 3.1 | 3.80 | 3.71 | 3.30 | 3.24 |
| 2 Controls | | 3.1 | 3.58 | | 3.23 | |
| 2 Controls | | 3.1 | 3.75 | | 3.19 | |
| 2 Controls | | 31 | 30.40 | 31.93 | 33.43 | 32.80 |
| 2 Controls | | 31 | 33.05 | | 31.94 | |
| 2 Controls | | 31 | 32.33 | | 33.04 | |
| 2 Controls | | 312 | 253.80 | 251.72 | 252.86 | 259.54 |
| 2 Controls | | 312 | 256.29 | | 277.33 | |
| 2 Controls | | 312 | 245.06 | | 248.44 | |
| week | Nominal metformin (µg/L) | PRE - Three replicate measurements (µg/L) | PRE - Mean metformin concentration (µg/L) | POST - Three replicate measurements (µg/L) | POST - Mean metformin concentration (µg/L) |
|------|--------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|
| 3    | Controls                 | 0.04                                      | 0.02                                      | 0.08                                      | 0.07                                      |
| 3    | Controls                 | 0.02                                      |                                           | 0.05                                      |                                           |
| 3    | Controls                 | 0.00                                      |                                           | 0.07                                      |                                           |
| 3    | Controls                 | 0.00                                      | 0.003                                    | 0.00                                      | 0.02                                      |
| 3    | Controls                 | 0.00                                      |                                           | 0.05                                      |                                           |
| 3    | Controls                 | 0.01                                      |                                           | 0.02                                      |                                           |
| 3    | 3.1                      | 3.26                                      | 3.27                                      | 3.37                                      | 3.39                                      |
| 3    | 3.1                      | 3.31                                      |                                           | 3.21                                      |                                           |
| 3    | 3.1                      | 3.24                                      |                                           | 3.59                                      |                                           |
| 3    | 31                       | 33.14                                    | 33.59                                    | 34.91                                    | 34.85                                    |
| 3    | 31                       | 34.76                                    |                                           | 35.13                                    |                                           |
| 3    | 31                       | 32.88                                    |                                           | 34.51                                    |                                           |
| 3    | 312                      | 264.31                                   | 273.83                                   | 278.24                                   | 270.71                                   |
| 3    | 312                      | 283.53                                   |                                           | 262.16                                   |                                           |
| 3    | 312                      | 273.65                                   |                                           | 271.73                                   |                                           |
Table S4. Survival and hatch parameters by replicate beaker for embryo-larval fathead minnows exposed to metformin.

| Beaker | Rep | Metformin (µg/L) | Hatchability (%) | Hatch success (%) | Survival from egg to 9 dph (%) | Survival from egg to 16 dph (%) | Deformed at hatch (%) | Time to hatch (days) |
|--------|-----|-----------------|------------------|------------------|------------------|------------------|-----------------|------------------|
| 1      | A   | Controls        | 95.0             | 95.0             | 95.0             | 95.0             | 0.0             | 4.0              |
| 2      | A   | Controls        | 100.0            | 95.0             | 95.0             | 95.0             | 5.0             | 4.1              |
| 6      | A   | Controls        | 100.0            | 95.0             | 95.0             | 95.0             | 5.0             | 4.1              |
| 10     | A   | Controls        | 95.0             | 95.0             | 95.0             | 95.0             | 0.0             | 4.0              |
| 11     | A   | Controls        | 100.0            | 100.0            | 100.0            | 100.0            | 5.0             | 4.0              |
| 13     | A   | Controls        | 100.0            | 90.0             | 90.0             | 90.0             | 10.0            | 4.1              |
| 16     | B   | Controls        | 100.0            | 90.0             | 90.0             | 90.0             | 10.0            | 4.1              |
| 18     | B   | Controls        | 95.0             | 90.0             | 90.0             | 90.0             | 5.3             | 4.3              |
| 21     | B   | Controls        | 100.0            | 90.0             | 90.0             | 90.0             | 10.0            | 4.4              |
| 22     | B   | Controls        | 100.0            | 100.0            | 100.0            | 100.0            | 0.0             | 4.3              |
| 25     | B   | Controls        | 100.0            | 95.0             | 95.0             | 95.0             | 0.0             | 4.2              |
| 29     | B   | Controls        | 100.0            | 90.0             | 90.0             | 90.0             | 10.0            | 4.2              |
| 31     | C   | Controls        | 100.0            | 100.0            | 100.0            | 100.0            | 0.0             | 4.2              |
| 32     | C   | Controls        | 100.0            | 100.0            | 100.0            | 100.0            | 0.0             | 4.3              |
| 34     | C   | Controls        | 100.0            | 90.0             | 90.0             | 90.0             | 10.0            | 4.2              |
| 37     | C   | Controls        | 100.0            | 95.0             | 95.0             | 95.0             | 0.0             | 4.7              |
| 42     | C   | Controls        | 100.0            | 100.0            | 100.0            | 100.0            | 0.0             | 4.2              |
| 43     | C   | Controls        | 100.0            | 100.0            | 95.0             | 95.0             | 5.0             | 4.4              |

| Mean   |      |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|------|
| s.d.   | 1.9  | 4.2  | 4.0  | 4.0  | 4.3  | 0.2  |      |      |
| Beaker | Rep | Metformin (µg/L) | Hatchability (%) | Hatch success (%) | Survival from egg to 9 dph (%) | Survival from egg to 16 dph (%) | Deformed at hatch (%) | Time to hatch (days) |
|--------|-----|------------------|------------------|-------------------|--------------------------------|-------------------------------|---------------------|---------------------|
| 3      | A   | 3.4              | 95.0             | 95.0              | 90.0                           | 90.0                          | 0.0                 | 4.2                 |
| 7      | A   | 3.4              | 95.0             | 85.0              | 85.0                           | 85.0                          | 10.5                | 4.0                 |
| 9      | A   | 3.4              | 100.0            | 85.0              | 85.0                           | 85.0                          | 15.0                | 4.1                 |
| 23     | B   | 3.4              | 100.0            | 100.0             | 100.0                          | 100.0                         | 0.0                 | 4.4                 |
| 24     | B   | 3.4              | 100.0            | 100.0             | 100.0                          | 100.0                         | 5.3                 | 4.0                 |
| 27     | B   | 3.4              | 100.0            | 100.0             | 100.0                          | 100.0                         | 0.0                 | 4.4                 |
| 35     | C   | 3.4              | 100.0            | 100.0             | 100.0                          | 100.0                         | 0.0                 | 4.9                 |
| 39     | C   | 3.4              | 100.0            | 95.0              | 90.0                           | 90.0                          | 5.0                 | 4.0                 |
| 44     | C   | 3.4              | 90.0             | 90.0              | 90.0                           | 90.0                          | 0.0                 | 4.2                 |
| Mean   |     |                  |                  |                   |                                |                               |                     | 4.0                 |
| s.d.   |     |                  |                  |                   |                                |                               |                     | 0.3                 |

| Beaker | Rep | Metformin (µg/L) | Hatchability (%) | Hatch success (%) | Survival from egg to 9 dph (%) | Survival from egg to 16 dph (%) | Deformed at hatch (%) | Time to hatch (days) |
|--------|-----|------------------|------------------|-------------------|--------------------------------|-------------------------------|---------------------|---------------------|
| 4      | A   | 33.6             | 95.0             | 95.0              | 95.0                           | 95.0                          | 5.3                 | 4.1                 |
| 8      | A   | 33.6             | 100.0            | 95.0              | 95.0                           | 95.0                          | 5.0                 | 4.2                 |
| 15     | A   | 33.6             | 100.0            | 95.0              | 95.0                           | 95.0                          | 5.0                 | 4.1                 |
| 19     | B   | 33.6             | 100.0            | 95.0              | 95.0                           | 95.0                          | 5.0                 | 4.3                 |
| 26     | B   | 33.6             | 100.0            | 95.0              | 95.0                           | 95.0                          | 5.0                 | 4.1                 |
| 30     | B   | 33.6             | 100.0            | 100.0             | 100.0                          | 100.0                         | 0.0                 | 4.4                 |
| 33     | C   | 33.6             | 100.0            | 100.0             | 95.0                           | 95.0                          | 0.0                 | 4.3                 |
| 36     | C   | 33.6             | 100.0            | 100.0             | 95.0                           | 95.0                          | 5.0                 | 4.4                 |
| 40     | C   | 33.6             | 100.0            | 100.0             | 100.0                          | 100.0                         | 10.0                | 4.2                 |
| Mean   |     |                  |                  |                   |                                |                               |                     | 4.5                 |
| s.d.   |     |                  |                  |                   |                                |                               |                     | 3.0                 |
| Beaker | Rep | Metformin (µg/L) | Hatchability (%) | Hatch success (%) | Survival from egg to 9 dph (%) | Survival from egg to 16 dph (%) | Deformed at hatch (%) | Time to hatch (days) |
|--------|-----|-----------------|------------------|------------------|-------------------------------|-------------------------------|----------------------|---------------------|
| 5      | A   | 269             | 100.0            | 95.0             | 95.0                          | 95.0                          | 5.0                  | 4.0                 |
| 12     | A   | 269             | 100.0            | 95.0             | 95.0                          | 95.0                          | 5.0                  | 4.1                 |
| 14     | A   | 269             | 100.0            | 100.0            | 100.0                         | 100.0                         | 0.0                  | 4.0                 |
| 17     | B   | 269             | 100.0            | 90.0             | 90.0                          | 90.0                          | 10.0                 | 4.5                 |
| 20     | B   | 269             | 100.0            | 100.0            | 100.0                         | 100.0                         | 0.0                  | 4.2                 |
| 28     | B   | 269             | 100.0            | 100.0            | 100.0                         | 100.0                         | 0.0                  | 4.2                 |
| 38     | C   | 269             | 100.0            | 95.0             | 95.0                          | 95.0                          | 5.3                  | 4.7                 |
| 41     | C   | 269             | 100.0            | 95.0             | 95.0                          | 95.0                          | 5.0                  | 4.2                 |
| 45     | C   | 269             | 100.0            | 95.0             | 95.0                          | 95.0                          | 5.0                  | 4.3                 |
| Mean   |     |                 | 100.0            | 96.1             | 96.1                          | 96.1                          | 3.9                  | 4.2                 |
| s.d.   |     |                 | 0.0              | 3.3              | 3.3                           | 3.3                           | 3.3                  | 0.2                 |
Table S5. Growth parameters at by replicate beaker for 9 dph larval fathead minnows exposed to metformin.

| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 9 dph | Wet weight at 9 dph (mg) | Total length at 9 dph (mm) | Condition factor at 9 dph | Tail length at 9 dph (mm) |
|--------|-----|------------------|--------------------------------|--------------------------|---------------------------|--------------------------|---------------------------|
| 1      | A   | Controls         | 9                              | 4.10                     | 9.61                      | 0.458                    | 1.13                      |
| 2      | A   | Controls         | 9                              | 4.80                     | 9.99                      | 0.480                    | 1.23                      |
| 6      | A   | Controls         | 9                              | 3.91                     | 9.53                      | 0.447                    | 1.08                      |
| 10     | A   | Controls         | 9                              | 3.90                     | 9.26                      | 0.444                    | 1.05                      |
| 11     | A   | Controls         | 9                              | 4.22                     | 9.57                      | 0.465                    | 1.25                      |
| 13     | A   | Controls         | 8                              | 4.98                     | 9.89                      | 0.504                    | 1.20                      |
| 16     | B   | Controls         | 8                              | 4.65                     | 10.17                     | 0.439                    | 1.22                      |
| 18     | B   | Controls         | 8                              | 4.58                     | 10.02                     | 0.450                    | 1.21                      |
| 21     | B   | Controls         | 8                              | 5.32                     | 10.36                     | 0.476                    | 1.32                      |
| 22     | B   | Controls         | 10                             | 5.11                     | 10.12                     | 0.490                    | 1.25                      |
| 25     | B   | Controls         | 9                              | 5.27                     | 10.13                     | 0.504                    | 1.29                      |
| 29     | B   | Controls         | 8                              | 5.60                     | 10.23                     | 0.515                    | 1.35                      |
| 31     | C   | Controls         | 10                             | 4.65                     | 10.20                     | 0.438                    | 1.21                      |
| 32     | C   | Controls         | 10                             | 4.46                     | 9.73                      | 0.468                    | 1.15                      |
| 34     | C   | Controls         | 8                              | 4.63                     | 9.90                      | 0.457                    | 1.16                      |
| 37     | C   | Controls         | 9                              | 4.02                     | 9.59                      | 0.452                    | 1.13                      |
| 42     | C   | Controls         | 9                              | 4.42                     | 9.83                      | 0.427                    | 1.20                      |
| 43     | C   | Controls         | 8                              | 4.39                     | 9.83                      | 0.418                    | 1.18                      |

| Mean   | 8.8 | 4.61 | 9.89 | 0.463 | 1.20 |
| s.d.   | 0.7 | 0.50 | 0.29 | 0.027 | 0.08 |
| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 9 dph | Wet weight at 9 dph (mg) | Total length at 9 dph (mm) | Condition factor at 9 dph | Tail length at 9 dph (mm) |
|--------|-----|-----------------|-------------------------------|-------------------------|---------------------------|---------------------------|--------------------------|
| 3      | A   | 3.4             | 8                             | 4.54                    | 9.73                      | 0.469                     | 1.10                     |
| 7      | A   | 3.4             | 7                             | 4.24                    | 9.67                      | 0.466                     | 1.13                     |
| 9      | A   | 3.4             | 7                             | 4.37                    | 9.71                      | 0.474                     | 1.21                     |
| 23     | B   | 3.4             | 10                            | 4.72                    | 10.02                     | 0.465                     | 1.23                     |
| 24     | B   | 3.4             | 10                            | 5.22                    | 10.06                     | 0.500                     | 1.31                     |
| 27     | B   | 3.4             | 10                            | 5.09                    | 9.98                      | 0.510                     | 1.26                     |
| 35     | C   | 3.4             | 10                            | 4.49                    | 9.83                      | 0.450                     | 1.18                     |
| 39     | C   | 3.4             | 8                             | 5.09                    | 10.06                     | 0.485                     | 1.28                     |
| 44     | C   | 3.4             | 7                             | 4.48                    | 10.14                     | 0.427                     | 1.24                     |
| Mean   |     |                 |                               |                         |                           |                           |                          |
| s.d.   |     |                 |                               |                         |                           |                           |                          |
| 4      | A   | 33.6            | 9                             | 4.03                    | 9.38                      | 0.462                     | 1.02                     |
| 8      | A   | 33.6            | 8                             | 3.53                    | 9.10                      | 0.447                     | 1.11                     |
| 15     | A   | 33.6            | 9                             | 4.06                    | 9.49                      | 0.472                     | 1.16                     |
| 19     | B   | 33.6            | 9                             | 5.16                    | 10.21                     | 0.479                     | 1.29                     |
| 26     | B   | 33.6            | 9                             | 5.66                    | 10.23                     | 0.523                     | 1.24                     |
| 30     | B   | 33.6            | 9                             | 5.31                    | 10.03                     | 0.526                     | 1.31                     |
| 33     | C   | 33.6            | 9                             | 4.54                    | 9.94                      | 0.464                     | 1.20                     |
| 36     | C   | 33.6            | 9                             | 4.42                    | 9.85                      | 0.452                     | 1.26                     |
| 40     | C   | 33.6            | 10                            | 4.27                    | 9.80                      | 0.435                     | 1.05                     |
| Mean   |     |                 |                               |                         |                           |                           |                          |
| s.d.   |     |                 |                               |                         |                           |                           |                          |

| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 9 dph | Wet weight at 9 dph (mg) | Total length at 9 dph (mm) | Condition factor at 9 dph | Tail length at 9 dph (mm) |
|--------|-----|-----------------|-------------------------------|-------------------------|---------------------------|---------------------------|--------------------------|
| 4      | A   | 33.6            | 9                             | 4.03                    | 9.38                      | 0.462                     | 1.02                     |
| 8      | A   | 33.6            | 8                             | 3.53                    | 9.10                      | 0.447                     | 1.11                     |
| 15     | A   | 33.6            | 9                             | 4.06                    | 9.49                      | 0.472                     | 1.16                     |
| 19     | B   | 33.6            | 9                             | 5.16                    | 10.21                     | 0.479                     | 1.29                     |
| 26     | B   | 33.6            | 9                             | 5.66                    | 10.23                     | 0.523                     | 1.24                     |
| 30     | B   | 33.6            | 9                             | 5.31                    | 10.03                     | 0.526                     | 1.31                     |
| 33     | C   | 33.6            | 9                             | 4.54                    | 9.94                      | 0.464                     | 1.20                     |
| 36     | C   | 33.6            | 9                             | 4.42                    | 9.85                      | 0.452                     | 1.26                     |
| 40     | C   | 33.6            | 10                            | 4.27                    | 9.80                      | 0.435                     | 1.05                     |
| Mean   |     |                 |                               |                         |                           |                           |                          |
| s.d.   |     |                 |                               |                         |                           |                           |                          |

Mean 8.6 4.69 9.91 0.472 1.22
s.d. 1.4 0.36 0.18 0.025 0.07

Mean 9.0 4.55 9.78 0.474 1.18
s.d. 0.5 0.69 0.38 0.032 0.10
| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 9 dph | Wet weight at 9 dph (mg) | Total length at 9 dph (mm) | Condition factor at 9 dph | Tail length at 9 dph (mm) |
|--------|-----|------------------|-------------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| 5      | A   | 269              | 9                             | 3.95                    | 9.50                     | 0.456                    | 1.07                     |
| 12     | A   | 269              | 9                             | 4.82                    | 9.81                     | 0.508                    | 1.22                     |
| 14     | A   | 269              | 10                            | 4.55                    | 9.76                     | 0.487                    | 1.20                     |
| 17     | B   | 269              | 8                             | 5.50                    | 10.44                    | 0.479                    | 1.26                     |
| 20     | B   | 269              | 10                            | 5.48                    | 10.63                    | 0.454                    | 1.38                     |
| 28     | B   | 269              | 10                            | 5.29                    | 10.21                    | 0.494                    | 1.34                     |
| 38     | C   | 269              | 9                             | 3.95                    | 9.58                     | 0.438                    | 1.08                     |
| 41     | C   | 269              | 9                             | 4.30                    | 9.98                     | 0.427                    | 1.17                     |
| 45     | C   | 269              | 9                             | 4.79                    | 10.16                    | 0.452                    | 1.22                     |
| Mean   |     |                  | 9.2                           | 4.74                    | 10.01                    | 0.466                    | 1.21                     |
| s.d.   |     |                  | 0.7                           | 0.60                    | 0.38                     | 0.027                    | 0.10                     |
Table S5 (cont). Growth parameters at by replicate beaker for 16 dph larval fathead minnows exposed to metformin for 21 d.

| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 16 dph | Wet weight at 16 dph (mg) | Total length at 16 dph (mm) | Condition factor at 16 dph | Tail length at 16 dph (mm) |
|--------|-----|-----------------|---------------------------------|--------------------------|----------------------------|---------------------------|-----------------------------|
| 1      | A   | Controls        | n/a                             |                          |                           |                           |                             |
| 2      | A   | Controls        | 10                              | 15.42                    | 13.72                     | 0.583                     | 2.42                        |
| 6      | A   | Controls        | 10                              | 14.81                    | 13.69                     | 0.553                     | 2.42                        |
| 10     | A   | Controls        | 10                              | 14.06                    | 13.51                     | 0.554                     | 2.36                        |
| 11     | A   | Controls        | 10                              | 15.33                    | 14.02                     | 0.540                     | 2.50                        |
| 13     | A   | Controls        | 10                              | 13.51                    | 13.63                     | 0.531                     | 2.53                        |
| 16     | B   | Controls        | n/a                             |                          |                           |                           |                             |
| 18     | B   | Controls        | 10                              | 17.07                    | 14.31                     | 0.572                     | 2.56                        |
| 21     | B   | Controls        | 9                               | 16.22                    | 14.19                     | 0.561                     | 2.51                        |
| 22     | B   | Controls        | 10                              | 14.60                    | 13.55                     | 0.572                     | 2.35                        |
| 25     | B   | Controls        | 10                              | 16.59                    | 14.29                     | 0.557                     | 2.56                        |
| 29     | B   | Controls        | 10                              | 17.58                    | 14.50                     | 0.564                     | 2.52                        |
| 31     | C   | Controls        | 10                              | 14.60                    | 13.98                     | 0.527                     | 2.48                        |
| 32     | C   | Controls        | 10                              | 15.22                    | 14.02                     | 0.550                     | 2.53                        |
| 34     | C   | Controls        | 10                              | 15.60                    | 14.08                     | 0.553                     | 2.54                        |
| 37     | C   | Controls        | 10                              | 14.65                    | 13.88                     | 0.535                     | 2.44                        |
| 42     | C   | Controls        | 10                              | 15.06                    | 14.11                     | 0.532                     | 2.41                        |
| 43     | C   | Controls        | 10                              | 15.10                    | 13.93                     | 0.534                     | 2.49                        |

Mean: 9.9 | 15.34 | 13.96 | 0.551 | 2.48
s.d.: 0.3 | 1.08 | 0.29 | 0.017 | 0.07
| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 16 dph | Wet weight at 16 dph (mg) | Total length at 16 dph (mm) | Condition factor at 16 dph | Tail length at 16 dph (mm) |
|--------|-----|----------------|---------------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| 3      | A   | 3.4            | 10                              | 15.00                     | 13.53                       | 0.576                       | 2.31                        |
| 7      | A   | 3.4            | 10                              | 14.34                     | 13.71                       | 0.551                       | 2.43                        |
| 9      | A   | 3.4            | 10                              | 15.10                     | 14.12                       | 0.530                       | 2.52                        |
| 23     | B   | 3.4            | 10                              | 15.22                     | 13.71                       | 0.563                       | 2.40                        |
| 24     | B   | 3.4            | 10                              | 17.27                     | 14.64                       | 0.544                       | 2.67                        |
| 27     | B   | 3.4            | 10                              | 18.60                     | 14.71                       | 0.571                       | 2.63                        |
| 35     | C   | 3.4            | 10                              | 15.39                     | 13.93                       | 0.560                       | 2.48                        |
| 39     | C   | 3.4            | 10                              | 13.61                     | 13.70                       | 0.518                       | 2.36                        |
| 44     | C   | 3.4            | 10                              | 17.18                     | 14.29                       | 0.584                       | 2.58                        |
| Mean   |     | 10.0           | 15.74                           | 14.04                     | 0.555                       | 2.49                        |
| s.d.   |     | 0.0            | 1.60                            | 0.43                      | 0.022                       | 0.12                        |

| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 16 dph | Wet weight at 16 dph (mg) | Total length at 16 dph (mm) | Condition factor at 16 dph | Tail length at 16 dph (mm) |
|--------|-----|----------------|---------------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| 4      | A   | 33.6          | 10                              | 15.06                     | 13.64                       | 0.583                       | 2.58                        |
| 8      | A   | 33.6          | 10                              | 15.25                     | 13.80                       | 0.574                       | 2.39                        |
| 15     | A   | 33.6          | 10                              | 15.69                     | 13.93                       | 0.566                       | 2.50                        |
| 19     | B   | 33.6          | 10                              | 17.34                     | 14.23                       | 0.594                       | 2.48                        |
| 26     | B   | 33.6          | 10                              | 16.95                     | 14.67                       | 0.529                       | 2.61                        |
| 30     | B   | 33.6          | 10                              | 17.75                     | 14.38                       | 0.587                       | 2.58                        |
| 33     | C   | 33.6          | 10                              | 15.05                     | 13.98                       | 0.547                       | 2.47                        |
| 36     | C   | 33.6          | 10                              | 17.26                     | 14.43                       | 0.568                       | 2.59                        |
| 40     | C   | 33.6          | 10                              | 16.05                     | 14.05                       | 0.563                       | 2.49                        |
| Mean   |     | 10.0          | 16.27                           | 14.12                     | 0.568                       | 2.52                        |
| s.d.   |     | 0.0           | 1.07                            | 0.33                      | 0.021                       | 0.07                        |
| Beaker | Rep | Metformin (µg/L) | N fish in growth means at 16 dph | Wet weight at 16 dph (mg) | Total length at 16 dph (mm) | Condition factor at 16 dph | Tail length at 16 dph (mm) |
|--------|-----|------------------|---------------------------------|---------------------------|-----------------------------|---------------------------|-----------------------------|
| 5      | A   | 269              | 10                              | 15.14                     | 13.80                       | 0.571                     | 2.44                        |
| 12     | A   | 269              | 10                              | 17.00                     | 14.21                       | 0.572                     | 2.57                        |
| 14     | A   | 269              | 10                              | 14.79                     | 13.73                       | 0.564                     | 2.43                        |
| 17     | B   | 269              | 10                              | 17.01                     | 14.21                       | 0.579                     | 2.53                        |
| 20     | B   | 269              | 10                              | 16.02                     | 14.11                       | 0.564                     | 2.46                        |
| 28     | B   | 269              | 10                              | 17.26                     | 14.45                       | 0.570                     | 2.57                        |
| 38     | C   | 269              | 10                              | 13.48                     | 13.38                       | 0.545                     | 2.28                        |
| 41     | C   | 269              | 10                              | 15.43                     | 14.07                       | 0.534                     | 2.46                        |
| 45     | C   | 269              | 10                              | 15.08                     | 13.86                       | 0.558                     | 2.47                        |
| Mean   |     |                  | 10.0                            | 15.69                     | 13.98                       | 0.562                     | 2.47                        |
| s.d.   |     |                  | 0.0                             | 1.25                      | 0.32                        | 0.014                     | 0.09                        |