

## SUPPLEMENTARY MATERIAL

### Grouping of chemicals into mode of action classes by automated effect pattern analysis using the zebrafish embryo toxicity test

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#### Tables

Tables S1, S3-S4 and S6, S7-S16 are provided as an Excel file.

**Table S1.** List of chemicals and additional physicochemical properties (LogS, log  $K_{ow}$ ) and calculation of log  $D_{lipw}(pH)$ . Suppliers and purity. Calculated baseline toxicity and toxic ratio for chemicals.

**Table S2.** Parent and metabolite compound activity of the selected test compounds. All chemicals are primary toxicants and do not require metabolic activation.

| Chemical                | Type of chemical | Active metabolite identified                   | Comment                                                                                                                                                | Reference                    |
|-------------------------|------------------|------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| Loratadine              | Drug             | Descarboethoxyloratadine                       | 4 times more potent than loratadine                                                                                                                    | (Barenholtz and McLeod 1989) |
| Diclofenac              | Drug             | 4'-hydroxy diclofenac                          | Very weakly active (one thirtieth the activity of diclofenac)                                                                                          | (Davies 1998)                |
| Propafenone             | Drug             | 5-hydroxypropafenone and N-depropylpropafenone | These metabolites have antiarrhythmic activity comparable to propafenone but are present in concentrations less than 25% of propafenone concentrations | (Hii et al. 1991)            |
| Nortriptyline           | Drug             | 10-hydroxynortriptyline                        | 1/8 the activity of nortriptyline                                                                                                                      | (Nordin and Bertilsson 1995) |
| Daunorubicin            | Drug             | Daunorubicinol                                 | Display biological activity in addition to daunorubicin                                                                                                | (Takanashi and Bachur 1975)  |
| All-trans retinoic acid | Drug             | Hydroxylated metabolites and 4-oxo-atRA        | Display biological activity in addition to atRA                                                                                                        | (Topletz et al. 2015)        |
| Triclabendazole         | Drug             | Triclabendazole sulfoxide                      | Display biological activity in addition to TCBZ                                                                                                        | (Wishart et al. 2018)        |
| Acetaminophen           | Drug             | No active metabolite                           |                                                                                                                                                        | (Wishart et al. 2018)        |
| Betamethasone           | Drug             | No information found                           |                                                                                                                                                        |                              |
| Carbendazim             | Fungicide        | No active metabolite                           | Active metabolite of Benomyl                                                                                                                           | (JMPR, 1973)                 |

|                   |           |                                                      |                                  |                          |
|-------------------|-----------|------------------------------------------------------|----------------------------------|--------------------------|
| Celecoxib         | Drug      | No active metabolite                                 |                                  | (Wishart et al. 2018)    |
| Dexamethasone     | Drug      | No information found                                 |                                  |                          |
| Diflorasone       | Drug      | No information found                                 |                                  |                          |
| Diniconazole      | Fungicide | No information found                                 |                                  |                          |
| Fenbendazole      | Drug      | No active metabolite                                 |                                  | (Wishart et al. 2018)    |
| Firocoxib         | Drug      | No active metabolite                                 |                                  | (Kvaternick et al. 2007) |
| Fluazifop-p-butyl | Herbicide | No information found                                 |                                  |                          |
| Flusilazole       | Fungicide | No information found                                 |                                  |                          |
| Hexaconazole      | Fungicide | No information found                                 |                                  |                          |
| Methotrexate      | Drug      | 7-Hydroxymethotrexate                                |                                  | (Lankelma et al. 1980)   |
| Olanzapine        | Drug      | N-desmethyl olanzapine and 2-hydroxymethylolanzapine | Less active than parent compound | (Calligaro et al. 1997)  |
| Oxaprozin         | Drug      | No active metabolite                                 |                                  | (Wishart et al. 2018)    |
| Topiramate        | Drug      | No active metabolite                                 |                                  | (Wishart et al. 2018)    |
| Tralkoxydim       | Herbicide | No information found                                 |                                  |                          |
| Triadimenol       | Fungicide | No information found                                 |                                  |                          |

## References

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- Davies NM (1998) Clinical Pharmacokinetics of Oxaprozin. Clinical Pharmacokinetics 35:425–436.
- Hii JTY, Duff HJ, Burgess ED (1991) Clinical Pharmacokinetics of Propafenone. Clinical Pharmacokinetics 21:1–10.
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- Lankelma J, van der Klein E, Ramaekers F (1980) The role of 7-hydroxymethotrexate during methotrexate anti-cancer therapy. Cancer letters 9:133–142.
- Nordin C, Bertilsson L (1995) Active Hydroxymetabolites of Antidepressants: Emphasis on E-10-Hydroxy-Nortriptyline. Clinical Pharmacokinetics 28:26–40.

Takanashi S, Bachur NR (1975) Daunorubicin metabolites in human urine. *Journal of Pharmacology and Experimental Therapeutics* 195:41–49

Topletz AR, Tripathy S, Foti RS, et al (2015) Induction of CYP26A1 by metabolites of retinoic acid: Evidence that CYP26A1 is an important enzyme in the elimination of active retinoids. *Molecular Pharmacology* 87:430–441.

Wishart DS, Feunang YD, Guo AC, et al (2018) DrugBank 5.0: A major update to the DrugBank database for 2018. *Nucleic Acids Research*.

**Table S3.** Chemicals and renewal regime performed accordingly to the confirmation of stability of exposure solutions

**Table S4.** Concentration-response curves for mortality in zebrafish embryos exposed for 48 and 96 hours.  $LC_{10}$  and  $LC_{50}$ s were obtained from modelled concentration response curves. RSS (residual sum of square) is used as an indicator of the goodness of the fit. Modelling is only conducted in case of a concentration dependent increase of mortality.

**Table S5.** Settings of the VAST Bioimager and LAS software (Leica Biosystems). Software version 1.2.5.1.

| Loading setup                        |                           | Centering fish inside the capillary        |                                                  |
|--------------------------------------|---------------------------|--------------------------------------------|--------------------------------------------------|
| Max volume between flushes           | 300 $\mu$ L               | Displacement with 5 pump microsteps        | 1 mm                                             |
| Initial pluger position              | 1000 $\mu$ L              | Min. visible fish length                   | 3.9 mm                                           |
| Limit for loading volume             | 1000 $\mu$ L              | Delay before and after adjusting position  | 700 ms                                           |
| Attempt to re-load                   | off                       | Number of attempts                         | 2                                                |
| Re-loading backup volume             | 40 $\mu$ L                |                                            |                                                  |
| Unloading setup (one fish at a time) | Move forward after backup | <b>Backup</b>                              |                                                  |
| Push through volume                  | 80 $\mu$ L                | Max backup volume                          | 30 $\mu$ L                                       |
| Unloading volume 1                   | 30 $\mu$ L                | Min backup volume                          | 1 $\mu$ L                                        |
| Push-back volume                     | 40 $\mu$ L                | Volume of a first backup pulse             | 2 steps                                          |
| Unloading volume 2                   | 400 $\mu$ L               | Move forward after backup                  | head first 7 microsteps, tail first 7 microsteps |
| Unload multiple fish in bulk         | off                       |                                            |                                                  |
| Operational mode setup               | Rotational angle          | <b>Object detection and rotation setup</b> |                                                  |
| Rotational position                  | Auto                      | Drop of intensity threshold                | 20                                               |
| Hi-Resolution imaging                | Auto                      | Min average intensity                      | 130                                              |
|                                      |                           | Min intensity drop                         | 25%                                              |
| Output                               |                           | Bubbles and debris                         | detect and discard                               |
| Bubbles and debris                   | Manual                    | Manual                                     | 90                                               |
| Auto mixer                           | On                        | Minimum similarity                         | 0.6                                              |
| Speed                                | 10%                       | Rotational backlash                        | 5 degrees                                        |
| Oscil                                | 3 (sec)                   |                                            |                                                  |

| Camera settings              |             | Pump speeds           |      |
|------------------------------|-------------|-----------------------|------|
| Exposure (Manual)            | 380 $\mu$ s | Loading speed         | 120  |
| Gain (Manual)                | 1 dB        | Stepping speed        | 2    |
|                              |             | Backup speed          | 70   |
| White balance                |             | Unloading speed       | 2000 |
| Red Gain                     | 170%        | Stepping speed        | 50   |
| Blue Gain                    | 200%        | Flushing speed        | 4000 |
|                              |             | Priming speed         | 2000 |
| Imaging with external device |             | Wand aspirate speed   | 2000 |
| X-position                   | 290 $\mu$ m | Backlash (microsteps) | 14   |
| Rotation angle               | 180         |                       |      |
| Ctrl output                  | 1           |                       |      |
| Trigger output               | 1           |                       |      |
| Bright field                 | off         |                       |      |
| Tray LED                     | off         |                       |      |
| Trigger out delay            | 700 ms      |                       |      |
| Trigger timeout              | 30000 ms    |                       |      |
| Degree per rotation          | 360         |                       |      |
| Rotation speed               | 30          |                       |      |
| CSV file record              | on          |                       |      |

**Table S6.** Raw data on frequency of spontaneous tail coilings for all chemicals.

**Table S7.** Model equations used for modelling concentration-response curves.

| Model type                            | Equation                                   | Parameters                      |
|---------------------------------------|--------------------------------------------|---------------------------------|
| Hill (sigmoidal 4 parameter equation) | LL.4 See drc package in r <sup>a</sup>     |                                 |
| Gauss                                 | Gaussian see drc package in r <sup>a</sup> |                                 |
| Exponential                           | EXD.3 see drc package in r <sup>a</sup>    |                                 |
| Polynomial                            | $y = x + bx^2$                             | b slope                         |
| Linear                                | $y = d + bx$                               | b slope<br>d mean control value |

<sup>a</sup>drc package documentation: <https://cran.r-project.org/web/packages/drc/drc.pdf>

**Table S8.** Histograms and Shapiro-Wilk-T test results from control data at 2 and 4 dpf.

**Table S9.** Magnitude of effect concentrations used according to endpoint

**Table S10.** Effect concentrations and concentration-response curves parameters in zebrafish embryos for all endpoints and chemicals.

**Table S11.** Sensitivity ratios for all chemicals across all evaluated endpoints

**Table S12:** Comparison of EC10 for morphological (4 dpf) and behaviour endpoints (LMR and STC)

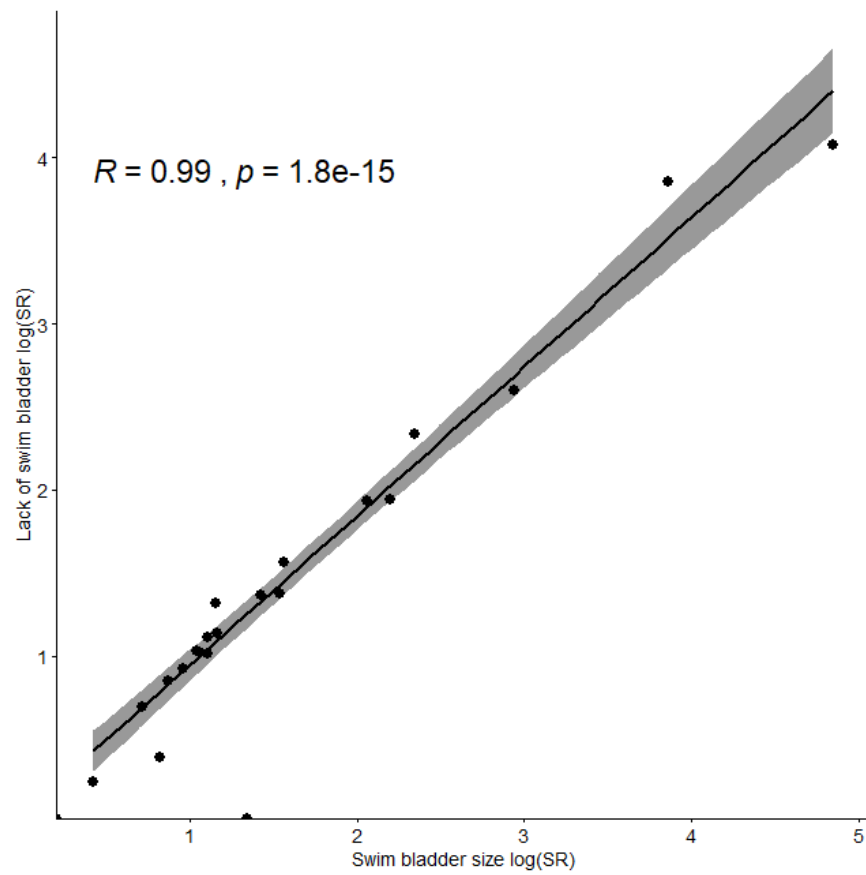
**Table S13:** Comparison of EC50 for morphological (4 dpf) and behaviour endpoints (LMR and STC)

**Table S14.** Calculated  $SR_{Cytotoxicity}$  for each assay of the Comptox library included in the analysis

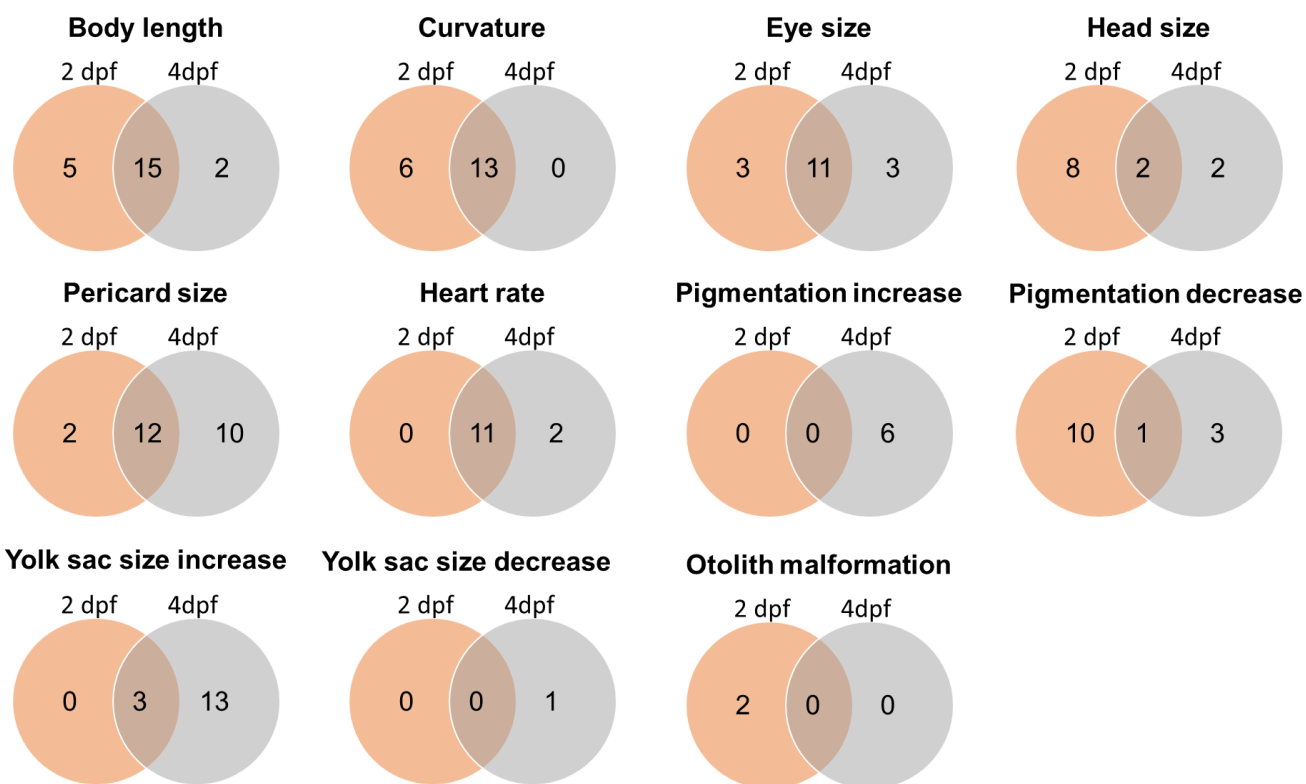
**Table S15.** Heatmap of the mean  $SR_{Cytotoxicity}$  values of each MoA group for each target subfamily  
ToxCast assay included

**Table S16.** Activity reported for our set of chemicals on the COX-1 and COX-2 activity assays in the Comptox library

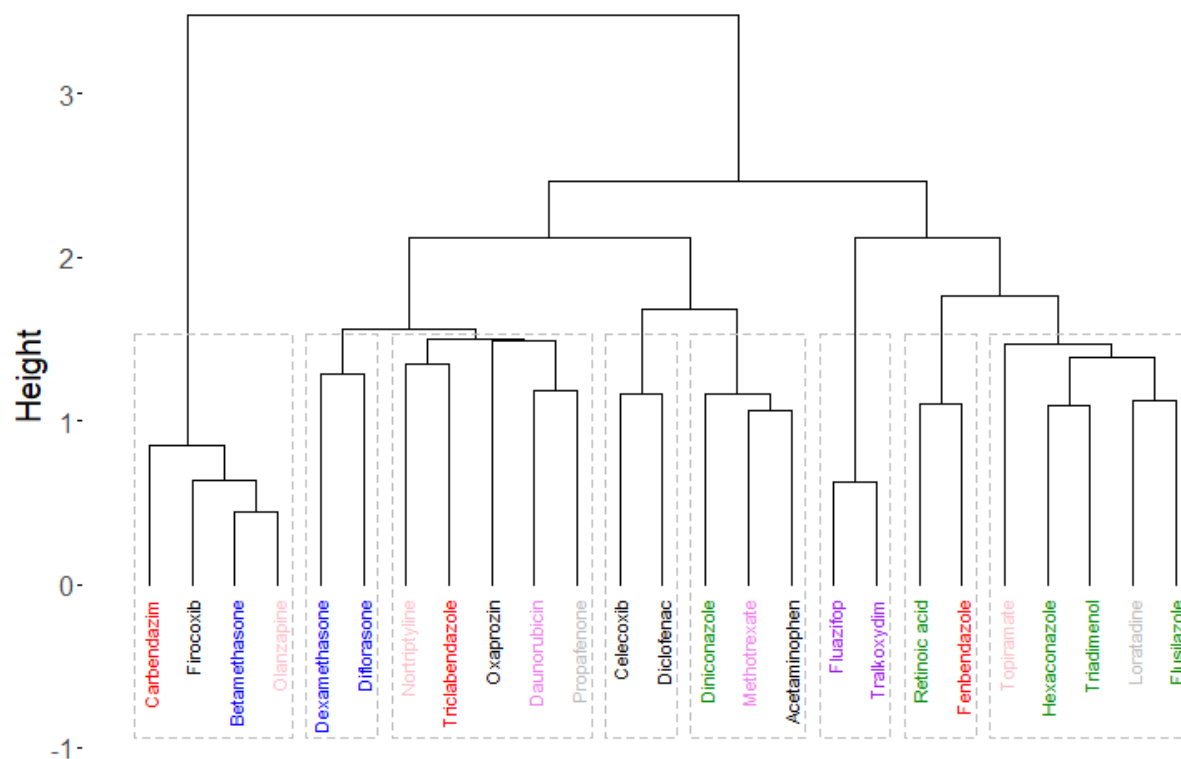
## Figures



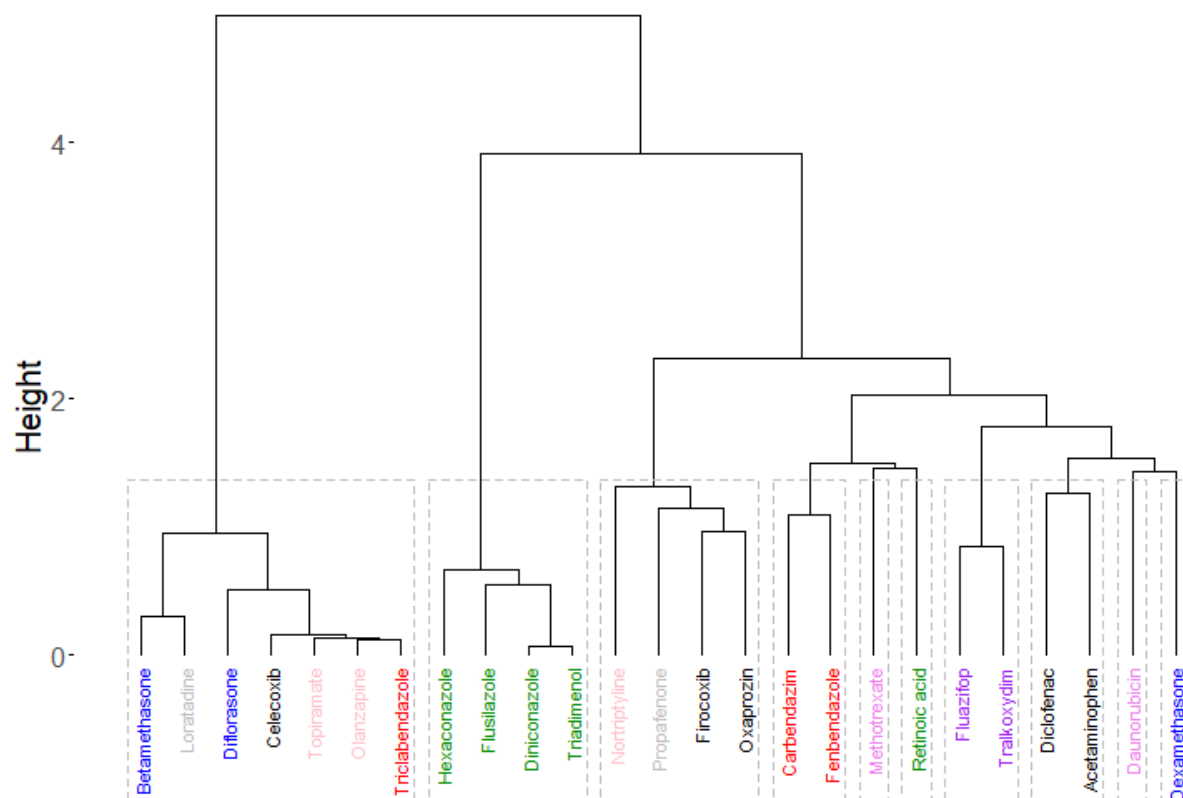
**Fig. S1.** Correlation between SR of swim bladder size and lack of swim bladder for all chemicals.



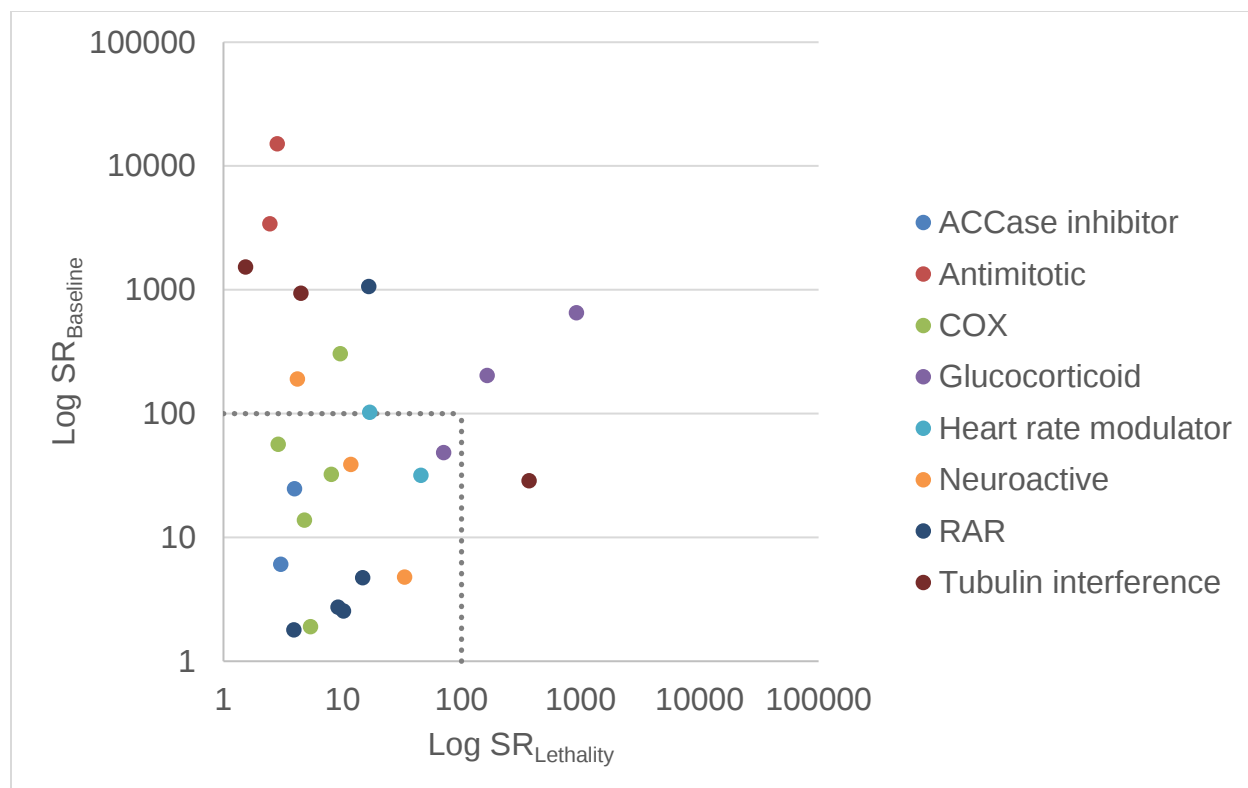
**Fig. S2.** Venn diagrams of chemicals showing effects at 2 dpf and 4 dpf. The number of chemicals in each time point and the overlap between time points for each endpoint analysed are shown in the Venn diagrams. The only chemical showing a yolk sac size decrease at 4 dpf was dexamethasone and the two chemicals showing otolith malformations at 2 dpf were the Acetyl CoA Carboxylase (ACCase) inhibitors (tralkoxydim and fluazifop).



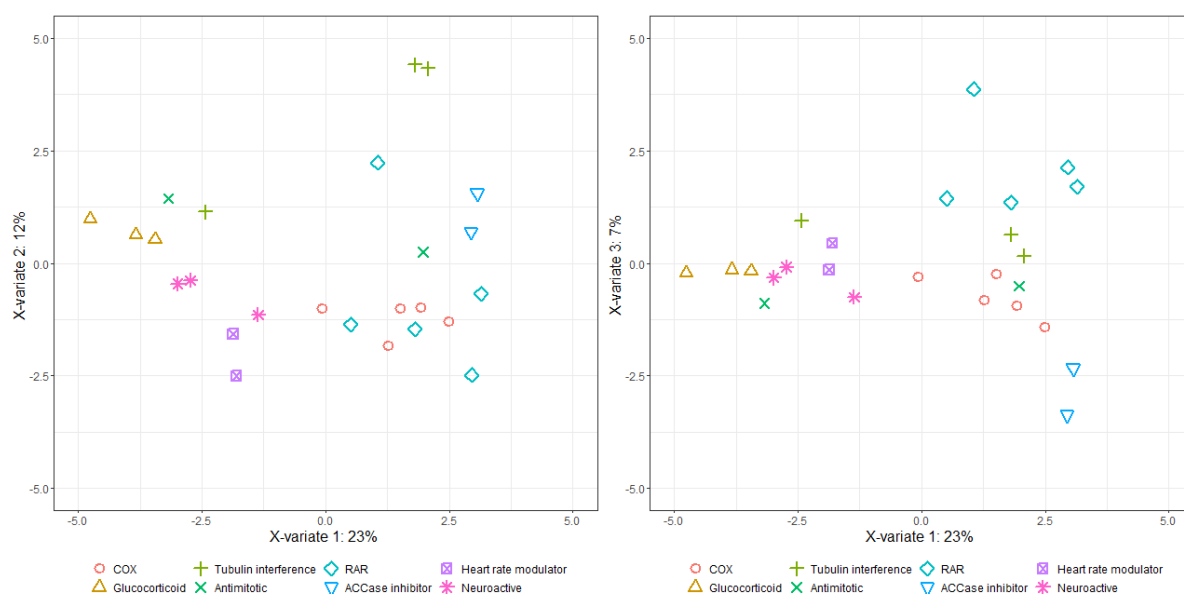
**Fig. S3.** Dendrogram of the cluster analysis using chemical  $SR_{Lethality}$  based on the chord distance and ward method. Chemical names are colored according to their MoA group. (black: COX inhibitors, blue: glucocorticoids, red: tubulin inhibitors, purple: ACCase inhibitors, violet: antimitotics, grey: heart rate modulators, green: retinoic signaling interfering compounds, pink: neuroactive)



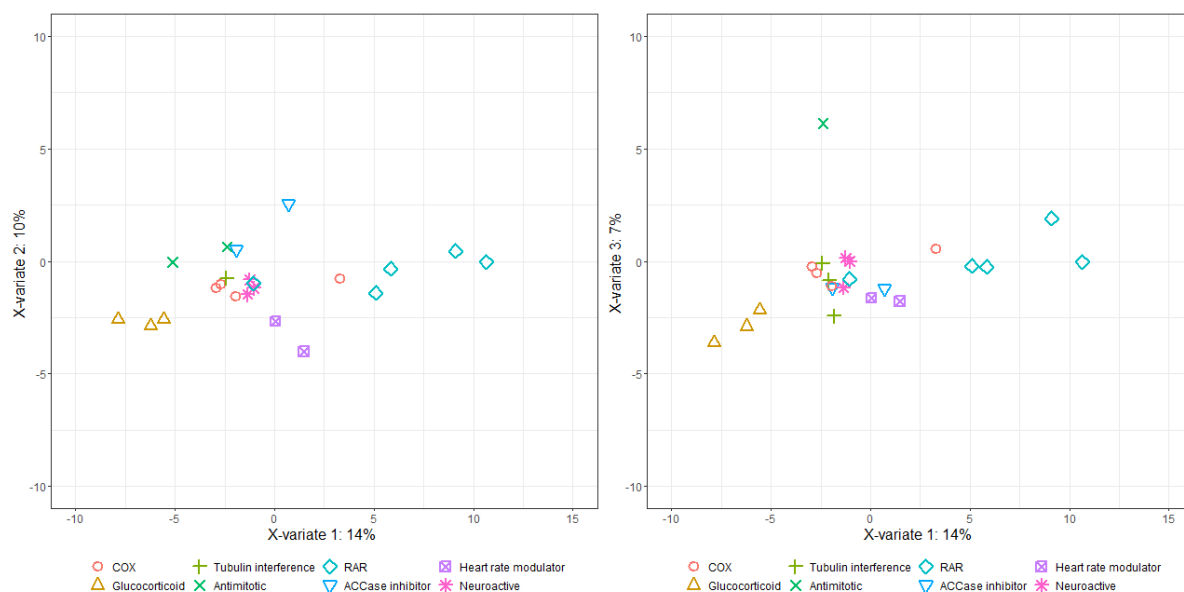
**Fig. S4.** Dendrogram of the cluster analysis using chemical  $SR_{Baseline}$  based on the chord distance and ward method. Chemical names are colored according to their MoA group. (black: COX inhibitors, blue: glucocorticoids, red: tubulin inhibitors, purple: ACCase inhibitors, violet: antimitotics, grey: heart rate modulators, green: retinoic signaling interfering compounds, pink: neuroactive)



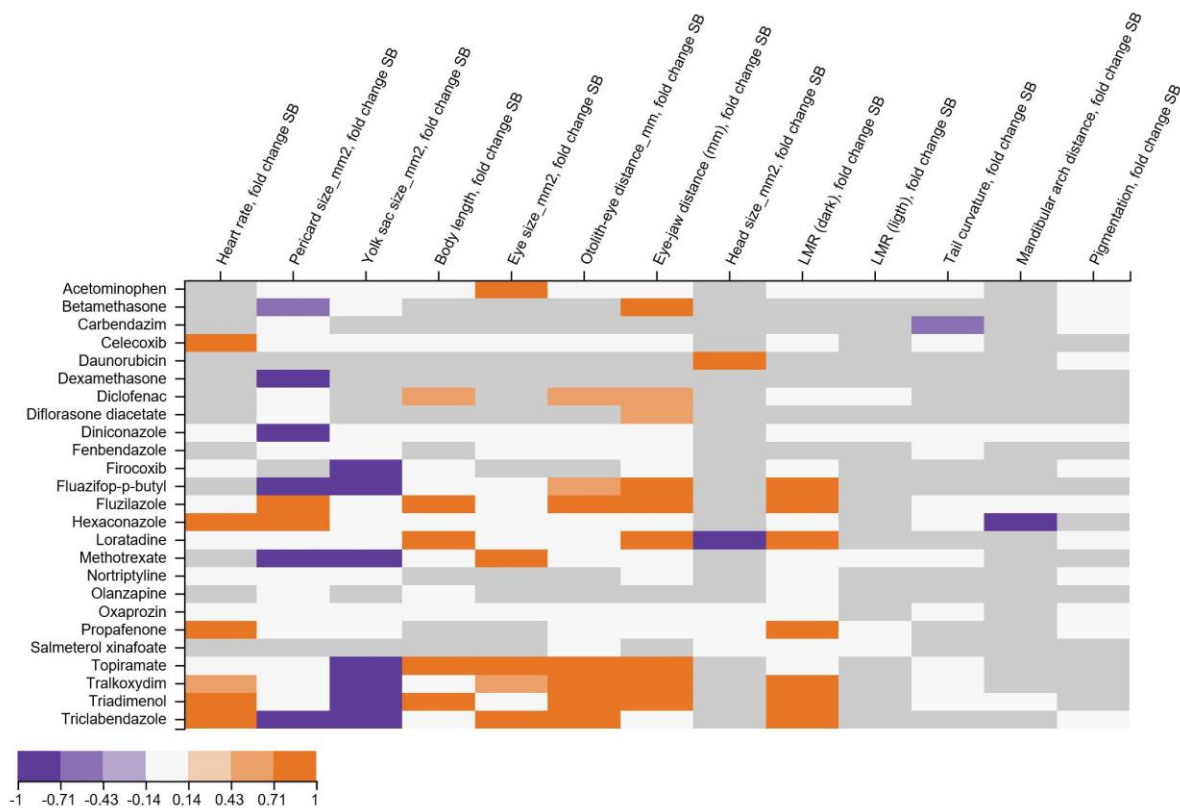
**Fig. S5.** Comparison of SR<sub>Baseline</sub> and SR<sub>Lethality</sub> of the most sensitive endpoint for each chemical tested in the study. Points are colored according to the chemical MoA group. RAR: chemicals interfering with the retinoic acid signaling. COX: cyclooxygenase inhibitors



**Fig. S6.** PLS-DA score plots from the 30 endpoints analyzed with zebrafish using the  $SR_{Baseline}$ . Left figure shows score plot between component 1 and 2 and right figure between the component 1 and 3. Each dot represents a chemical and are grouped in 8 broad MoA classes. Accuracy of the model: 42.8%. RAR: chemicals interfering with the retinoic acid signaling. COX: cyclooxygenase inhibitors.



**Fig. S7.** PLS-DA score plot from the 30 endpoints analyzed with zebrafish using the  $SR_{Lethality}$  combined with the 124 in vitro assays of ToxCast library using the  $SR_{Cytotoxicity}$ . Left figure shows score plot between component 1 and 2 and right figure between the component 1 and 3. Each dot represents a chemical and are grouped in 8 broad MoA classes. RAR: chemicals interfering with the retinoic acid signaling. COX: cyclooxygenase inhibitors



**Fig. S8.** Correlation between fold change in swim bladder size and other morphological and functional endpoints for each chemical (y-axis). Correlation is indicated as a color scale from purple (-1, negative correlation) to orange (1, positive correlation). Grey values depict no affected morphological and functional endpoints for each chemical.