Supplementary information

The thyroid hormone system disrupting potential of resorcinol in fish

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1. Supplement to introduction

Table S1: Overview of the available mortality data following resorcinol exposure in different fish species. NA = not available

Species	Life stage	Exposure duration (days)	Type of exposure	LC50		Reference
				µM	mg/L	
Danio rerio	Embryo	3	Semi static	4995	550	Thienpont et al. (2011)
Danio rerio	Embryo	2	Static	3849	424	Achenbach et al. (2020)
Danio rerio	Larvae	7	Semi static	2379	262	Van Leeuwen et al. (1990)
Oncorhynchus mykiss	Larvae	60	Semi static	2906	320	Van Leeuwen et al. (1990)
Oncorhynchus mykiss	NA	4	Flow through	908	100	DeGraeve et al. (1980a) [1]
Pimephales promelas	Adult	4	Flow through	243	26.8	Koppers Company (1981) [2]
Pimephales promelas	Adult	4	Flow through	268	29.5	Koppers Company (1981) [2]
Pimephales promelas	NA	4	Flow through	908	100	DeGraeve et al. (1980a) [1]
Pimephales promelas	NA	4	Static	363-545	40-60	Koppers Company (1981) [2]
Pimephales promelas	Adult	4	Static	545	60	Curtis and Ward (1981)
Pimephales promelas	Juvenile	1	Static	805	88.6	Curtis et al. (1979)
Pimephales promelas	Juvenile	2	Static	695	72.6	Curtis et al. (1979)
Pimephales promelas	Juvenile	4	Static	485	53.4	Curtis et al. (1979)
Leuciscus idus	NA	4	NA	315	34.7	Unpublished study report (1981) [3]

[1] Source: Hahn et al. (2006)

[2] Source: ECHA (2023)

[3] Source: Tukes (2017)

2. Supplement to materials and methods

2.1 Zebrafish housing and egg production

Table S2: Overview of the different housing conditions of the adult zebrafish population at the University of Antwerp (UA) and the University of Southern Denmark (SDU). RO = reverse osmosis water.

	University of Antwerp	**University of Southern Denmark**
Strain	AB strain	In house wild type strain from the University of Heidelberg
System	ZebTec standalone system	Zebcare circulating husbandry system
Water composition	Reconstituted fresh water: RO water with Instant Ocean® Sea Salt and $NaHCO_3$	Reconstituted fresh water: RO water with 294 mg/L $CaCl_2$ x 2 H_2O, 123.3 mg/L $MgSO_4$ x 7 H_2O, 63 mg/L $NaHCO_3$, 5.5 mg/L KCl (ISO, 2007)
Temperature	28.0 ± 0.2 °C	26 ± 1°C
Conductivity	500 ± 50 µS	500 ± 20 µS
pH	7.5 ± 0.3	7.7 ± 0.2
Light/dark cycle	14/10 h	14/10 h
Monitoring ammonia, nitrite, nitrate levels	Twice per week	Weekly
Ammonia levels	0 mg/L	< 0.05 mg/L
Nitrite levels	< 0.3 mg/L	< 0.3 mg/L
Nitrate levels	< 12.5 mg/L	< 12.5 mg/L
Feeding regime	Weekdays: Twice: granulated food (Zebrafeed) Once: *Daphnia* sp. nauplii, *Artemia* sp. nauplii, Chironomidae or Chaoboridae larvae Weekend days: Once: granulated feed (Gemma)	Morning: ad libitum granulated feed (SDS400) Afternoon: *Artemia* sp. nauplii
Breeding groups	1 female + 1-2 males	5-7 females + 5 males

2.2 Zebrafish embryo exposures

Table S3: Overview of the different housing conditions and water parameters during the zebrafish embryo exposure experiments at the University of Antwerp (UA) and the University of Southern Denmark (SDU). RO = reverse osmosis water.

	University of Antwerp	**University of Southern Denmark**
Water composition	Reconstituted fresh water: RO water with Instant Ocean® Sea Salt and $NaHCO_3$	Reconstituted fresh water: RO water with 294 mg/L $CaCl_2$ x 2 H_2O, 123.3 mg/L $MgSO_4$ x 7 H_2O, 63 mg/L $NaHCO_3$, 5.5 mg/L KCl (ISO, 2007)
Temperature	28.5 ± 0.2 °C	26 ± 1°C
Conductivity	500 ± 10 µS	500 ± 20 µS
pH	7.5 ± 0.1	7.8 ± 0.2
Light/dark cycle	14/10 h	14/10 h

Table S4: Overview of the different zebrafish embryo exposures that were performed at the University of Antwerp (UA) and the University of Southern Denmark (SDU). dpf = days post fertilization, SB = swim bladder

Exposure for assessment of	Performed at	Nominal resorcinol concentration		Exposure duration (dpf)	Temperature (°C)
		µM	mg/L		
Swim bladder inflation	SDU (experiment 1)	0, 28, 57, 114, 227, 454	0, 3.125, 6.25, 12.5, 25,50	0-8	26
	SDU (experiment 2)	0, 182, 363, 545, 727, 908	0, 20, 40, 60, 80, 100	0-8	26
	UA (experiment 3)	0, 454, 908	0, 50, 100	0-5	28.5
	UA (experiment 4)	0, 363, 545, 727	0, 40, 60, 80	0-5	28.5
	UA (experiment 5: SB inflation + swimming activity[1])	0, 182, 363, 545, 727	0, 20, 40, 60, 80	0-7	28.5
	SDU (experiment 6: T4 supplementation)	0, 727	0, 80	0-8	26
Eye development	SDU	0, 4.5, 45, 454	0, 0.5, 5, 50	0-5	26
Whole-body TH levels	UA	0, 9, 91, 182, 363, 545	0, 1, 10, 20, 40, 60	0-5	28.5
qPCR analysis	UA	0, 363, 727	0, 40, 60	0-5	28.5

[1] Final experiment as reported under section 2.4 of the manuscript

2.3 Assessment of effect on swim bladder inflation (Experiments 1-4)

Table S5: Overview of the preliminary experiments performed at the University of Southern Denmark (SDU) to determine the final exposure concentrations. Mortality and swim bladder inflation were determined at 7 dpf for experiment 1 and 8 dpf for experiment 2. For experiment 1, n=24 for the control and n=20 for all other groups. For experiment 2, n=44 for the control and n=20 for all other groups. Experiment numbers correspond with those in Table S4. Values that differ significantly from the controls are indicated with an asterisks. SB: swim bladder.

Nominal resorcinol concentration		Mortality (%)	Non-inflated SB (%)
µM	**mg/L**		
Experiment 1			
0	0	4.2	13.0
28	3.125	10.0	11.1
57	6.25	10.0	5.6
114	12.5	15.0	5.9
227	25	5.0	26.3
454	50	25.0*	100.0*
Experiment 2			
0	0	6.8	19.5
182	20	10.0	77.8*
363	40	5.0	89.5*
545	60	5.0	100.0*
727	80	5.0	100.0*
908	100	25.0	100.0*

Table S6: Overview of the preliminary experiments performed to determine the final exposure concentrations at UA. Mortality, swim bladder inflation, larval length and swim bladder surface were determined for each experiment at 5 dpf. Two 24-well plates were used in each experiment (n=40 for all groups). Experiment numbers correspond with those in Table S4. SB: swim bladder, NA: not available. Values that differ significantly from the controls are indicated with an asterisk. Values in italic indicate that statistical analysis was not possible due to sample size.

Nominal resorcinol concentration		Mortality (%)	Non-inflated SB (%)	Average larval length ± SD (mm)	Average SB surface ± SD (mm²)
µM	**mg/L**				
Preliminary experiment 3					
0	0	0	0	4.018 ± 0.210	0.084 ± 0.008
454	50	2.5	79.5*	4.107 ± 0.127	0.079 ± 0.013
908	100	5.0	100.0*	3.802 ± 0.166*	NA
Preliminary experiment 4					
0	0	5.0	21.1	4.083 ± 0.189	0.084 ± 0.008
363	40	2.5	53.9*	4.012 ± 0.296	0.069 ± 0.016*
545	60	2.5	94.9*	4.036 ± 0.265	*0.060 ± 0.020*
727	80	0	100.0*	4.040 ± 0.271	NA

2.4 Swimming activity – internal plate controls (experiment 5)

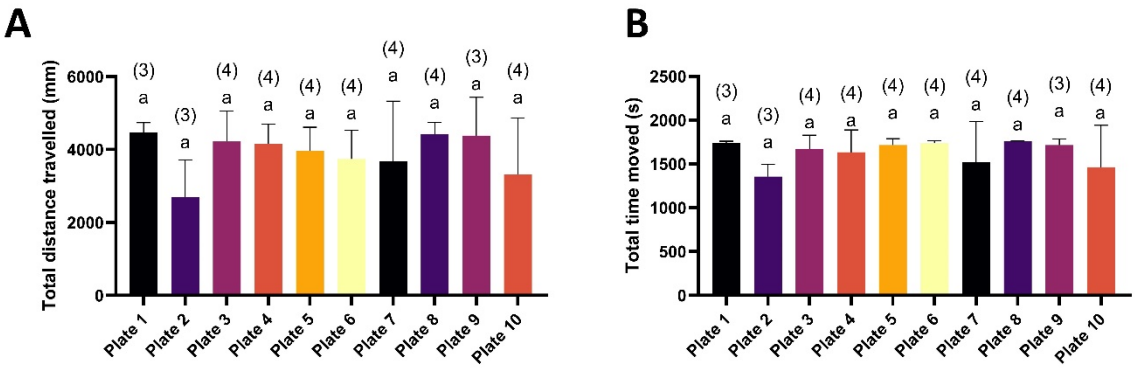

Figure S1: Swimming activity of the internal plate controls at 5 dpf. Total distance travelled (A) and total time moved (B) of the internal control embryos for the six plates that were used for analysis of swimming activity. Sample sizes are indicated between parentheses and different letters indicate significant differences.

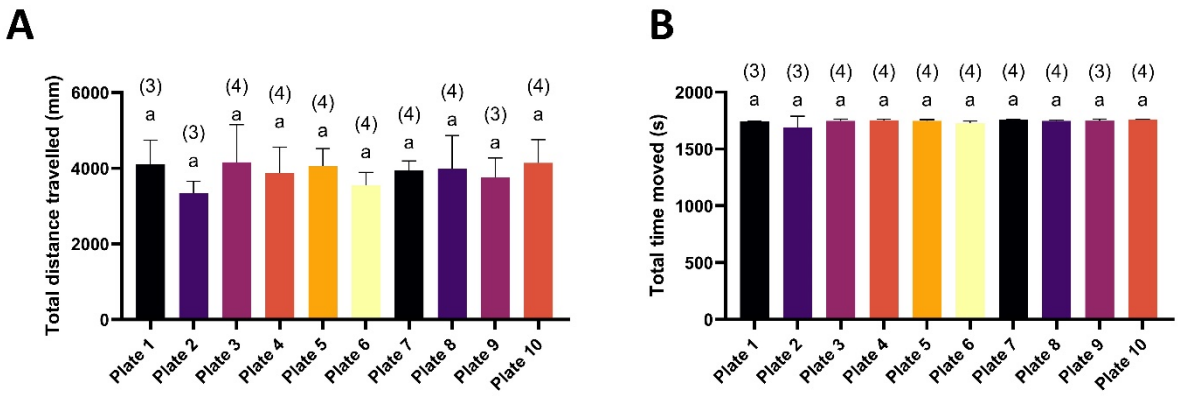

Figure S2: Swimming activity of the internal plate controls at 7 dpf. Total distance travelled (A) and total time moved (B) of the internal control embryos for the six plates that were used for analysis of swimming activity. Sample sizes are indicated between parentheses and different letters indicate significant differences.

2.5 Zebrafish exposures for thyroid hormone measurements

Separate exposures were carried out at UA. Zebrafish embryos were exposed until 5 dpf to either 0, 9, 91, 182, 363 or 545 µM resorcinol as described in section 2.4 of the main text (daily medium renewal) but were housed in 960 mL polypropylene containers instead of 24-well plates to achieve a larger sample size. Each container was filled with 200 mL of medium and 100 embryos. For each test concentration, four replicates were sampled over two exposures (each exposure included 2 replicates). At 5 dpf, 100 embryos per sample were euthanized in 1 g/L ethyl 3-aminobenzoate methanesulfonate (MS-222, Sigma-Aldrich, CAS: 886-86-2, buffered to pH 7.5) and rinsed twice with reconstituted fresh water. Embryos were transferred to a cryovial, snap frozen in liquid nitrogen, and kept at -80°C until further analysis.

2.6 Eye histology

Zebrafish larvae were euthanized by hypothermic shock and fixed overnight in 10% formalin buffer (CellPath, Newton Powys, United Kingdom). Larvae were stored in 70% ethanol until further processing. Molds prepared from 1 % Agarose (UltraPureTM, Invitrogen, USA) were used for pre-embedding the larvae. The agarose mold containing the larvae was dehydrated in an ascending series of ethanol (70, 96, and 99 %), cleared in Tissue Clear (Sakura Finetek Europe B.V., the Netherlands), and embedded in paraffin. Coronal sections (3 µm) were prepared employing a rotating microtome (Thermo Scientific, Microm HM 355S) and transferred on glass slides. Sections were stained with the hematoxylin-eosin stain and analyzed using a Nikon Eclipse Ti microscope (Nikon Europe B.V., the Netherlands) equipped with an DFK 33UX250 camera (The Imaging Source Europe GmbH, Germany). Pictures were taken from sections showing the optic nerve entering the retina and eye diameter was measured at the widest part of the eye using Image J 1.52n (Schneider et al., 2012). For determining thicknesses of photoreceptor and inner plexiform layers, the retina was divided into quarters and thicknesses of the layers were measured once in each of the quarters.

2.7 qPCR analysis

A separate exposure was carried out at UA. Zebrafish embryos were exposed to 363 and 727 µM resorcinol until 5 dpf as described in section 2.4 of the main text (daily medium renewal) but were housed in 960 mL polypropylene containers instead of 24-well plates to achieve a larger sample size. Each container was filled with 200 mL and 40 embryos. At 5 dpf, 10 embryos were pooled per sample, euthanized in 1 g/L MS-222, rinsed with reconstituted fresh water and snap frozen in liquid nitrogen. For the embryos exposed to 363 µM, independent samples of embryos with either an inflated or non-inflated swim bladder were collected (Table S8). Five replicate samples were collected for each treatment.

Table S7: Primer sequences used in quantitative polymerase chain reaction (qPCR) analyses. Ta = annealing temperature

Gene	Full name	Primer sequence (5' → 3')	Ta (°C)	Source
Target genes				
dio1	iodothyronine deiodinase type I	F: GTTCAAACAGCTTGTCAAGGACTTC R: AGCAAGCCTCTCCTCCAAGTT	63	Walpita et al. (2007)
dio2	iodothyronine deiodinase type II	F: GCTTTCTTCTGGATGCCTAC R: TTGTGGGTCTTACCGCTG	63	Liu et al. (2011)
dio3a	iodothyronine deiodinase type III a	F: GCGCGTACGGAGCTTACTTC R: AGCTCGGAGATGCGGAATCC	66	Heijlen et al. (2014); Houbrechts et al. (2016)
dio3b	iodothyronine deiodinase type III b	F: CCGACAGCAACAAGATGTTCAC R: CGCTCTTGAAGAAGTCCAGCTT	64	Walpita et al. (2010)
thraa	thyroid hormone receptor alpha	F: GGCTCGGAGTGGTTTCTGA R: CTTGCGGTGGTTGATGTAGTG	60	Horie et al. (2022); Liang et al. (2015)
thrβ	thyroid hormone receptor beta	F: TGGGAGATGATACGGGTTGT R: ATAGGTGCCGATCCAATGTC	60	Yu et al. (2010)
tpo	thyroperoxidase	F: CCAGCCAGACCTCGTTC R: CGGAGATGAGCGGAAGAAG	60	Baumann et al. (2016)
ttr	transthyretin	F: GTATCGGGTGGAGTTTGACA R: CTCAGAAGGAGAGCCAGTGT	62	Liu et al. (2011)
Reference genes				
18S	18S ribosomal RNA	F: CGGAGAGGGAGCCTGAGAA R: AGTCGGGAGTGGGTAATTTGC	60	Biga et al. (2005)
actb1	actin, beta 1	F: AAGTGCGACGTGGACA R: GTTTAGGTTGGTCGTTCGTTTGA	60	Gonzalez et al. (2006)
alas2	aminolevulinate, delta-, synthase 2	F: CAGTGCGAGTGTTGAAGA R: TTATCGCCTGCACGTAGA	60	Van Dingenen et al. (2023)
arnt2	aryl-hydrocarbon receptor nuclear translocator 2	F: ACCAACGTGATTGGCTAC R: CCACTCTCGGTTCTTCAT	60	Initial preparation (NCBI blast)
hprt1	hypoxanthine phosphoribosyl-transferase 1	F: CAAAATGACCAGTCCACAGG R: TGTCCTCTTCACCAGCAAACT	64	Liu et al. (2011)

Purity of the RNA was confirmed using a BioDrop spectrophotometer (Biochrom Ltd., Cambridge, UK) to determine A260/230 and A260/280 ratios. Minimum A260/230 values were 1.8 and minimum A260/280 values were 2 (Table S8). RNA integrity was measured using a Fragment Analyzer (Agilent Technologies, Diegem, Belgium). RNA quality number (RQN) was 10 for all samples (Table S8).

Table S8: Average RNA concentration (ng/µL), A260/230, A260/280 ratios and RNA quality number (RQN) values for all RNA samples. SB = swim bladder

	Replicate	Average RNA concentration (ng/µL)	A260/230 ratio	A260/280 ratio	RQN
0 µM resorcinol	**1**	172.0	2.2	2.0	10
	2	147.2	2.3	2.0	10
	3	155.2	2.0	2.0	10
	4	157.6	2.1	2.0	10
	5	156.0	2.1	2.0	10
363 µM resorcinol - inflated SB	**1**	172.8	2.2	2.0	10
	2	150.4	2.3	2.0	10
	3	161.6	2.3	2.0	10
	4	156.8	2.0	2.0	10
	5	145.6	2.2	2.0	10
363 µM resorcinol - uninflated SB	**1**	152.8	2.1	2.0	10
	2	164.0	2.3	2.0	10
	3	153.6	2.3	2.0	10
	4	160.0	2.3	2.0	10
	5	145.6	1.8	2.0	10
727 µM resorcinol	**1**	154.4	2.2	2.0	10
	2	164.8	2.2	2.0	10
	3	159.2	2.2	2.0	10
	4	162.4	2.3	2.0	10
	5	155.2	2.2	2.0	10

Using the geNorm algorithm (Vandesompele et al., 2002), the two most stable reference genes were chosen out of a list of five candidate reference genes (*alas2, 18S, hprt1, actb1* and *arnt2*). The two genes with the lowest score are the most stable and were therefore selected (Figure S1).

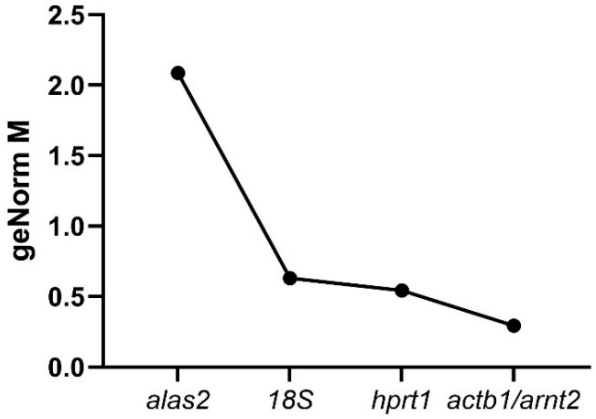

Figure S3: geNorm M-score of the candidate reference genes.

2.8 Analytical measurements

The actual resorcinol concentrations in water samples were analyzed using a high-performance liquid chromatography method (HPLC 1220 Infinity, Agilent Technologies). For each sample, 20 µl was injected on an Ascentis C18 column (15 cm x 2.1 mm, 5µm (model 581304-U)) with a flow of 0.5 ml/min and an isocratic elution using 70% buffer A (ASTM 1a, 0.2% Acetic Acid) and 30% buffer B (MeOH, 0.2% Acetic Acid). The detection UV wavelength was 280 nm and the resorcinol standards were made from resorcinol purchased from Sigma Aldrich (CAS 108-46-3, batch No. 307521). Linear range of the standard curve was 0.1-100 mg/L and limit of detection (LOD) was 0.1 mg/L.

Table S9: Overview of sampling of the medium from each exposure for analytical measurements. Fresh medium is the freshly made medium right after renewal, old medium represents the same medium after 24h right before renewal.

Timepoint (hpf)	Fresh/Old medium	Number of replicates
Swim bladder inflation experiment (UA)		
24	Fresh	1
48	Old	3
144	Fresh	1
168	Old	3
TH measurements experiments (two exposures)		
0	Fresh	3
24	Old	3
96	Fresh	3
120	Old	3
qPCR experiment		
0	Fresh	3
24	Old	3
96	Fresh	3
120	Old	3

Table S10: Average resorcinol concentrations ± standard deviation (SD) measured in the water samples. As on average no major differences were found between exposure 1 and 2 for the TH measurements, values reported in this table are the average values derived from both exposures.

Nominal concentration		Average measured concentration ± SD		Average % of nominal concentration ± SD
mg/L	µM	mg/L	µM	
Swim bladder inflation experiment (UA)				
20	182	17.4 ± 1.6	158 ± 14.9	87.2 ± 8.1
40	363	35.3 ± 3.5	321 ± 31.4	88.2 ± 8.6
60	545	54.3 ± 2.5	493 ± 22.5	90.5 ± 4.1
80	727	72.5 ± 2.3	658 ± 21.3	90.6 ± 2.9
Exposure for TH measurements				
1	9	0.05 ± 0.02*	5 ± 1.7*	52.8 ± 18.5*
10	91	8.2 ± 0.4	74 ± 3.4	81.6 ± 3.7
20	182	17.2 ± 1.3	156 ± 11.7	85.9 ± 6.4
40	363	35.7 ± 1.4	324 ± 12.4	89.1 ± 3.4
60	545	52.6 ± 2.8	478 ± 25.7	87.7 ± 4.7
Exposure for qPCR analysis				
40	363	41.8 ± 0.6	380 ± 5.0	104.5 ± 1.4
80	727	82.4 ± 2.3	748 ± 20.8	103.0 ± 2.9

* In the 9 µM treatment, unexpectedly low concentrations (around 0.9 µM) were detected during the second exposure in the samples of the old medium at 5 dpf (Figure S6A). The 9 µM samples from the second exposure were, therefore, excluded.

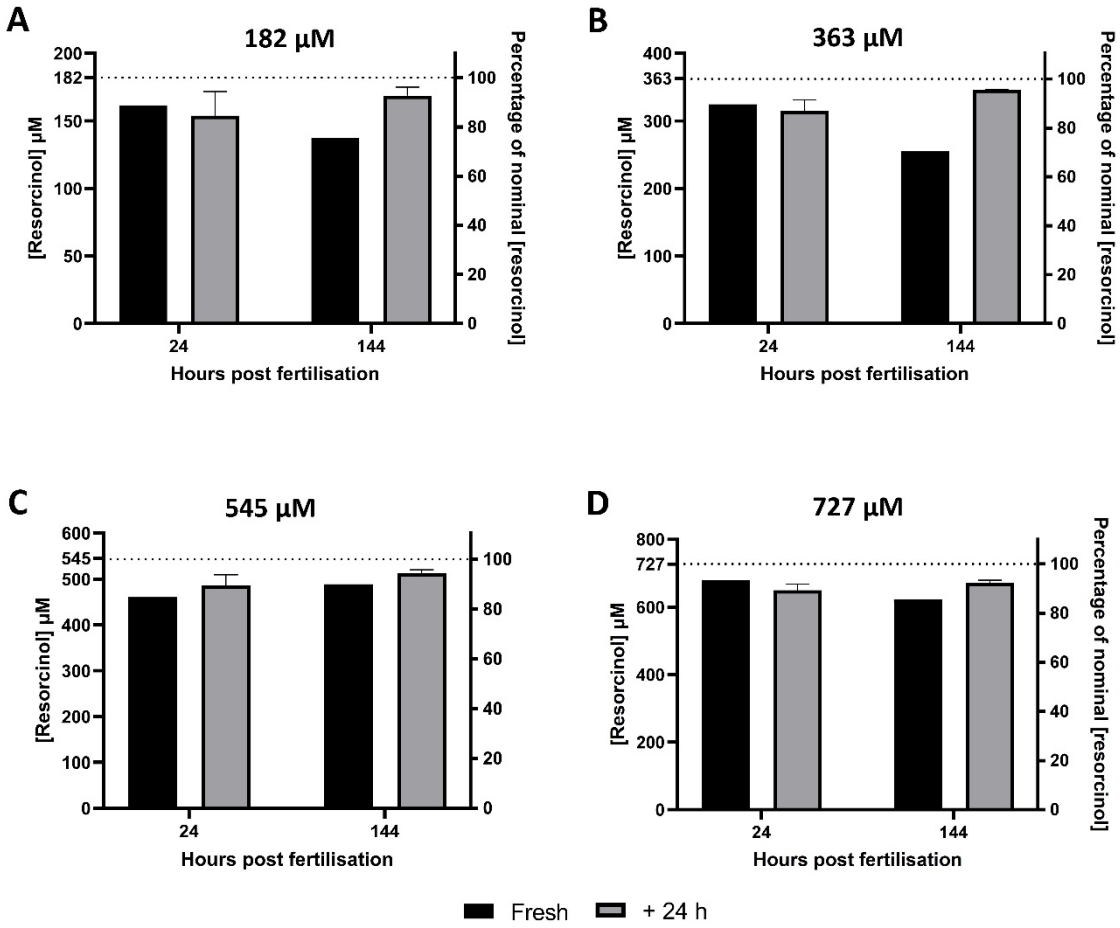

Figure S4: Resorcinol measurements in the medium for the exposure to assess swim bladder inflation. Average resorcinol concentrations are represented as a function of time. Right y-axes represent the percentage of the nominal concentration, the dotted line corresponds to 100% of the nominal concentration: 182 µM (A), 363 µM (B), 545 µM (C) and 727 µM (D). Black bars show fresh medium, grey bars represent the medium after 24h just before renewal. Error bars show standard deviation; only one sample was taken for the fresh medium.

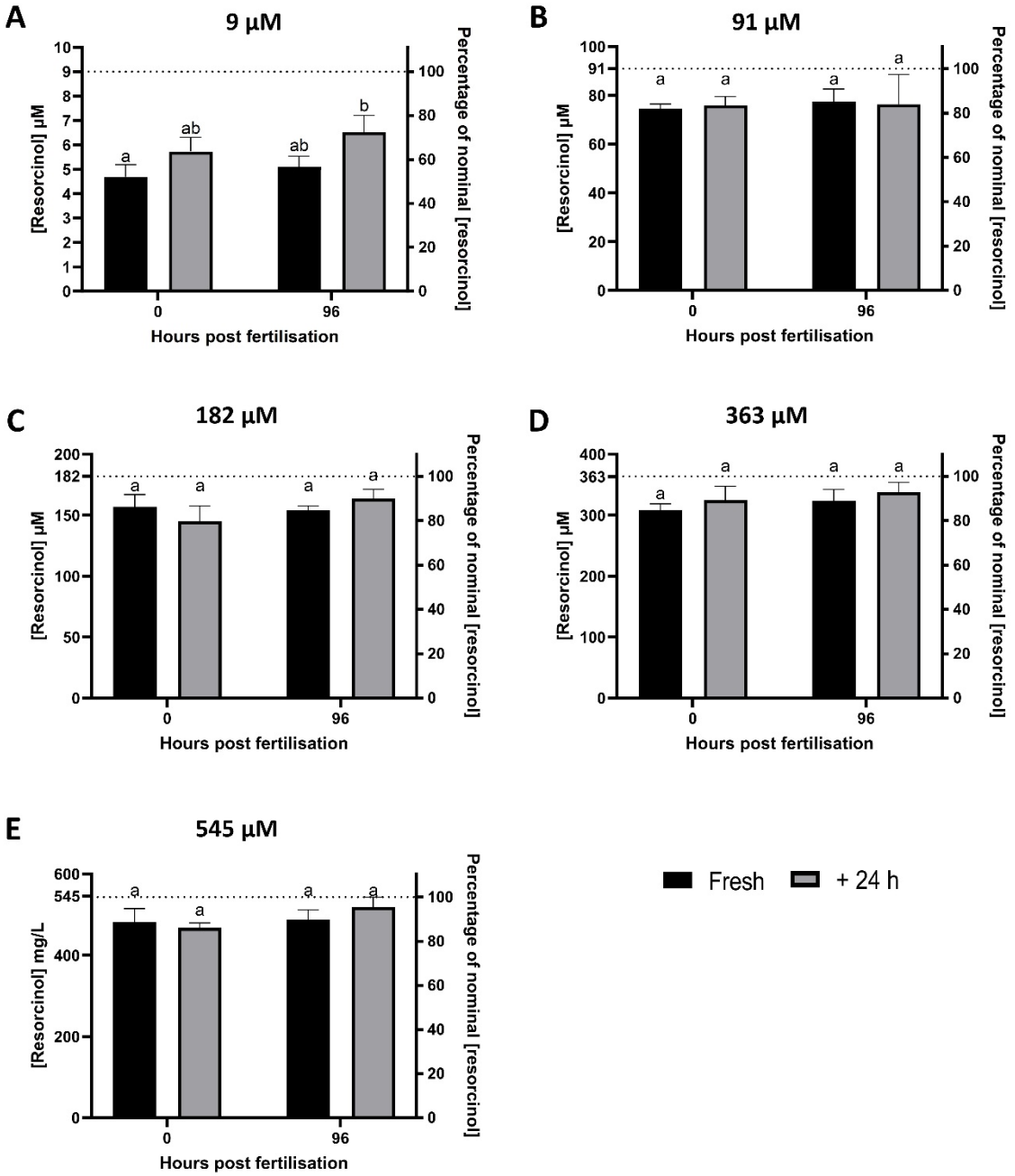

Figure S5: Resorcinol measurements in the medium for the first exposure for thyroid hormone measurements. Average resorcinol concentrations are represented as a function of time. Right y-axes represent the percentage of the nominal concentration, the dotted line corresponds to 100% of the nominal concentration: 9 µM (A), 91 µM (B), 182 µM (C), 363 µM (D) and 545 µM (E). Black bars show fresh medium, grey bars represent the medium after 24h just before renewal. Error bars show standard deviation. Statistical differences are indicated by different letters.

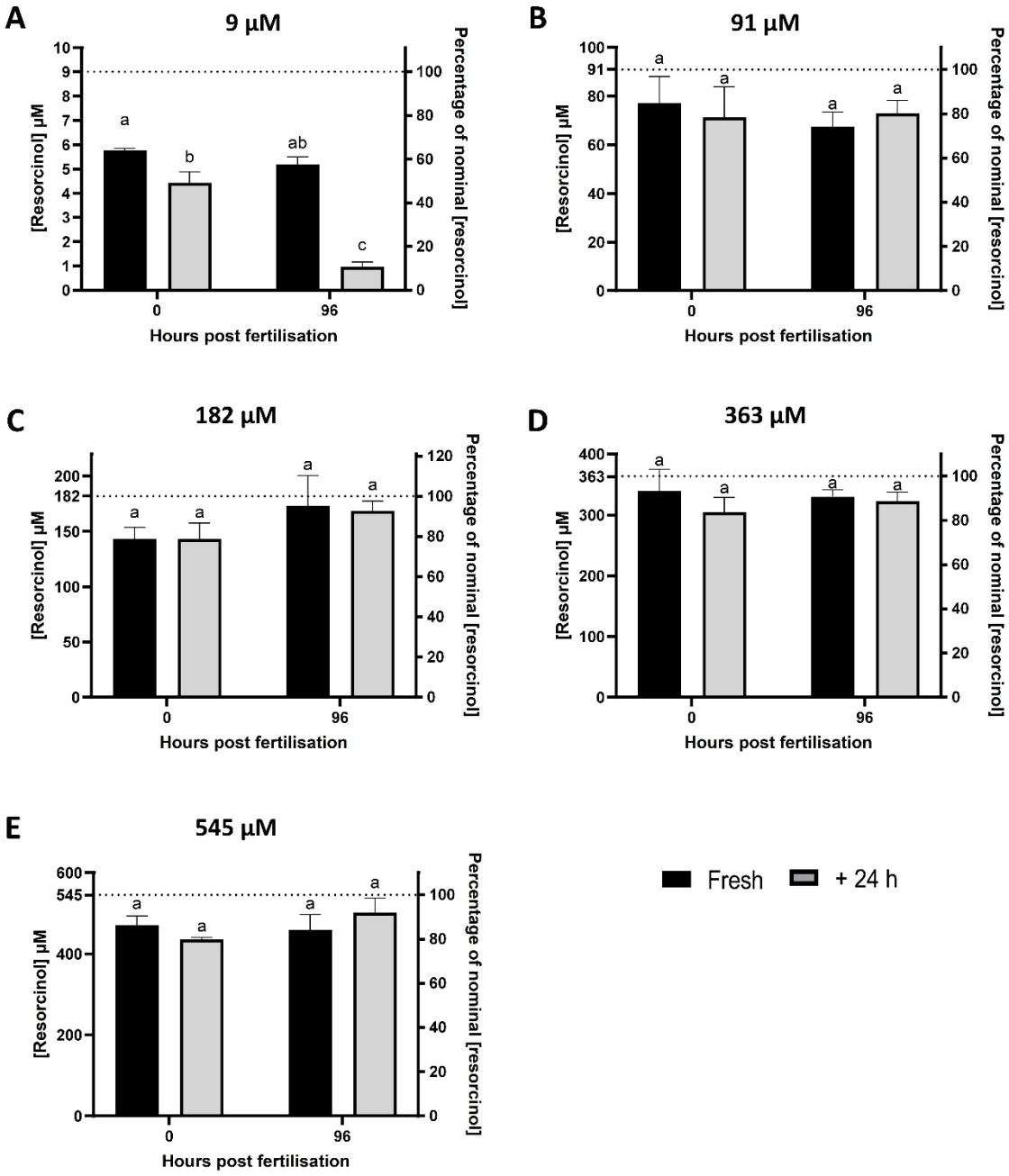

Figure S6: Resorcinol measurements in the medium for the second exposure for thyroid hormone measurements. Average resorcinol concentrations are represented as a function of time. Right y-axes represent the percentage of the nominal concentration; the dotted line corresponds with 100% of the nominal concentration: 9 µM (A), 91 µM (B), 182 µM (C), 363 µM (D) and 545 µM (E). Black bars show fresh medium, grey bars represent the medium after 24h just before renewal. Error bars show standard deviation. Statistical differences are indicated by different letters. In panel A, in the 9 µM treatment, unexpectedly low concentrations (around 0.9 µM) were detected in the samples of the old medium at 5 dpf (Figure S6A). The 9 µM samples from the second exposure were, therefore, excluded from analysis of the whole-body TH levels.

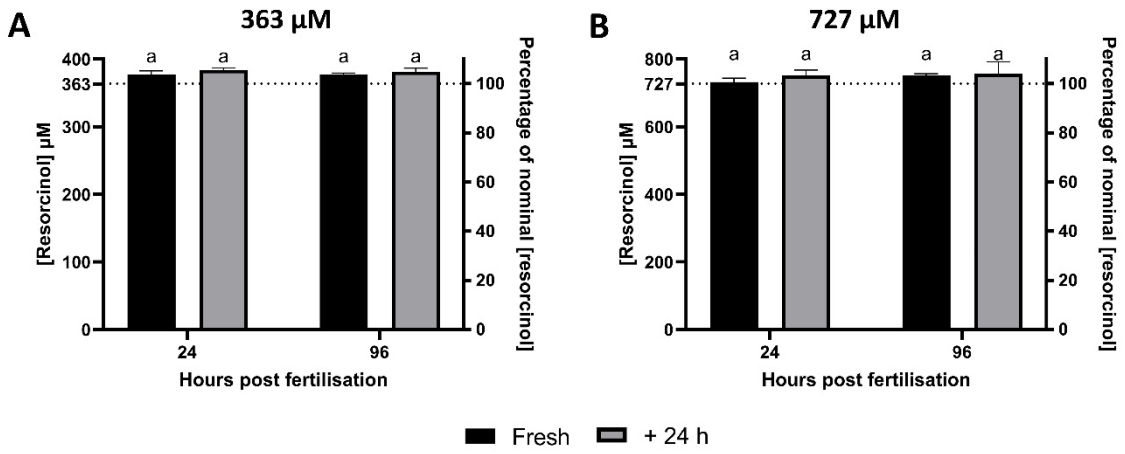

Figure S7: Resorcinol measurements in the medium of the exposure for qPCR analysis. Average resorcinol concentrations are represented as a function of time. Right y-axes represent the percentage of the nominal concentration, the dotted line corresponds to 100% of the nominal concentration: 363 µM (A) and 727 µM (B). Black bars show fresh medium, grey bars represent the medium after 24h just before renewal. Error bars show standard deviation. Statistical differences are indicated by different letters.

2.9 In vitro assays

2.9.1 Thyroperoxidase (TPO) inhibition

TPO inhibition was assessed using rat thyroid microsomes prepared according to Tater et al. (2021). The TPO activity was detected using two distinct assays, either by fluorometric assessment of peroxidase activity using Amplex Ultrared (AUR) as described previously in (Dong et al., 2020) or by the assessment of TPO-mediated tyrosine iodination (TYR-IOD) with the detection of its product 3-iodo-L-tyrosine (MIT). Its concentration was determined by high pressure liquid chromatography Agilent 1260 Infinity II HPLC in combination with mass spectrometry with inductively coupled plasma (Agilent 8900 ICP-MS/MS). Separation of analytes was conducted on reverse phase column Agilent Eclipse XDB-C18 (4.6 × 150 mm, 5 µm). Mobile phase for gradient elution contained 0.1% solution of trifluoroacetic acid in deionized water and 0.1% solution of trifluoroacetic acid in methanol. The detection of analytes was based on the ion with m/z 127 amu (iodine ion).

2.9.2 Sodium/iodide symporter (NIS) inhibition

NIS inhibition assay was based on HEK293T cells overexpressing functional NIS protein. The detection of NIS activity was based on the assessment of the uptaken iodide levels measured by spectrophotometric Sandell-Kolthoff reaction as described previously (Dong et al., 2019). Briefly, cells were routinely cultivated in DMEM supplemented with 10% FBS and 4 µg/mL puromycin (Sigma-Aldrich) at 37°C and 5% CO_2 level. For the experiment, cells were seeded to poly L-lysine (Sigma-Aldrich)- coated 96-well plates at a density of 15000 cells/well and left to grow for 48 h, after which the cells were exposed to dilution series of the model chemicals with potassium iodide for 2 h. After the exposure, cells were washed and lysed with ceric acid solution (25mM $(NH_4)_4Ce(SO_4)_4$, 0.5M H_2SO_4) and sodium arsenate solution (25mM $NaAsO_4$, 0.5M H_2SO_4, 0.2M NaCl). After 20 min the absorbance was recorded using a plate reader (Biotek Synergy Mx) at 415 nm.

2.9.3 Iodotyrosine deiodinase 1 (DIO1) inhibition

DIO1 inhibition was assessed by the detection of iodide cleaved from T3 substrate by Sandell-Kolthoff reaction as described previously (Renko et al., 2015; Weber et al., 2022) in HepG2 cell line homogenate. Briefly, the cells were grown in Ham's F12 with 10% FBS and sodium selenide (100 µM). Before the harvesting, the cells were rinsed with ice-cold PBS, homogenized and sonicated in lysis buffer consisting of 250 mM sucrose, 20 mM HEPES and 2 mM EDTA. Protein-containing lysate is then mixed in a ratio 1:1 (200 µL total) with substrate buffer (pH 6.8) containing 20 µM thyronine, 0.2 M KPO_4, 2 mM EDTA and 80 mM DTT as a cofactor of DIO reaction. The lysate and substrate were incubated for 3 hours at 37°C in a 96-well plate, the plate was subsequently cooled down to 4°C to terminate the DIO reaction and centrifuged. Released iodine was then separated from the protein/substrate by filtration through DOWEX W50-X2 resin with 10% acetic acid co-elution. The released iodide levels were determined as described for NIS inhibition.

2.9.4 Transthyretin (TTR)-binding inhibition

TTR-binding inhibition was assessed using a method based on the detection of release of fluorometrically-labeled T4 from TTR that was originally developed by Ren and Guo (2012) and then optimized and modified into a 96-well plate method by Hamers et al. (2020) and Ouyang et al. (2017). Briefly, the fluorescent conjugate of T4 with fluorescein 5-isothiocyanate (fT4) releases high fluorescence when it is bound to TTR, which is decreased by competing ligands.. The assay was performed in low-binding 96-well plate (Corning). A dilution series of model chemical was exposed to fT4 with and without TTR for 2 h and the difference of fluorescence was recorded (Biotek Synergy Mx).

2.9.5 Aryl hydrocarbon receptor (AhR) and thyroid hormone receptor (TR) mediated activity

AhR- and TR-mediated activity was assessed using human reporter gene cell models AZ-AhR (Novotna et al., 2011) and PZ-TR (Illés et al., 2015), respectively. The procedure is described in detail in Nováková et al. (2020). Briefly, the cells were grown in DMEM +10% FBS. Cells were exposed to the dilution series of the chemical and reference compounds, 2,3,7,8-tetrachlordibenzo-*p*-dioxin and T3 for AZ-AhR and PZ-TR, respectively, for 24 h. After that, cells were lysed with lysis buffer (Promega) and luciferase activity was detected on a plate reader (Biotek Synergy Mx) using substrate buffer described previously (Pavlíková et al., 2012).

3. Supplement to results and discussion

3.1 Thyroid hormone measurements at 5 dpf

Table S11: Whole-body T4 concentration (ng/g) following resorcinol exposure (n=4, except for 9 µM where n=2) at 5 days post fertilization (dpf). Values that differ significantly from the controls are indicated with an asterisks.

Nominal concentration		Whole-body T4 levels (ng/g)
µM	mg/L	
0	0	3.501 ± 0.562
9	1	0.928 ± 0.945*
91	10	0.053 ± 0.038*
182	20	0.172 ± 0.198*
363	40	0.035 ± 0.002*
545	60	0.036 ± 0.001*

3.2 Effects on larval length and swim bladder surface at 5 and 7 dpf

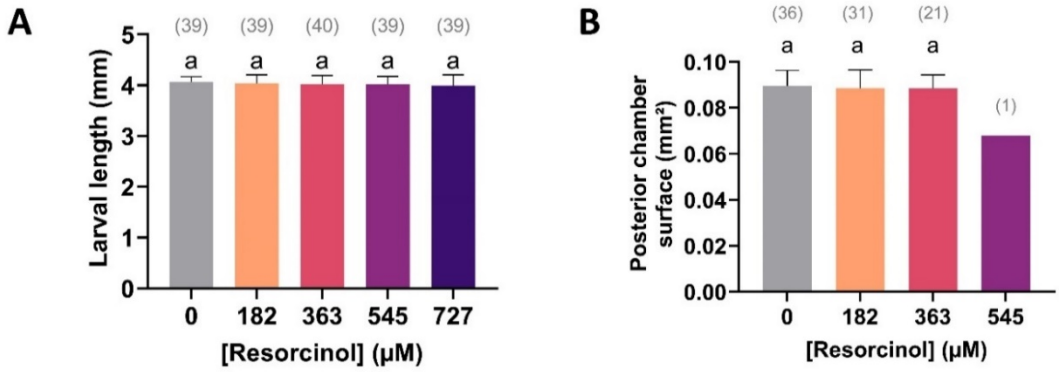

Figure S8: Effect of resorcinol exposure on larval length and swim bladder surface at 5 dpf (Experiment 5). Larval length (A) and swim bladder surface area (B) following resorcinol exposure. Error bars show standard deviation. Sample sizes are given in parentheses and significant differences are indicated with different letters. Swim bladder surface area was only determined for larvae with an inflated swim bladder.

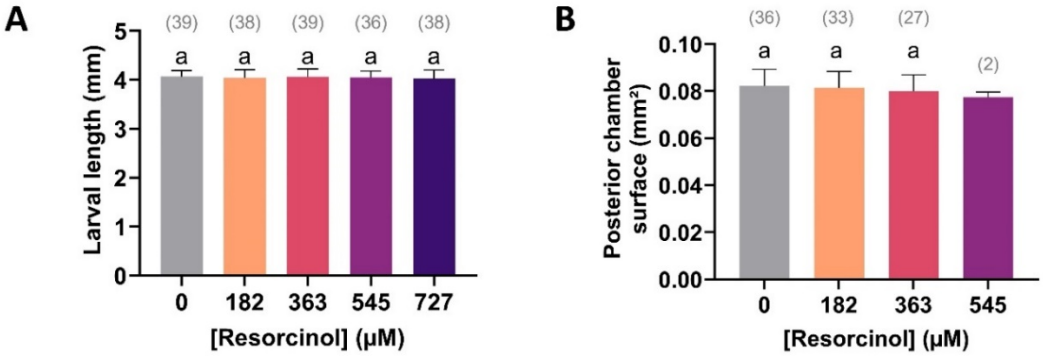

Figure S9: Effect of resorcinol exposure on larval length and swim bladder surface at 7 dpf (Experiment 5). Larval length (A) and swim bladder surface area (B) following resorcinol exposure. Error bars show standard deviation. Sample sizes are given in parentheses and significant differences are indicated with different letters. Swim bladder surface area was only determined for larvae with an inflated swim bladder.

3.3 Swimming behavior at 7 dpf

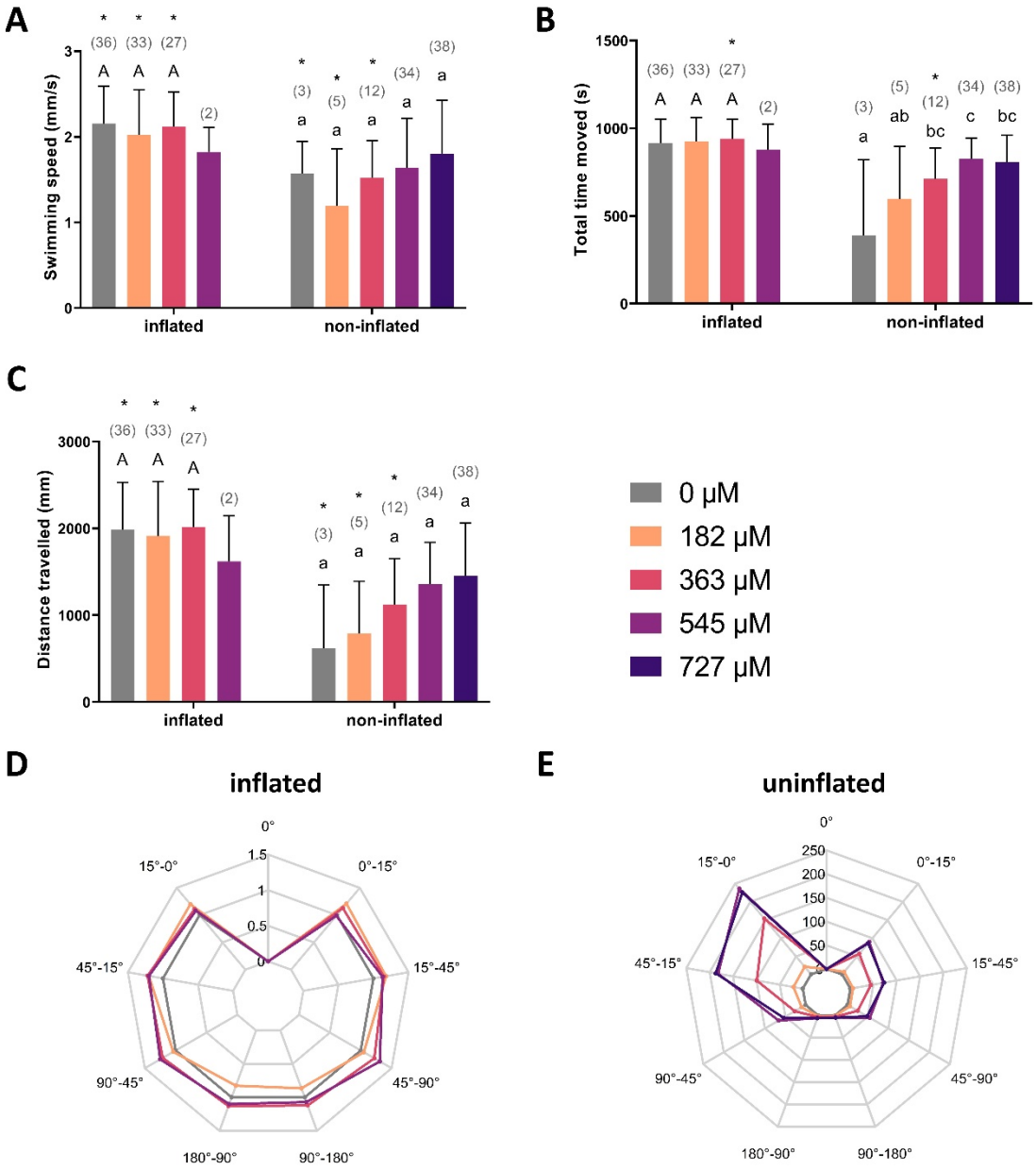

Figure S10: Effect of resorcinol exposure on swimming activity and turning angles at 7 dpf. Effects of resorcinol on swimming speed (A), total time moved (B) and the total distance travelled (C). Error bars show standard deviation and sample sizes are given in parentheses. Different letters indicate significant differences. Capital and lowercase letters indicate the significant differences within inflated or uninflated swim bladders respectively. Significant differences within a concentration (inflated vs non-inflated) are indicated with asterisks. Effect on turning angles in larvae with inflated (D) and uninflated swim bladders (E) illustrated using radar plots. Each corner of the radar plot represents a range of different turning angles. The y-axis is represented by the concentric circles showing the average turning angle normalized to both the sum of swimming distance and the controls.

3.4 Eye morphology at 5 dpf

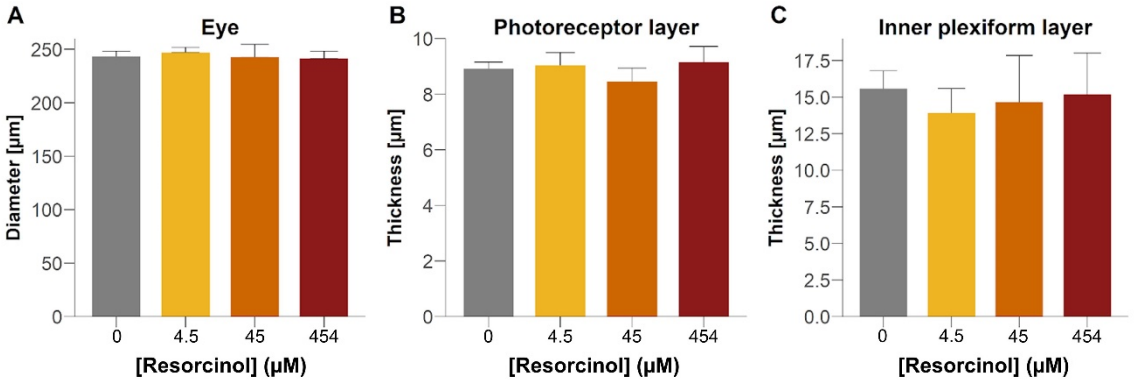

Figure S11: Eye morphology at 120 hpf following resorcinol exposure. Eye diameter (A), photoreceptor layer thickness (B), and thickness of the inner plexiform layer (C) following resorcinol exposure. Data is presented as the average + standard deviation. n = 4 replicates with 8-12 individual larvae. There were no statistically significant differences between the treatments.

3.5 In vitro assays

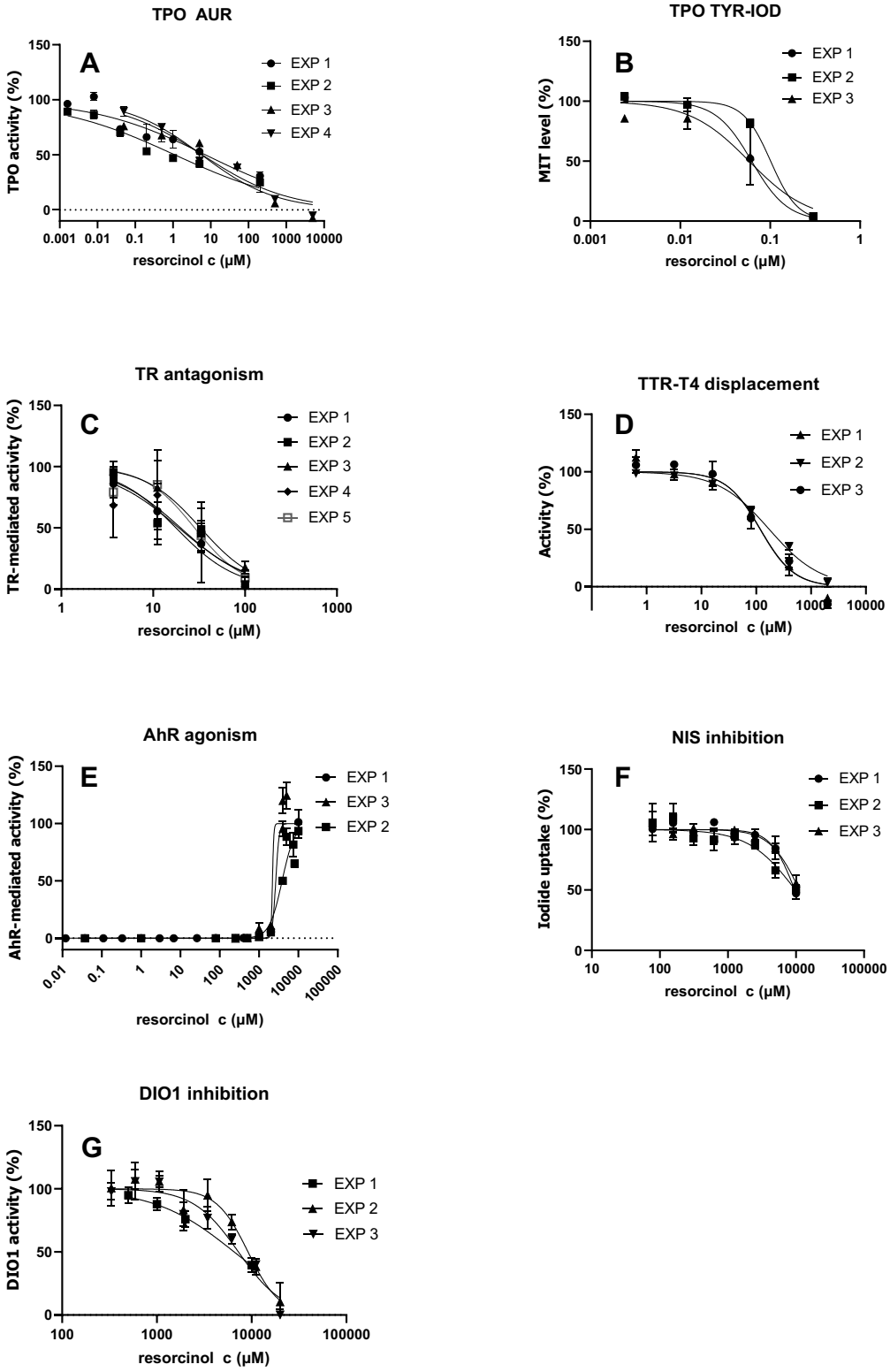

Figure S12: Dose-response relationship of resorcinol in the in vitro battery of bioassays. Effect on the peroxidation step in thyroperoxidase activity detected by Amplex Ultrared assay (A); inhibition of TPO-mediated tyrosine iodination detected by ICP-MS (B); Antagonistic effect on Thyroid hormone receptor (C); Interaction with transthyretin (D); Aryl hydrocarbon receptor-mediated activity (E); Inhibition of iodide uptake by Na-I symporter (F); Inhibition of deiodinase 1 (G).

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