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BIOCONCENTRATION, METABOLISM AND EXCRETION OF  
TRICLOCARBAN IN LARVAL QURT MEDAKA (*ORYZIAS*  
*LATIPES*)

**SUPPLEMENTARY MATERIAL**

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## Experimental

### Online-SPE-LC-MS/MS analysis of TCC and its metabolites

Online SPE-LC analysis was performed with in back-flush mode on an Agilent 1200 LC system (Agilent, Palo Alto, CA). In detail, the system was comprised of two G1379B degasers, two G1312B gradient HPLC pumps and a high-pressure two-position six port valve attached in a G1316B column oven set to 40°C. Samples were kept at 4°C in a LEAP HTC-PAL auto sampler (Leap Technologies, Carrboro, NC) equipped with a 100 µL sample loop and a 100 µL syringe. The samples (100 µL) were injected into a flow of 1500 µL/min 0.1% acetic acid (AA) in water delivered by pump 1 (Fig. S3). For tissue samples, 5 µl were injected in a 20 µl injection loop. The injected solution was mixed with the solvent by a 50 X 4.6 mm column filled with 5 µm stainless steel particles (Agilent solvent mixer, G1312-87330). Subsequently, the analytes were extracted from the solvent stream by a Cyclone RP-18 column (Thermo Fisher Scientific, Waltham, MA) with the dimensions of 50 X 0.5 mm, a particle size of 50 µm and a pore size of 10 nm (Figure S3A). After 0.5 min, the 6 port valve was switched, and the analytes were back flushed by the flow of 300 µL/min delivered by pump 2 (Fig. S3) into a Kinetex solid-core (RP) column (Phenomenex, Torrance, CA) with the dimensions of 2.1 X 50 mm, a particle size of 1.7 µm and a pore size of 10 nm. The analytes were separated by a binary gradient of 25 mM ammonium acetate containing 0.1 % acetic acid (HAc) as solvent A and 95/5 ACN/water (v/v). The gradient, the flow rates and the six port switching times are displayed in Figure S3 and in detail in Table S1.

Mass spectrometric detection was carried out on an ABI 4000 TRAP tandem mass spectrometer equipped with a pneumatically assisted "turbo V" electrospray ionisation (ESI)-source (Applied Biosystems, Foster City, CA). The instrument was operated in negative ion mode with an ion-spray voltage of -2500V, using 25 psi curtain gas, 40 psi nebulizer gas and 70 psi drying gas at a temperature of 450°C. The <sup>35</sup>Cl isotopes of the analytes were detected in unit resolution in the scheduled selected reaction monitoring mode (SRM) with a detection window of 30 s and a circle time of 0.5 sec. The transitions and the specific electronic parameters are listed in table S2. The collision-activated dissociation gas was set to "medium" and the entrance potential was 10 V for all analytes. All source parameters were optimized for TCC under LC conditions and the electronic parameters were optimized for each analyte by direct infusion. Analyst Software (version 1.5.1, Applied Biosystems) was used for controlling the online-SPE-LC-ESI-MS/MS system, data acquisition, integration and quantification. The analyte concentrations of the samples were calculated directly by comparison of the analyte peak area detected with that of the I.S. For calibration, the analyte to I.S. ratios were fitted linear reciprocally weighted by concentration. Two separate calibration curves were prepared for 100 µL injections (0.3 pM-100 nM) and for 5 µL injections (0.3 nM – 3 µM).

To enable semi-quantitative detection of further glucuronic acid conjugates of which no authentic reference compounds were available, biological samples were used to optimize detection conditions.

Urine of an exposed human diluted 1:1 (v:v) with ACN was used as source of *N*-Gluc-TCC and *N*-Gluc-TCC utilizing optimized ESI-MS/MS conditions as previously described <sup>1</sup>. The glucuronides of 3'-OH-TCC and 6-OH-TCC were generated by microsomal incubation with activated UDP- glucuronic acid as co-substrate as described elsewhere <sup>2</sup> and detected with similar ESI-MS/MS conditions as 2'-Gluc-O-TCC (Table 2). Analysis for further metabolites was carried out under LC conditions operating the mass spectrometer with the same source conditions as for SRM detection and a declustering potential of -70 V: (i) In full scan mode (150-650 amu with a cycle time of .3 sec), (ii) Selected ion monitoring (SIM) on *m/z* 295; 297, 329; 331; 333; 345, 347; 409; 411 and 413 or (iii) in enhanced product ion mode (EPI) on the same ions respectively generating high quality fragment spectra (spread collision energy (CE) -5--35, scan range 100-300 amu) by operating the third quadruple as linear ion trap.

## Results

### Online-SPE-LC-MS/MS analysis of TCC and its metabolites

In order to quickly analyze TCC and its metabolites in water and fish tissue samples, we utilized a recently described online SPE-LC-ESI-MS/MS method. This approach provides excellent accuracy, precision and robustness for the direct analysis of biological samples <sup>1</sup>. In order to detect low concentrations of TCC and metabolites in water samples, the sensitivity of this method was improved by (i) a larger injection volume, (ii) an increased separation efficiency and (iii) an improved MS detection in scheduled SRM mode. Enhanced chromatographic resolution was gained by the introduction of a mixer prior to the online-SPE column (Fig. S3). The mixing of the organic solvent containing sample with the polar eluent causes an efficient trapping of the analytes in the first segment of the SPE column and thus less tailing in elution in back-flush mode. Further increase of the separation efficacy could be achieved by utilization of a solid-core particle column and improved gradient (Fig. S3). Compared to the previously described method we included the simultaneous detection of further metabolites: The glucuronic acid conjugate of 2'-OH-TCC (2'-O-Gluc-TCC), which elutes first at 1.27±0.01 min, the hydroxylated TCC metabolite 6-OH-TCC eluting at 4.11±0.01 min and the recently described oxidative metabolite 3',4'-dichloro-4'-hydroxy-carbanilide (DHC) <sup>3</sup> with a retention time of 2.24±0.01 min. Except for 2'-OH-TCC and 6-OH-TCC, which were not separated on the RP column used all compounds show baseline separation (Fig. 2). The co-elution does not interfere the quantification, because the MS/MS detection is carried out on specific isocyanate ions of the fragmentation of the urea moiety, as previously described in detail <sup>1,3</sup>. All analytes eluted in narrow Gaussian shaped peaks with full width at half maximum height of 4 sec or less (Fig. 2, Table S3). Due to this chromatographic resolution an excellent signal to noise ratio (s/n) was observed for low concentration of the analytes with a detection limit (LOD, s/n = 3) as low as 0.3 pM (30 amol on column) in 100 µL injections for most of the analytes (Table S3). With a dynamic range of 3-5 orders of magnitude (Table S3) and accuracy of 100±20% over the whole calibration range,

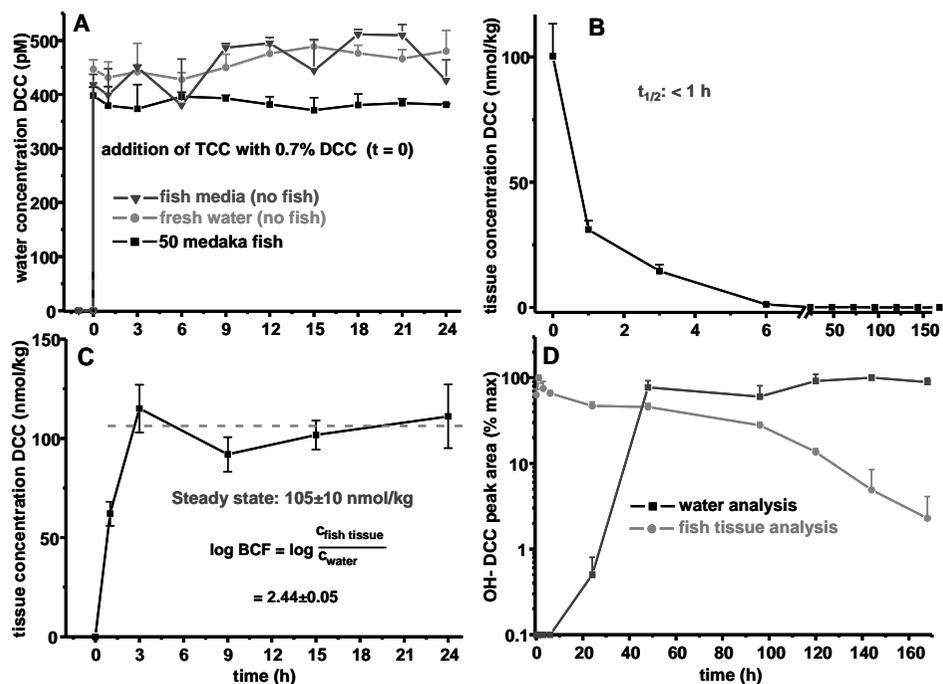
this method is ideally suited for the investigation of low concentrated samples. For highly concentrated samples such as tissue from exposed fish the method was adapted by using a lower injection volume of 5-20 µL.

In order to allow the evaluation of the formation of further glucuronides known from the mammal metabolism of TCC <sup>4</sup> biological samples were analysed. Human urine was used as source of *N*-Gluc-TCC and *N*-Gluc-TCC <sup>1</sup>. The 3'-O-Gluc-TCC and 6-O-Gluc-TCC were generated by microsomal incubations of 3'-OH-TCC and 6-OH-TCC. As shown in Figure 2B, the *N*-glucuronides elute at around 1.08 minutes slightly after the void volume of the analytical column. 3'-O-Gluc-TCC formed the same characteristic fragment ion as 2'-O-Gluc-TCC of the chlorophenyl isocyanat ion at *m/z* 168 and eluted at 1.12 min (Figure 2C). 6-O-Gluc-TCC gave rise to intense fragment ions at *m/z* 202 (dichlorophenyl-isocyanate ion) and thus could be independently detected from the co-eluting 2'-O-Gluc at a retention time of 1.27 min (Figure 2A, Figure 2D). Although these metabolites cannot be quantitatively measured because of the lack of authentic reference compounds all known glucuronic acid conjugates are covered by the method. Thus, their formation could be monitored in a semi-quantitativ fashion based on the peak areas.

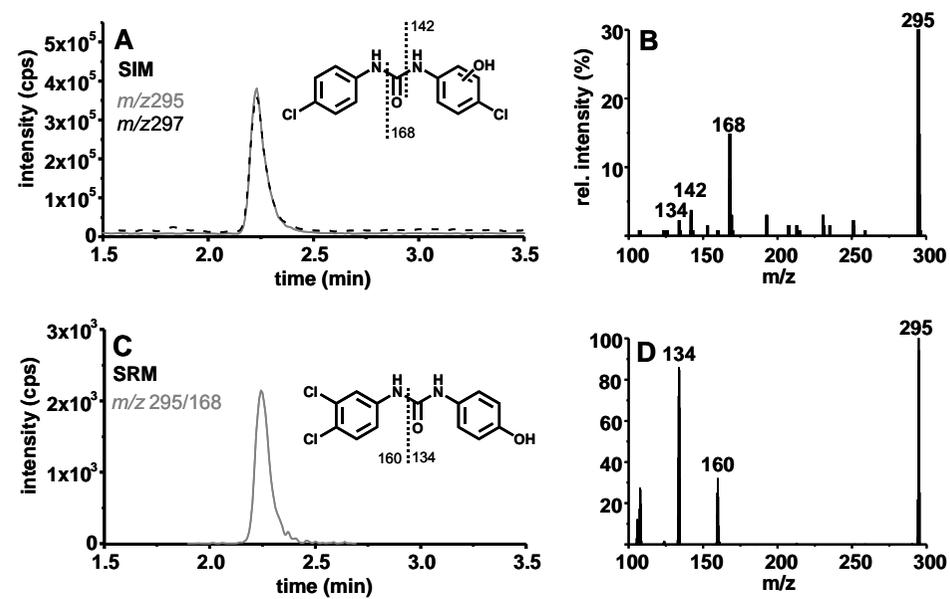
## References

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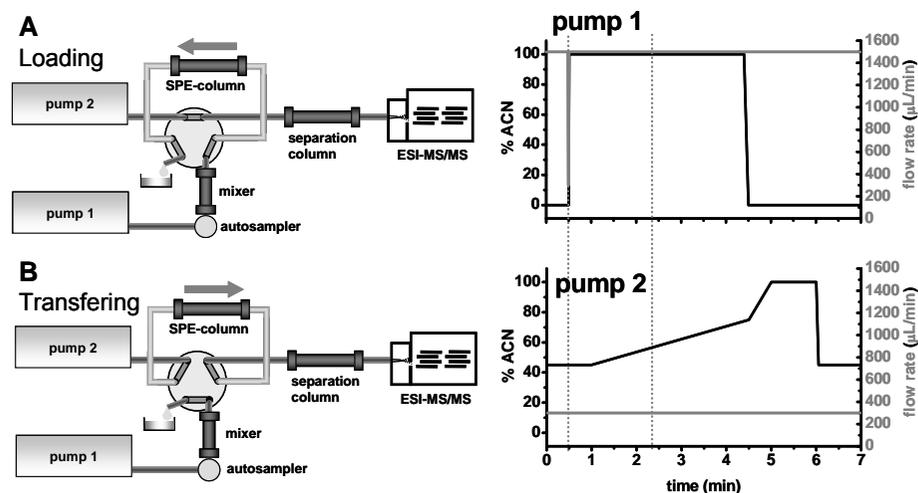
## Supplementary Figures



**Figure S 1** Accumulation, metabolism and elimination of DCC by medaka fish. DCC was present as impurity in the TCC used for medaka fish exposure (0.7%). A: Time course of DCC water concentration (300 mL) with 50 medaka fish and used media and fresh water as control. C: DCC concentration in fish tissue during 24 h incubation with 20 ppb TCC (0.7% DCC) B: DCC concentration in fish tissue after transferring the fish in fresh water. D: Half quantitative time course of the peak areas of the tentatively identified DCC metabolite OH-DCC (Supplementary Data, Fig. S2) in water and fish tissue after transferring the fish in fresh water. Note the peak areas are normalized as percentage of the highest area in each analysis and do not reflect absolute concentrations. All concentrations/peak areas are shown as mean and of three independent incubations. No metabolites were detected in water and fish samples from control experiment. The DCC concentration in the water after transferring exposed fish in fresh water was below the limit of detection.



**Figure S 2** LC-MS characterization of new metabolite. A LC-ESI(-)-MS extracted ion chromatogram (XIC) of at  $m/z$  295 and 297. B MS/MS spectrum of peak at  $m/z$  295. The MS/MS spectrum of the peak at  $m/z$  297 gave rise to ions at  $m/z$  142/144 and  $m/z$  168/170 in the ratio 1:1, indicating the presence of two chlorine atoms in the molecule. For comparison the SRM chromatogram (0.1 nM) and the MS/MS spectrum of DHC is shown in panel C-D. Insert. The suggested structure with sites of fragmentation leading to the most abundant fragments is shown as insert in panel A and the structure and site of fragmentation of DHC is shown in panel C.



**Figure S 3** Scheme of the online-SPE-LC-MS/MS set up. The sample (100 µL) is transferred onto the SPE column by pump 1 through a mixing column (A). After this loading step, the six port valve is switched (0.5 min) so that the analytes are eluted from the SPE column towards the separation column by pump 2 (B). The valve is switched back immediately after (2.4 min). The analytes elute, while the SPE column is cleaned and regenerated. In the diagrams the applied gradients (black line) and flow rates (gray line) of the LC-pumps are shown. The switching points of the six port valve are indicated by the dashed lines.

## Supplementary Tables

**Table S 1** Gradients and flow rates of gradient pump 1 and pump 2 and time points the valve is switched.

time (min)	Pump 1 flow rate (µL/min)	% B	
0.00	1500	0	
0.48	1500	0	
0.49	300	0	
0.50	300	0	
0.51	1500	100	
2.75	1500	100	
4.40	1500	0	
4.50	1500	0	
7.00	1500	0	
time (min)	Pump 2 flow rate (µL/min)	% B	
0.00	500	45	
1.00	500	45	
4.50	500	75	
5.00	500	100	
6.00	500	100	
6.05	500	45	
7.00	500	45	
time (min)	Six Port Valve position	SPE column mode	separation column mode
0.0-0.50	A	loading	equilibrating
0.5-2.4	B	transferring	transferring
2.4-7.0	A	cleaning/ equilibrating	eluting/ cleaning

**Table S 2** Mass spectrometric parameters for the ESI(-)-MS/MS detection of the analytes and the I.S. The  $m/z$  values of the transitions used for quantification in SRM as well as the optimized potentials: Declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) are shown.

analyte	$m/z$ [M-H]	$m/z$ product ion	DP (V)	CE (V)	CXP (V)
N-Gluc-TCC	489	336	-70	-15	-9
N-Gluc-TCC	489	302	-70	-15	-9
3'-O-Gluc-TCC	505	168	-65	-38	-9
2'-O-Gluc-TCC	505	168	-65	-38	-5
6-OH-TCC	505	202	-65	-38	-5
2'-O-SO <sub>3</sub> -TCC	409	168	-60	-32	-11
OH-DCC	295	168	-65	-15	-9
DHC	295	134	-90	-16	-1
3'-OH-TCC	329	168	-75	-16	-9
DCC	279	126	-70	-20	-7
6-OH-TCC	329	202	-60	-16	-7
2'-OH-TCC	329	168	-65	-18	-9
TCC	313	160	-75	-20	-9
3'-Cl-TCC	347	160	-80	-22	-11
I.S.	319	160	-70	-20	-7

**Table S 3** Performance of the online solid phase extraction-LC-MS/MS method with 100 µL injection volume. The observed retention times, the peak width, the used dynamic range of for each analyte and the limit of detection (LOD) are presented.

analyte	retention time <sup>a</sup> (min)	peak width (sec) <sup>a</sup>		Dynamic range (nM)	LOD [pM]; [amol on column]
		full width	FWHM		
2'-O-Gluc-TCC	1.27±0.01	10.54	3.26	0.1 – 100	30 [3000]
2'-O-SO <sub>3</sub> -TCC	2.65±0.01	16.66	3.34	0.001 – 30	0.3 [30]
DHC	2.24±0.01	15.29	4.06	0.003-30	1 [100]
3'-OH-TCC	3.18±0.01	17.42	3.89	0.003 – 100	1 [100]
DCC	3.69±0.01	20.29	4.01	0.001 – 30 <sup>c</sup>	0.3 [30]
2'-OH-TCC	4.08±0.01	20.17	4.01	0.001 – 30 <sup>c</sup>	0.3 [30]
6-OH-TCC	4.11±0.01	18.83	4.11	0.001 – 100	0.3 [30]
TCC	4.40±0.01	20.59	4.16	0.01 – 100 <sup>c</sup>	3 [300] <sup>c</sup>
3'-Cl-TCC	5.07±0.01	16.50	4.05	0.1 – 100 <sup>d</sup>	1 [100]

<sup>a</sup> Mean and SD determined of 20 injections of standard solutions (0.01-10 nM of analyte)  
<sup>b</sup> Both 2'-OH-TCC and 6-OH-TCC present in calibration mixture. Limited dynamic range probably caused by their co-elution.  
<sup>c</sup> TCC peak ubiquitous in blank injections, increasing LOD  
<sup>d</sup> Low amounts present in standards (impurity of internal standard (I.S.), increased LOQ.

**Table S 4a** Water concentrations of TCC and its metabolites during 24 hours incubations with 20 ppb TCC of 50 medaka fish, and control experiments. Results are presented as mean and SD of three independent experiments. Half quantitative values below the limit of quantification of the method (s/n = 9, accuracy ± 20%, Table S3) are displayed in grey color.

50 medaka fish														
Time h	TCC nM water		DCC pM water		2'-OH-TCC pM water		3'-OH-TCC pM water		6OH-TCC pM water		2SO3-O-TCC pM water		2-O-Gluc-TCC pM water	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
-1	< 0.1		< 0.6		< 0.6		< 2		< 0.6		< 0.6		< 60	
0,08	54,1	2,4	398	15	< 0.6		< 2		< 0.6		< 0.6		< 60	
1	51,1	2,7	379	35	< 0.6		< 2		< 0.6		1,0	1,6050	< 60	
3	51,1	6,1	373	45	1,2	0,0	< 2		0,7	0,52	0,7	0,5671	< 60	
6	51,3	2,4	396	8	0,8	0,1	< 2		0,8	0,48	1,4	1,4795	< 60	
9	49,7	1,3	393	5	1,2	0,8	< 2		0,4	0,17	2,0	0,1	51,9	8,4
12	47,9	2,0	381	15	0,9	0,6	< 2		0,4	0,18	0,4	0,4	98,5	2,5
15	45,6	3,5	371	23	2,7	2,2	4,5	4,4	0,7	0,38	2,7	2,4	155,9	9,8
18	45,5	1,9	381	20	3,9	1,2	10,7	1,4	1,4	0,94	5,5	1,8	235,3	6,1
21	45,5	1,6	384	8	5,4	3,5	16,8	10,3	1,7	0,73	3,3	2,0	242,9	63,8
24	43,6	1,4	381	2	9,8	5,1	25,8	14,5	2,0	1,47	6,9	2,0	280,7	9,2

fresh water														
Time h	TCC nM water		DCC pM water		2'-OH-TCC pM water		3'-OH-TCC pM water		6OH-TCC pM water		2SO3-O-TCC pM water		2-O-Gluc-TCC pM water	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
-1	< 0.1		< 0.6		< 0.6		< 2		< 0.6		< 0.6		< 60	
0,08	60,1	3,2	447	17	< 0.6		< 2		< 0.6		< 0.6		< 60	
1	57,9	3,2	431	29	< 0.6		< 2		< 0.6		< 0.6		< 60	
3	63,7	2,7	442	53	< 0.6		< 2		< 0.6		< 0.6		< 60	
9	60,5	1,6	450	24	< 0.6		< 2		< 0.6		< 0.6		< 60	
12	63,0	2,3	475	15	< 0.6		< 2		< 0.6		< 0.6		< 60	
15	64,1	0,4	489	11	< 0.6		< 2		< 0.6		< 0.6		< 60	
18	62,4	3,0	476	15	< 0.6		< 2		< 0.6		< 0.6		< 60	
21	62,2	2,8	466	17	< 0.6		< 2		< 0.6		< 0.6		< 60	
24	62,8	5,4	480	38	< 0.6		< 2		< 0.6		< 0.6		< 60	

used media														
Time h	TCC nM water		DCC pM water		2'-OH-TCC pM water		3'-OH-TCC pM water		6OH-TCC pM water		2SO3-O-TCC pM water		2-O-Gluc-TCC pM water	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
-1	< 0.1		< 0.6		< 0.6		< 2		< 0.6		< 0.6		< 60	
0,08	57,1	3,5	418	19	< 0.6		< 2		< 0.6		< 0.6		< 60	
1	55,3	4,8	399	49	< 0.6		< 2		< 0.6		< 0.6		< 60	
3	60,7	1,2	450	4	< 0.6		< 2		< 0.6		< 0.6		< 60	
6	56,7	1,0	380	85	< 0.6		< 2		< 0.6		< 0.6		< 60	
9	64,7	0,9	487	8	< 0.6		< 2		< 0.6		< 0.6		< 60	
12	65,7	2,4	495	11	< 0.6		< 2		< 0.6		< 0.6		< 60	
15	66,3	3,3	444	58	< 0.6		< 2		< 0.6		< 0.6		< 60	
18	66,8	1,0	511	9	< 0.6		< 2		< 0.6		< 0.6		< 60	
21	66,4	0,5	509	20	< 0.6		< 2		< 0.6		< 0.6		< 60	
24	57,3	1,6	426	38	< 0.6		< 2		< 0.6		< 0.6		< 60	

**Table S 4b** Water concentrations of TCC and its metabolites after transferring 50 medaka fish (exposed 24 hours incubations with 20 ppb TCC) in fresh water. Results are presented as mean and SD of three independent experiments. Half quantitative values below the limit of quantification of the method (s/n = 9, accuracy ± 20%, Table S3) are displayed in grey color

Time h	TCC		DCC		2'OH-TCC		3'OH-TCC		6OH-TCC		2SO3-O-TCC		2-O-Gluco-TCC		DHC	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
0,08	14,6	17,0	<2	<2	<0,6	<2	<2	<2	<0,6	<0,6	<0,6	<0,6	<60	<60	4,1	2,5
1	21,9	6,2	<2	<2	<0,6	<2	<2	<2	<0,6	<0,6	1,8	2,1	<60	<60	6,3	3,1
3	95,5	1,5	<2	<2	<0,6	<2	<2	<2	<0,6	<0,6	4,2	2,2	115,2	19,4	6,5	11,4
6	82,7	0,6	<2	<2	0,4	0,4	<2	<2	1,3	1,4	6,0	1,0	209,5	48,9	4,1	7,6
24	68,8	24,6	<2	<2	3,3	0,2	7,3	2,0	1,4	1,1	16,6	1,1	708,7	50,0	12,3	1,6
48	65,3	43,8	<2	<2	319,3	69,6	1081,3	256,0	25,7	3,2	20,7	3,6	367,2	192,2	601,3	148,9
96	61,4	99,8	<2	<2	89,3	38,8	652,0	179,0	8,0	2,8	69,1	7,0	5,6	2,3	438,7	110,0
120	90,8	20,0	<2	<2	101,4	48,9	734,0	165,7	8,6	5,0	374,3	202,6	17,7	17,0	527,3	49,7
144	42,6	25,0	<2	<2	86,6	40,7	841,6	178,6	8,9	4,5	597,9	344,9	15,7	11,6	582,0	46,9
168	30,7	16,3	<2	<2	79,2	27,3	675,3	152,0	8,0	3,6	1060,7	209,4	27,5	3,6	537,3	23,4

**Table S 4c** Fish tissue concentration of TCC and its metabolites during 24 hours incubations with 20 ppb TCC. Results are presented as mean and SD of the analysis of homogenates of 50 fish of three independent experiments.

Time h	number of living fish per beaker	weight fish per beaker [mg]	TCC		DCC		2'OH-TCC		3'OH-TCC		6OH-TCC		2SO3-O-TCC		2-O-Gluco-TCC		DHC	
			mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
1	49	20	18,38	1,53	62,04	6,08	30,19	5,23	74,37	8,72	7,90	1,08	67,27	4,21	0,22	0,02	not measured	not measured
3	50	0	34,81	3,56	115,01	12,02	142,0	21,90	195,7	28,66	45,80	8,11	262,0	33,59	1,00	0,14	not measured	not measured
9	50	0	34,63	2,86	91,93	8,74	211,4	17,86	186,3	10,70	72,12	7,01	534,6	45,33	2,40	0,14	not measured	not measured
15	50	0	34,97	2,53	101,72	7,27	563,8	102,3	444,1	57,56	189,2	33,59	804,3	85,46	4,65	0,09	not measured	not measured
24	50	0	34,77	5,00	111,12	16,00	965,8	23,78	624,0	11,77	332,4	11,79	914,2	31,89	4,82	0,56	417,85	69,25

Steady state (3-24 h): BCF 34,0 1,6 105,0 3,9

BCF (3-24 h)  
 3 681 2,83 308 2,50  
 9 636 2,80 244 2,37  
 15 767 2,88 274 2,44  
 24 764 2,88 292 2,47  
 Mean BCF 712 2,85 280 2,45  
 SD 64 0,04 27 0,06

DHC was included in the method after this analysis. Only the 0 hand 24-hours time point were repeated.

**Table S 4d** Fish tissue concentration of TCC and its metabolites after transferring 50 medaka fish (exposed 24 hours incubations with 20 ppb TCC) in fresh water. Results are presented as mean and SD of the analysis of homogenates of 50 fish of three independent experiments.

Time h	number of living fish per beaker	weight fish per beaker [mg]	TCC		DCC		2'OH-TCC		3'OH-TCC		6OH-TCC		2SO3-O-TCC		2-O-Gluco-TCC		DHC	
			mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
0,08	49	34	39508	4884	100,3	13,0	360,4	32,8	262,3	29,0	125,4	10,2	1200,2	96,0	1554,0	148,8	417,8	40,0
1	50	40	19221	1976	31,1	3,5	316,8	56,2	238,1	42,6	115,2	20,7	1036,0	167,4	1290,9	15,6	355,4	60,7
3	50	0	13879	2621	14,5	2,6	506,0	16,5	407,3	10,7	174,2	4,0	1331,5	100,5	1440,5	46,0	493,3	29,8
6	50	0	1698	12,9	1,2	0,2	349,9	58,4	311,8	69,2	136,1	23,3	1429,4	259,2	1429,4	287,2	40,0	40,0
24	50	0	119	52,4	0,1	0,0	304,1	16,7	252,5	13,9	119,0	7,0	852,2	64,9	520,2	76,9	261,4	23,5
48	50	0	43,6	1,9	0,1	0,1	227,4	31,0	199,4	35,2	85,6	9,9	632,0	33,2	180,8	50,9	194,8	18,9
72	50	0	35,2	3,6	0,1	0,1	232,6	9,4	145,6	7,1	84,9	5,6	504,5	23,7	306,8	76,5	206,7	22,9
96	50	0	42,3	19,7	0,0	0,0	149,4	1,7	102,0	2,5	50,6	1,9	562,5	35,3	190,4	54,6	156,8	19,0
120	50	0	32,9	11,9	0,0	0,1	82,6	14,1	64,4	9,4	27,8	6,6	426,5	71,1	68,5	14,9	66,6	19,5
144	21	5	14	5	0,0	0,0	31,7	24,9	17,7	11,6	8,6	6,3	90,6	24,2	40,1	12,4	30,1	15,0
168	25	16	23,8	8,5	0,0	0,0	14,4	10,1	15,3	11,5	4,1	3,2	72,2	18,4	67,3	60,4	21,8	13,1

BCF calculation:  
 h BCF Log Mean SD  
 48 668 2,82  
 96 688 2,84  
 120 363 2,56  
 144 642 2,81  
 168 776 2,89 2,78 0,13