Determining the toxicity of organic compounds to the nematode Caenorhabditis elegans based on aqueous concentrations

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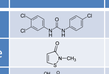
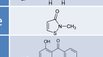
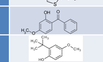
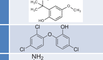
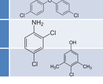
Background/Aims

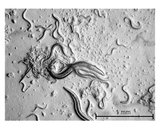
- *Caenorhabditis elegans* is used for assessing the toxicity of chemicals in aqueous medium. However, chemicals can absorb to the bacterial food, which reduces the freely dissolved concentrations of the tested compounds. Thus, based on total or nominal concentrations it is difficult to (1) compare the toxicity to other test organisms and (2) to compare effect mechanisms of chemicals with different properties.
- For seven compounds with different hydrophobicity, EC50 values for *C. elegans*' reproduction were determined based on aqueous concentrations and compared to EC50s based on modelled C_{free}.
- Toxicity thresholds based on chemical concentrations (EC) and activity (Ea) were used to evaluate the mode of action and to compare to the sensitivity of other relevant aquatic test organisms (*Daphnia magna*) .

Materials and Methods

Tab. 1: Properties of studied chemical compounds

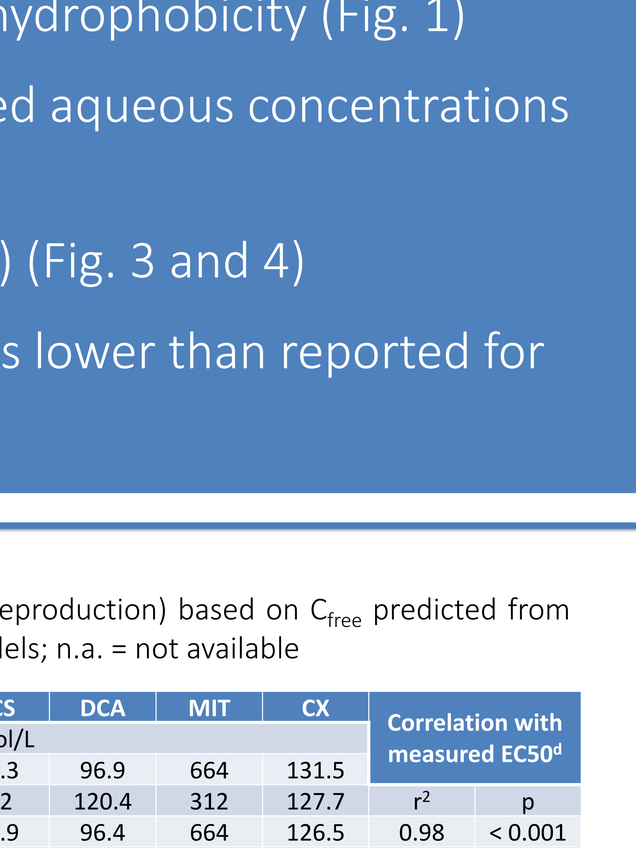
Compound	Structure		Predicted MoA	log K_{OW}	WS µmol/L	EC50 D. magna (µmol/L)
Trichlorocarbanilide		TCC	Non-Polar Narcosis	4.9	2.0	0.016
Methylisothiazolinone		MIT		-0.83	4.3×10^6	8.1
Benzophenone-3		BP-3	Polar Narcosis	3.52	26	8.2
Butylhydroxyanisole		BHA		3.5	3,384	18
Triclosan		TCS		4.76	22.5	0.66
3,4-Dichloroaniline		DCA		2.37	3,580	1.4
Chloroxylenol		CX		3.25	1,916	12.8

Toxicity Test



ISO 10872 with *Caenorhabditis elegans*
- 96h
- Water + Food bacteria
- Reproduction

Fig. 1: Study design



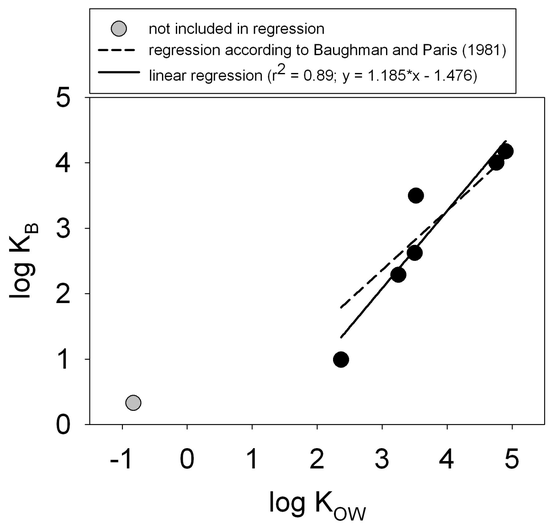
C_{free} = freely dissolved concentration
$C_{bacteria}$ = concentration of bacterial-bound fraction
K_B = Bacterial binding constant

Conclusions

- Constants for binding of organic chemicals to bacteria (K_B) correlated well with hydrophobicity (Fig. 1)
- Modeled freely dissolved concentrations (C_{free}) corresponded well with measured aqueous concentrations (Tab. 2)
- Most chemicals affected *C. elegans* by baseline toxicity (Exception: TCC and MIT) (Fig. 3 and 4)
- For most chemicals, toxicity (based on chemical activity; Ea50) for *C. elegans* was lower than reported for *Daphnia magna* (Fig. 4)

Results & Conclusions

Fig. 2: Measured log K_B (partitioning coefficient bacteria/water: $C_{bacteria}/C_{aq}$) plotted against log K_{OW} of the seven tested organic chemicals.



Tab. 2: EC50-values (µmol/l; C. elegans; reproduction) based on C_{free} predicted from nominal concentrations using various models; n.a. = not available

	TCC	BP-3	BHA	TCS	DCA	MIT	CX	Correlation with measured EC50[d]	
	µmol/L							r^2	p
Nominal	0.414	n.a.	566	29.3	96.9	664	131.5		
Measured	0.131	22.5	414	5.2	120.4	312	127.7		
Model 1	0.151	n.a.	529	12.9	96.4	664	126.5	0.98	< 0.001
Model 2	0.113	n.a.	495	9.8	95.6	664	121.2	0.98	< 0.001
Model 3	0.092	n.a.	518	8.3	96.6	663	126.0	0.99	< 0.001
BT-model[e]	3.67	54.7	56.9	4.83	519	2.7×10^5	92.8	0.43	>0.05

Model 1: simplified Armitage-model: $C_{free} = M/(V_{medium}+K_{OW} \times V_{lipid\_bacteria})$; *Armetage et al. 2014 (ES&T, 48, 9770-9779)*

Model 2: $C_{free} = C_{nom}/(K_B \times m_{bacteria}+1)$; using partitioning coefficient bacteria-water (K_B) calculated from K_{OW} according to Baughman and Paris 1981 *(Crit Rev Microbiol, 8, 205-227):*

Model 3: $C_{free} = C_{nom}/(K_B \times m_{bacteria}+1)$; using own experimentally determined K_B (Fig. 1).

BT-model = baseline toxicity model (Saleem et al. 2023; ES&T, 57, 1692-1700)

Fig. 3: EC50 (mol/L; log) for effects of seven organic chemicals on the reproduction of *C. elegans* (96h) plotted against the chemicals' hydrophobicities (log K_{OW})

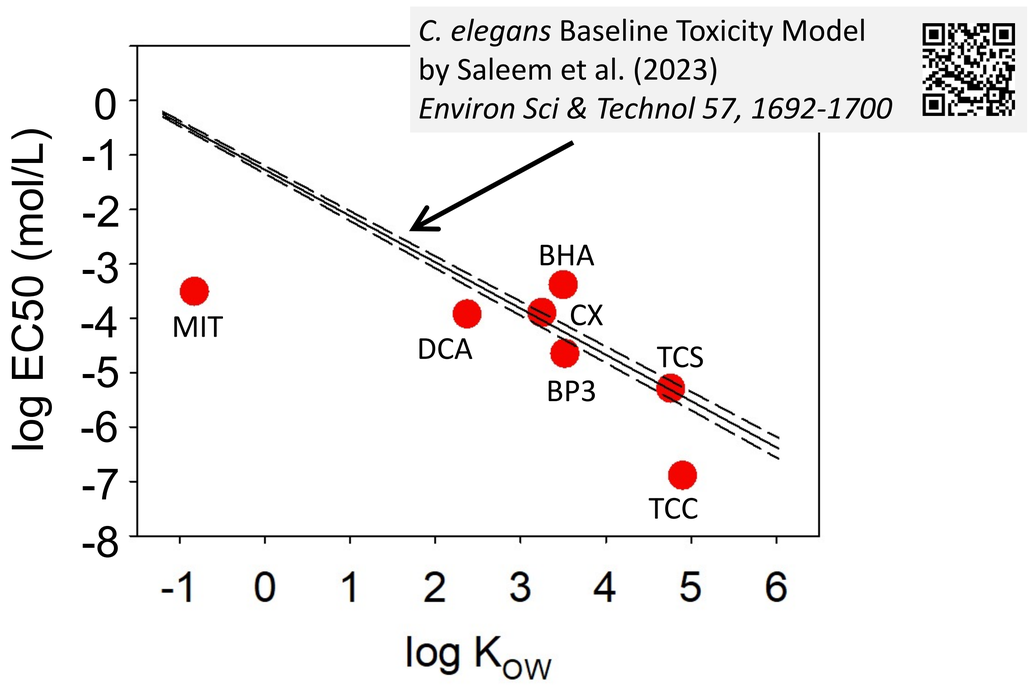


Fig. 4: Chemical activity based toxicity thresholds (Ea50) for *C. elegans* and *D. magna* (24h; immobilization) plotted against the (subcooled) liquid solubility (S_W) of the seven tested chemicals according to Mackay et al. 2014 (*SAR QSAR Environ Res,* 25, 343-355); baseline toxicity is expected to occur at chemical activities of 0.01 – 0.1.