A modified approach for the determination of bioconcentration factors (BCF) in fish

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Introduction

The method for the determination of bioconcentration factors (BCF) given in the OECD guideline 305 builds on the exposure of adult fish in dilute aqueous solution of the chemical at constant concentrations. This means that numerous samples of water and fish tissue have to be analyzed to calculate the BCF. Moreover, the test can take several weeks and is expensive. Miscellaneous methods to facilate the screening for BCF, e.g. with fish larvae [1] or with fertilized fish eggs and C14-labeled compounds [2] have been published.
Working groups of the Helmholtz Centre for Environmental Research (UFZ) and the Federal Environment Agency (UBA) tested a different approach to determine BCF at reduced costs and animal consumption. The Fish Embryo Toxicity Test (FET) according to DIN 38415-6 was modified in a way that the concentration of the chemical in the aqueous medium was kept in a range ≤ 1% of the acute toxicity. Compounds that could be analyzed both by gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) were selected for the experiments. Uptake of the chemical in fertilized fish eggs of the zebrafish (*Danio rerio*) as well as the decrease of the test substance in the aqueous medium was analyzed. BCF were calculated from the kinetics of the degradation in the aqueous solution and the internal concentration in the fish eggs.

Material and methods

Aqueous stock solutions were prepared by stirring defined amounts of the compounds in water of high purity grade for several days. From the stock solution aliquots were diluted with an artificial freshwater according to DIN EN ISO 7346-3 (medium) which had been aerated at 26 °C. Fertilized fish eggs were exposed to this solution as shown in Fig. 1. In a few cases, the experiment was extended to 72 hours.

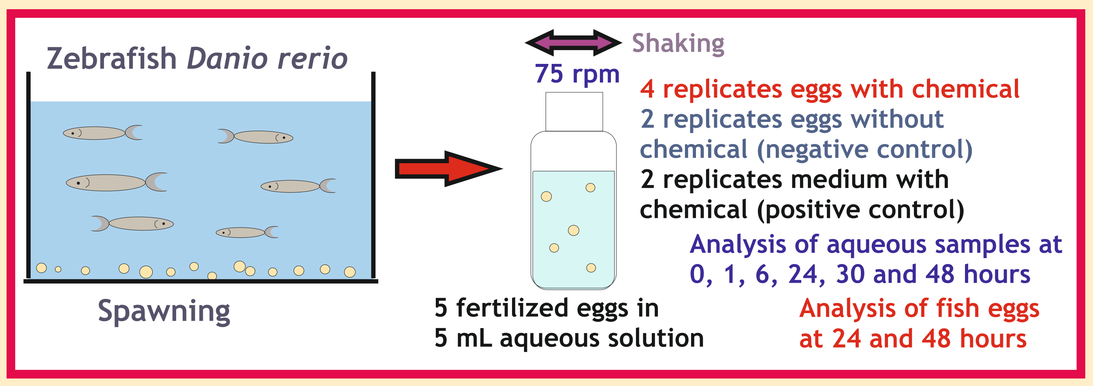


Figure 1: Exposure of the fish eggs, for each sampling a set of 8 bottles is prepared

Aqueous medium was decanted into a 10-mL-flask, the bottle rinsed with 1 mL of pure medium, this sample adjusted to pH 2 (10µL HCl [25%]), internal standard (see Table 1) added, the sample passed through a conditioned solid phase extraction (SPE) column (see Table 1), the column dried and eluted with organic solvent (see table 1), the solvent evaporated, residue dissolved in 100µL of ethyl acetate, analysis by GC-MS

Fish eggs adhering to the glass wall of the bottle were rinsed with 0.5 mL of ethanol, transferred into a 2-mL reaction vial, 0.5 mL of ethanol and internal standard (see Table 1) added, eggs smashed with a glass capillary, vial treated in a sonic bath 5 min, solvent evaporated, residue dissolved in 100µL of ethyl acetate, analysis by GC-MS

Table 1: Compounds tested for BCF, solid phase extraction (SPE) colums used, and internal standards used for analysis

Compound	CAS-No.	Internal Standard used, amount added	SPE column used	Elution solvent	Solvent volume [mL]
Phenanthrene	85-01-8	Phenanthrene-D10, 20 ng	Strata-X, 200mg/6mL (Phenomenex)	Acetone	5
Chlorpyrifos	2921-88-2	Chlorpyrifos-D10, 50 ng	ENV+, 50mg/3mL (IST Biotage)	Ethyl acetate	3
Methyltriclosan	4640-01-1	$^{13}C_{12}$-Methyl-triclosan, 20 ng	ENV+, 50mg/3mL (IST Biotage)	Ethyl acetate	3

When the uptake of the chemical in the fish eggs has reached the "steady state", i.e. the dissipation curve in the medium is declining only slightly, the BCF_{int} can be calculated from the concentration of the compound C_y [ng/µL] in the yolk of the fish egg (internal dose) and in the aqueous medium C_w [ng/µL]:

$$BCF_{int} = C_y/C_w \qquad \text{(Eq. 1)}$$

Assuming that all chemical dissipated from the aqueous medium is in the yolk or in the embryo, respectively, the BCF_{std} can be calculated from the difference of the concentrations in the medium (C_{wo} -C_w), its volume V_w [µL] and the volume of 5 yolks:

$$BCF_{std} = [(C_{wo}-C_w)V_w]/(V_yC_w) \qquad \text{(Eq. 2)}$$

The kinetic approach according to Banerjee [1] is

$$C_w/C_{wo} = [1/(k_1F + k_2)]\cdot\{k_2+k_1\exp[-(k_1F+k_2)\cdot t]\} \qquad \text{(Eq. 3)}$$

where F is a geometric factor, the quotient of the volume of the yolk and the volume of the medium. The kinetic BCF then is

$$BCF_{kin} = k_1/k_2 \qquad \text{(Eq. 4)}$$

Degradation curves according to Eq. 3 were fitted using the software Origin 8.1 (Origin Lab Corporation, USA).

References

[1] Banerjee, S., et al., *Env. Sci. Technol.* 1984 (18), 79 - 81
[2] Petersen, G.I., Kristensen, P., *Env. Tox. Chem.* 1988 (17), 1385 - 1395
[3] Schreiber, R., et al., *Chemosphere* 2009 (77), 928 - 933
[4] Welling, W., deVries, J.W., *Ecotox. Env. Safety* 1992 (23), 64 - 75
[5] Balmer, M.E., et al., *Env. Sci. Technol.* 2004 (38), 390 - 395

Results and discussion

All fish eggs of the *Danio rerio* used in our experiments had a mean diameter of 1.26mm and their yolks had a mean diameter of 0.75mm. This translates into a yolk volume of 0.221 µL resulting in a geometric factor (see Eq.3) of F = 0,000221 .

The BCF for phenanthrene found in our experiments were lower than those found in the experiments of the UFZ [3] and with C14-labeled phenanthrene [2]. The UFZ experiments lasted 72 h and the tested concentrations in the medium were higher. Our experiment with the lower concentration (2.16µg/L) was prolonged to 72 h, but then hatching appeared.The BCF_{int} determined by residue analysis are similar in both of our tests (Figure 2). Higher BCF_{std} and BCF_{kin} in the second test may have been found due to elevated blank values in the negative control.

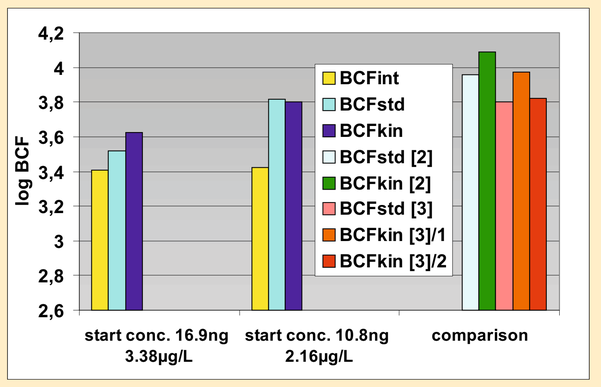
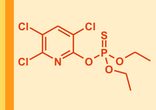


Figure 2: BCF of phenanthrene found in our experiments compared to published data



No acute toxicity of chlorpyrifos for the zebrafish (LC_{50} or $LC_{x<50}$) within the range of water solubility was found. For that reason a higher concentration (66µg/L) was chosen for the first experiment (Figure 3). After 48 hours, the dissipation curve in the aqueous medium still declined too strongly, and the content of chlorpyrifos in the eggs still increased, resulting in inplausible BCF. The experiment should have been prolonged at least to 72 h. The sensitivity of the compound to hydrolysis was obvious in the second experiment (13.8µg/L). At 48 h the sum of content of chlorpyrifos in the eggs and in the medium was considerably lower than the content in the positive control. This indicates a faster hydrolysis in the presence of fish eggs and resulting in a very high BCF_{std} (Figure 3).
In an experiment with juvenile guppies [4] a BCF about 1700 was found.

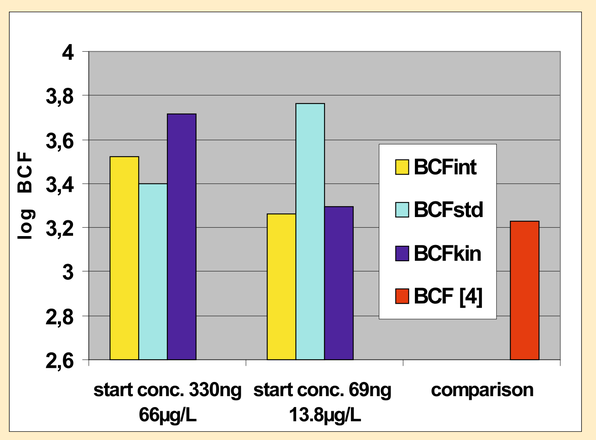
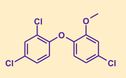


Figure 3: BCF of chlorpyrifos found in our experiments compared to published data



Also for methyltriclosan, no acute toxicity for the zebrafish was found within the range of water solubility. In the test with the higher concentration (80.8µg/l, Figure 4) the system was not yet close to the steady state, thus the BCF_{kin} calculated was not plausible. The other BCF as well as those from the experiment with lower concentration (8.55µg/L) were in accordance with BCF values reported from investigations of fish samples from lakes in Switzerland [5]. In those samples, BCF for methyltriclosan between 2000 and 5000 for the whole fish were found.

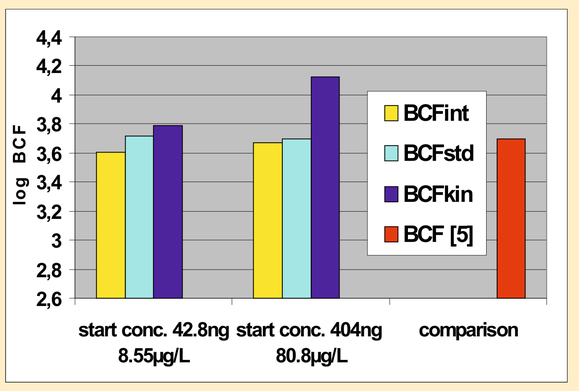


Figure 4: BCF of methyltriclosan found in our experiments compared to published data

Conclusions

The exposure of zebrafish embryos to diluted aqueous solutions of lipophilic chemicals is a suitable method for the screening of BCF in fish embryo. For further improvements the following points must be taken into consideration:

- For tests with ubiquitously occurring chemicals blank values must be checked and contamination must be strictly avoided.
- If a test of 48 h shall give satisfactorily results, the start concentration of the compound must not be chosen too high, even if acute toxicity is not found for the *Danio rerio* within the range of solubility in the aqueous medium.
- If a test is extended to 72 h or longer, hatching of the embryos will occur which means handling of fish larvae. Then local regulations of animal protection have to be regarded.
- If a compound is sensitive to hydrolysis, a different set of equations must be used to calculate the BCF_{kin}.

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