VARIATION OF ACCUMULATION AND CLEARANCE OF THE PREDIOXIN 5-CHLORO-2-(2,4-DICHLOROPHENOXY)-PHENOL (IRGASAN DP 300, TRICLOSAN) WITH THE PH OF WATER.

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Introduction

Bioconcentration factors (BCFs) are one tool in assessing the environmental fate of chemicals. According to international test guidelines they are measured in a fish/water system (1). For stable, non-polar compounds BCFs are correlated to octanol-water partition coefficients (2), but for compounds undergoing dissociation, for example phenols, BCFs are dependent on the degree of ionisation as well (3). Furthermore, it may be assumed that the kinetics of accumulation and clearance of dissociating compounds is dependent on environmental conditions. This is proofed by bioconcentration experiments with the predioxin 5-chloro-2-(2,4-dichlorophenoxy)-phenol also known as „Irgasan DP 300“ or „Triclosan“ (figure 1) varying the pH of the test water.

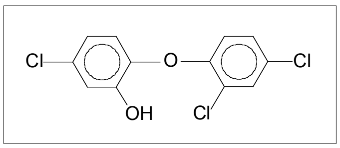

Figure 1: Structure of Triclosan

Materials and Methods

Bioconcentration experiments were performed according to test schedule No. 305 E of the OECD (1). The test fish was zebra fish (*Brachydanio rerio*) bearing an age of 4 - 6 months, a length of 2.5 - 3 cm and a weight of 0.15 - 0.25 g. The test water was tap water of pH 8, for bioconcentration experiments of other pH (6, 7, 9) phosphate buffer (pH 6 and 7) borate buffer (pH 9), respectively, were mixed into the test water by a peristaltic pump. Triclosan was mixed into the flow through aquaria (30 L) by a pump as well. Oxygen content (7 - 8 mg/l), pH and temperature (20 - 21 °C) of the water were monitored continuously. During the accumulation phase the fish were swimming in an aquarium containing Triclosan (35 - 50 µg/l). The clearance phase was started transferring the fish to other aquaria which contained no phenol but only buffered water (figure 2). At certain intervals water and fish samples were taken to follow the kinetics of accumulation and clearance.

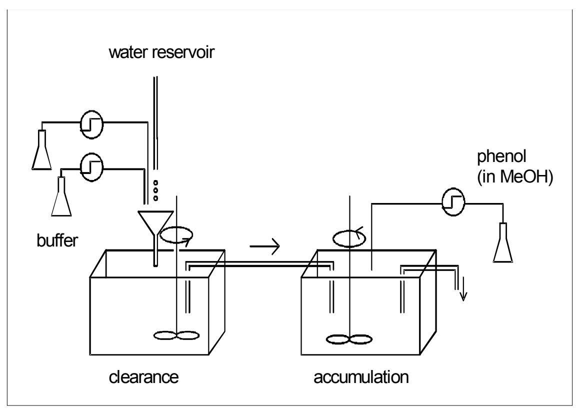

Figure 2: Flow through system for the fish test

Concentrations of Triclosan in fish and water were analysed by GC-ECD extracting the water with toluene the fish with hexane/2-propanol, respectively, derivatising the 5-chloro-2-(2,4-di-chlorophenoxy)-phenol by triethylsulfonium hydroxide TESH (4).
Calculations of BCFs and kinetic parameters were performed by non linear regression analysis applying the two-compartment model (5).

Results and Discussion
Figure 3 displays the kinetics of uptake and clearance of Triclosan in fish at pH 7.

Figure 3: Kinetics of accumulation and clearance of Triclosan in zebra fish at pH 7.
Triclosan concentration in water: 50 µg/l. Top right: clearance displayed in logarithmic scale

The rate constants and the BCFs obtained with varying pH of the test water are compiled in table 1.
Uptake rates (k_1) decreased with an increase of the pH of the test water whereas the clearance rates (k_2) showed no significant variation (table 1). As the BCF calculated from kinetic data is defined as BCF = k_1/k_2 (6) the BCFs were also decreasing with the increase in the pH (figure 4).
Transport of compounds through cell membranes like gills of fish is accomplished by passive diffusion; for dissociating substances like Triclosan (pK_a: 7.9) transport is further influenced by the pH of the medium (7). With water of pH 6 the compound is nearly undissociated resulting in a much quicker uptake rate than in water of pH 9 with about 10 % of undissociated phenol. During the clearance phase the concentration gradient results in a depuration of the compound from fish into the water. As the blood pH of fish (~ 7.4) is independent on the pH of the surrounding water, clearance rates are always identical.

Table 1: Bioconcentration factors (BCF) and rate constants for uptake (k_1) and clearance (k_2) of Triclosan including standard deviations (s_{k1} , s_{k2} , s_{BCF}) in dependence on the pH of water

pH	k_1 [h^{-1}]	s_{k_1}	k_2 [h^{-1}]	s_{k_2}	BCF	s_{BCF}
6	356	72	0.0410	0.0093	8700	2632
7	288	35	0.0354	0.0044	8150	1417
8	262	28	0.0413	0.0045	6350	963
9	129	30	0.0347	0.0083	3700	1232

Figure 4: Uptake rate (left) and bioconcentration factor (right) versus pH of water for Triclosan

Bioaccumulation in aquatic biota is a function of the physico-chemical properties of substances; from our experiments it may be concluded that for compounds undergoing dissociation bioaccumulation is a function of environmental conditions as well.

Acknowledgement
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