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BIOACCUMULATION OF LIPOPHILIC SUBSTANCES IN FISH EARLY LIFE STAGES

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Abstract—Accumulation of ^{14}C-labeled polycyclic aromatic hydrocarbons, naphthalene, phenanthrene, pyrene, and benzo(a)pyrene and polychlorinated biphenyl (PCB) congeners PCB 31 and PCB 105 with a log octanol/water partition coefficient (K_{ow}) range from 3.37 to 6.5 was investigated in eggs and larvae of zebra fish (*Brachydanio rerio*), and in larvae of cod (*Gadus morhua*), herring (*Clupea harengus*), and turbot (*Scophthalmus maximus*). Significant differences in the uptake and elimination rate constants between eggs and larvae of zebra fish were seen. The low rate of uptake and the lower elimination rate of eggs did, however, lead to bioconcentration factors (BCFs) comparable to those for larvae. As biotransformation of xenobiotics in embryonic and larval stages was indicated to be insignificant compared to juvenile/adult stages, body burdens of readily biotransformed chemicals may be higher in fish early life stages. Because weight and lipid content did not differ much between the investigated species, the main reason for the variability in BCFs between marine species (cold water species) and freshwater species (warm water species) was considered to be caused by differences in exposure temperatures that affect the degree of biotransformation. Due to the smaller size of larvae and thus an increased total surface of the membranes per unit fish weight, steady-state conditions were reached at a faster rate in early life stages than in juvenile/adult life stages. The lipid-normalized bioconcentration factors (BCF_L) were linearly related to K_{ow}, but BCF_L was, in general, higher than K_{ow}, indicating that octanol is not a suitable surrogate for fish lipids. Differences in bioconcentration kinetics between larvae and juvenile/adult life stages are considered to be the main reason for the higher sensitivity, with respect to external effect concentrations, generally obtained for early life stages of fish.

Keywords—Bioaccumulation Fish early life stages Polycyclic aromatic hydrocarbons Polychlorinated biphenyls

INTRODUCTION

For determination of the potential of bioaccumulation of a chemical, bioaccumulation studies are normally performed with juvenile and most often warm water fish species [1]. Existing correlations between the bioconcentration factor (BCF) and the octanol/water partitioning coefficient (K_{ow}), which are used for chemical regulations, have all been developed for juvenile and/or adult fish. The validity of these correlations for covering early life stages of fish is not known.

Early life stages of fish are considered to be the most sensitive life stages. This is due to the many critical events that take place in a very short time span [2,3]. Differences in sensitivity between early life stages and juvenile/adult stages of fish may partly be caused by differences in levels of accumulation. Before a substance induces harmful effects, it has to reach the ''site of toxic action.'' The time needed to reach the site of toxic action depends on the barriers to be passed. Due to the small size of fish in their early life stages, it is expected that this time is considerably shorter for early life stages than the time needed in juvenile/adult fish. Furthermore, difference between sensitivity to toxic effects and total accumulation may be explained by the specific accumulation of xenobiotics in the yolk tissue, with the yolk acting as a temporary toxicant sink. The yolk absorption process is believed to be a major factor in the redistribution of chemicals that results in toxic effects in later development stages [4].

In early life stages of fish (embryos and larvae), the metabolic capability is not yet or only slightly developed [5,6]. This is why higher concentrations of readily metabolized chemicals are expected in early life stages compared to those in juvenile/adult fish. As lipophilic chemicals are mainly accumulated in the lipids of the organisms, comparison of BCF in different fish species should be performed on basis of lipid-normalized BCF values [7,8]. It is well known that the relative lipid content in early life stages is higher than in juvenile/adult stages, and for this reason it can be expected that early life stages will accumulate higher concentrations of lipophilic chemicals per kilogram of total weight. Therefore, due to the low metabolic capability and the relatively high lipid content, elevated body burdens of readily metabolized chemicals may be achieved in eggs and larvae with a potential for developing chronic effects in later developmental stages.

Previous studies with early life stages of fish have shown that the rate of elimination of lipophilic xenobiotics is dependent on the developmental stage [3,4,9]. Significant differences in the rates of uptake and elimination of xenobiotics by eggs and larvae of fish have been demonstrated. The rates of accumulation and elimination are generally much lower in eggs than in larvae. The differences in the rates of uptake between eggs and larvae appear to be related to the lower rate of transport of xenobiotics across the chorion compared to the rate of transport across the gill epithelium of the larvae. Although the chorion does not totally prevent an uptake of xenobiotics, the penetration of xenobiotics into the embryo tissues is probably low. One reason is that eggs have a smaller surface area exposed to the environment than do larvae. Another reason is that embryos do not actively circulate fluids through or near the chorion, whereas larvae circulate blood through the gills [3]. Due to the expected low accumulation in eggs, the embryonic stage is not included in the U.S. Environmental Pro-

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tection Agency standard guide for early life-stage toxicity test with fish [10].

In addition to the exposure of waterborne xenobiotics, early life stages of fish may be exposed to xenobiotics by a transfer from parental fish to the developing gametes. Previous studies have shown relatively high percentages of unmetabolized naphthalene and benzo[a]pyrene in fish ovaries compared to other tissues. This may indicate that the deposition of lipid in the oocyte strongly favors accumulation of lipophilic parent compounds [11,12]. The transfer of xenobiotics from parental fish to developing gametes may thus represent an important role in the exposure of early life stages to xenobiotics [13–15].

In the present study, bioaccumulation experiments with polycyclic aromatic hydrocarbons (PAHs) known to be readily metabolized in juvenile/adult fish and PCBs known to be more recalcitrant with respect to metabolism were performed with eggs and larvae of zebra fish (*Brachydanio rerio*). For comparison with more relevant Danish wildlife fish species, bioaccumulation experiments were also performed with cod (*Gadus morhua*), herring (*Clupea harengus*), and turbot (*Scophthalmus maximus*). The role of lipid content, the effect of temperature differences on the toxicokinetic parameters, and the metabolic capability in egg and larvae stages have been evaluated.

MATERIALS AND METHODS

Organisms

Adult zebra fish were obtained from a local supplier and were acclimatized under flow-through conditions for a minimum of 12 d, to exclude fish with abnormal behavior and to achieve fish in good breeding condition. The fish were fed with TetraMin Hauptfutter (Tetrawerke, Melle, Germany) and *Artemia nauplii* (San Francisco Bay, San Francisco, CA, USA) twice a day. The temperature was kept at 27 ± 1°C and the fish loading was kept below 2 g fish per liter. After acclimatization, eight females and 16 males were placed in a 100-L breeding aquarium (glass), covered with black plastic. When breeding was started, a spawning tray was placed in the aquarium. The spawning tray consisted of a stainless steel frame (18.5 × 30 × 7 cm) covered with a net to prevent the adults from eating the eggs. Spawning substrate, consisting of four to six breeding trees (unraveled nylon rope), was attached to the tray. The breeding tray was placed in the aquarium late in the afternoon and removed the following day approximately 3 h after the light had been switched on in accordance with previous studies [16]. To check the success of fertilization, the eggs were removed from the spawning vessels and placed by means of a glass pipette (inner diameter ≤ 4 mm) in petri dishes containing filtered aquarium water. Viable eggs were selected for the tests by means of a binocular microscope (12–25× magnification). Under the conditions used, the duration of the embryonic stage was approximately 72 h. After hatching, the larvae were successfully nourished by the yolk for at least 7 d.

Mature Baltic cod were caught in the Bornholm Basin in the Baltic Sea (May 1995 and June 1996). Ripe females and males were stripped of eggs and sperm, and the eggs were fertilized with sperm from several males by the method described by Westin and Nissling [17]. The eggs were kept at a temperature of 6.5 ± 1°C. Hatching was achieved 12 to 13 d after fertilization. After hatching, the larvae were nourished by the yolk for at least 6 d. Mature herrings were caught in Præstø Bay in April 1995, and the fertilization of the eggs followed the procedures described by Munk and Rosenthal [18]. The eggs were maintained at a temperature of 7.5 ± 1°C, and hatching was achieved approximately 14 d after fertilization. After hatching, the larvae were nourished by the yolk for at least 4 d. Turbot eggs were obtained from a turbot hatchery in Denmark (Maximus). The eggs were kept at a temperature of 15 ± 1°C and hatched after 3 to 5 d. After hatching, the larvae were nourished by the yolk for at least 2 d. The experiments with cod, herring, and turbot were performed in natural seawater with a salinity of 28 to 30‰ collected from the Kattegat, Denmark, west of Læsø at a depth of approximately 30 m.

Table 1. Physicochemical properties of the investigated substances[a]

Substance	Molecular weight (g)	Solubility (μmol/L)	log K_{ow}	Reference
Naphthalene	128.18	244.19	3.37	[41,42]
Phenanthrene	178.24	8.977	4.57	[42,43]
Pyrene	202.26	0.667	5.18	[42,43]
Benzo[a]pyrene	252.32	0.024	6.50	[42,44]
PCB 31	257.5	1.55[b]	5.7 ± 0.2	[45]
PCB 105	326.4	0.178[b]	6.33[c]	[45]

[a] PCB = polychlorinated biphenyl.
[b] Calculated from: log $S_w = -1.40 - 0.47N$ (mmol/L), N = number of chlorine [45].
[c] Calculated from: log $K_{ow} = 4.33 + 0.40N$, N = number of chlorine [45].

Chemicals

Radiolabeled [1-^{14}C]naphthalene (8.9 mCi/mmol, radiopurity ≥98%); [9-^{14}C]phenanthrene (8.3 mCi/mmol, radiopurity ≥98%); [4,5,9,10-^{14}C]pyrene (32.3 mCi/mmol, radiopurity ≥98%), and [7-^{14}C]benzo[a]pyrene (26.6 mCi/mmol, radiopurity ≥98%) were obtained from Sigma Radiochemical (St. Louis, MO, USA). Analytical grade naphthalene (>99%) and benzo[a]pyrene (>98%) were obtained from Merck (Darmstadt, Germany), whereas phenanthrene (>98%) and pyrene (>96%) were obtained from Sigma. Radiolabeled 2,4′,5-trichlorobiphenyl (PCB 31) (19 mCi/mmol) and 2,3,3′,4,4′-pentachlorobiphenyl (PCB 105) (26.5 mCi/mmol) were kindly provided by the Wallenberg Institute, Stockholm, Sweden. Analytical grade PCB 31 and 105 were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Authentic standards for identification of lipids were obtained from Sigma. All other chemicals were readily available and of analytical grade. The physicochemical properties of the investigated chemicals are given in Table 1.

Test solutions

Stock solutions of radiolabeled (2 μCi/ml) and nonlabeled chemicals in acetone were prepared. The exposure concentrations were achieved by mixing radiolabeled and nonlabeled chemicals, giving a ^{14}C activity of approximately 1.1 × 10^5 disintegration per minute (DPM) per liter of test solution. The acetone concentration in the test solutions did not exceed 119 mg/L. Concurrent control experiments were performed with acetone (119 mg/L) together with controls without any chemical added.

Bioconcentration experiments

Bioconcentration experiments were performed with eggs of zebra fish and larvae of zebra fish, cod, herring, and turbot.

Egg stages of cod, herring, and turbot were not tested due to the long duration of the embryo stage of these species. The experiments were performed in a semistatic test system with renewal of test solutions every 24 h. All experiments were performed in climate rooms with a maximum variation in temperature range of ±1°C. For each exposure, up to approximately 200 organisms were placed in 1-L glass beakers. For body burden analysis, each sample consisted of up to five pooled eggs or larvae. During the first day of exposure, up to eight samples were taken at 1-h intervals. In the remaining part of the exposure period, the sampling frequency was reduced to two samples each 24 h. In depuration experiments, organisms were transferred to a medium without test substance. During the first day of depuration, up to six samples for body burden determination were taken. In the remaining part of the depuration period, the sampling frequency was reduced to two samples each 24 h. The duration of the exposure and the depuration period varied in accordance with specific survival time of the yolk sac larvae of each of the investigated species.

Water analysis

Every 24 h, samples of old test solution and freshly prepared solution, respectively, were withdrawn from each test concentration. The concentrations of the radiolabeled chemicals were determined by mixing a 10-ml sample with 10 ml of scintillation cocktail (Insta-gel Plus®) in 20-ml vials. The ^{14}C activity was determined by the International Agency for ^{14}C Determination, VKI, by liquid scintillation counting (LSC). The LSC (Wallac 1409 LSC, Wallac, Turku, Finland) measurements are checked every 3 months with a set of standards and meets the following requirements: counting efficiency ±2% in relation to previous countings and a standard deviation less than 1%. Blanks are measured every week and are below 50 counts per minute (CPM). The specific activity obtained by mixing labeled and nonlabeled substances was measured once for each test concentration and for each chemical by gas chromatography according to the method outlined by Madsen and Kristensen [19]. From this specific activity and the ^{14}C activity measured every 24 h, the actual test concentration during the test period was estimated. Temperature, pH, oxygen saturation, and salinity were measured at the start and end of the experiments.

Fish analysis

Dry weight determinations were performed on 10 samples, each consisting of 10 pooled zebra fish eggs prior to hatching, and on a second 10 samples, each consisting of 10 pooled larvae at the end of the yolk sac stage, of zebra fish, cod, herring, and turbot, respectively. Wet weight determinations of five samples, each consisting of five larvae, and lipid analysis of 10 samples, each consisting of five pooled larvae, were performed for each species collected at the end of the yolk sac stage. For lipid analysis, an internal standard, *n*-hexane-3-one (25 μl), was added to each of the samples, and the tissue was extracted in 1 ml of chloroform:methanol (2:1, v/v) for 24 h at −20°C [20]. Nitrogen was flushed over the samples in the vials before sealing to create an anoxic atmosphere. The tissue/solvent solution was centrifuged for 5 min at 5,000 *g*, and the solvent containing the lipid was evaporated to dryness under a stream of nitrogen. The remaining lipid fraction was dissolved in hexane. Lipid determinations on the extracts were performed by thin-layer chromatography/flame ionization detection (TLC/FID) by an Iatroscan® Mk-5 (Bioscan, Washington, DC, USA) following the procedures described by Ackman [21].

The samples were rinsed by successive transfer to beakers with water containing no test substance to remove radioactivity adhering to the surface of the organisms. The rinsing was limited to less than 1 min to minimize the depuration from the organism. After rinsing, organisms were transferred to glass vials with 50 μl tissue solubilizer (Packard). After 30 min at room temperature, 10 ml Ultima Gold LSC cocktail was added, and the radioactivity was determined by LSC as previously described. The content of PAH and PCB in eggs/larvae tissue (millimoles per kilogram dry weight) was calculated from the specific activity of the test solutions.

Toxicokinetics and statistics

A first-order, one-compartment bioaccumulation model was used for describing the uptake and elimination rates of the tested chemicals from water by eggs and larvae [22]:

$$dC_f/dt = k_1C_w - k_2C_f \quad (1)$$

In this equation, C_f is the concentration of the chemical in eggs/larvae (millimoles per kilogram dry weight), t is the time (h), k_1 is the first-order uptake rate constant (liter per kilogram dry weight per hour), C_w is the concentration of the substance in water (millimolar), and k_2 is the first-order elimination rate constant (per hour). k_1 and k_2 are conditional rate constants and thus dependent upon experimental factors such as pH, temperature, etc. Assuming that the initial concentration in eggs/larvae is zero at the start of the exposure and that the concentration of the chemical in the water is constant, Equation 1 integrates to

$$C_f = (k_1/k_2)C_w(1 - e^{-k_2t}) \quad (2)$$

In the experiments in which steady-state or ''near'' steady-state conditions were achieved, the rate constants could be estimated from the uptake curves. When both exposure and elimination experiments were performed, the rate constants were estimated by use of the entire data set composing both uptake and elimination. The BCF was determined by the mean measured concentrations in fish and water at steady state (BCF_{exp}) and calculated from the ratio of k_1 to k_2 (BCF_k). Lipid-normalized bioconcentration factors were calculated as the ratio of BCF_k to total lipid fraction of the fish. Wet weight-normalized bioconcentration factors (BCF_{ww}) used for comparison with literature data were calculated as the ratio of BCF_k to the dry weight fraction.

The values k_1 and k_2 were estimated by nonlinear regression by use of the software package Sigma Plot [23]. The program uses weighted minimum least squares optimization by means of the Marquardt–Levenberg optimization algorithm [24] with numerical estimates of partial derivatives. For this application, the variance of the data points was assumed to be constant, and regressions were performed without weighting. The program output includes estimates of rate constants as well as the associated standard deviations. Comparison of the rate constants between experiments should ideally be made by performing a paired t test. Since the calculation procedure prevents test for equality of variance and exact calculations of degrees of freedom (df), a more indicative method for a paired comparison of the rate constants was used. An approximation to the 95% confidence intervals was calculated as the rate constant ± (1.96 SE) (Student's t for $\alpha = 0.05$ and $df = \infty$).

Table 2. Lipid content (±SD) in eggs and larvae of fish

Species	Dry weight (μg per egg or larva)	Total lipid (μg per egg or larva)	Percentage of dry weight	Reference
Zebra fish				
Eggs	63.2 ± 8.1	6.5 ± 1.3	10.3	This study
Larvae	39.1 ± 2.3	7.7 ± 1.7	19.6	This study
Juveniles	$63 \times 10^3 \pm 12.5 \times 10^3$[a]	$7.1 \times 10^3 \pm 0.5 \times 10^3$	11.2	[34]
Herring				
Larvae	113.7 ± 11.7	17.2 ± 5.7	15.2	This study
Eggs	—	—	11.4	[26]
Larvae	—	—	19.4	[26]
Cod				
Larvae	59.6 ± 9.9	8.0 ± 3.5	13.5	This study
Eggs	115	11.7	10.2	[25]
Larvae	67	11.0	21.2	[25]
Turbot				
Larvae	57.9 ± 5.7	10.5 ± 4.1	18.2	This study
Larvae	44	11.5	26.0	[27]

[a] Recalculated to dry weight by a dry weight percent of 0.26 ± 1.3 obtained for juvenile zebra fish in the present study.

Comparison of two rate constants was simply made by noting whether the intervals were overlapping. It should be emphasized that conclusions based on this procedure are only indicative.

RESULTS AND DISCUSSION

Throughout the exposure periods of the experiments with naphthalene, benzo[a]pyrene, PCB 31, and PCB 105, the larvae did not show any toxic responses, and no toxicity was observed in controls or in acetone controls. The larvae of all species in experiments with phenanthrene and pyrene showed slight toxic responses observed as malformation (bilaterally bent chorda) toward the end of the exposure period. For all experiments performed, the oxygen saturation was above 70%, and pH was 7.86 ± 0.5 throughout the test period. In the experiments with the marine species, the salinity did not change during the test period.

Dry weights and lipid contents together with literature values are given in Table 2. The lipid content in the larvae was found to be 1.2 to 1.8 times higher than in juvenile/adult stages (zebra fish). The lipid content in the larvae was 1.9 times higher than in the embryonic stages, which is in agreement with other findings [25,26]. For a comparison of the obtained results with literature values, transformation of the data from dry weight to wet weight was necessary. A dry weight of approximately 20% was found for the larvae, but with a pronounced variability due to the small size of the larvae. A dry weight percentage of 20 was found to be comparable to previous findings (21 ± 5% dry weight) in early life stages of marine species [25–27] and is in the following used for all species investigated.

Table 3 gives a summary of exposure temperatures and 24-h mean measured exposure concentrations together with toxicokinetic parameters and BCF values obtained. Uptake and depuration curves are shown graphically in Figure 1 together with the corresponding exposure concentrations. In the accumulation experiments with PCB 31, a slight decline in water concentration toward the end of the uptake phase may have led to a slight underestimation of the BCF. However, the toxicokinetic parameters obtained and BCF levels estimated for PCB 31 in both zebra fish and cod fit in well with the correlations relating BCF, k_1, and k_2 to K_{ow} discussed in the following.

For validation of the model used for determination of k_1 and k_2 from the uptake phase only, depuration experiments were performed in accordance with some of the experiments in which steady-state or near steady-state conditions were achieved. Values of k_1 and k_2 determined from uptake and depuration experiments for phenanthrene (zebra fish), pyrene (zebra fish), PCB 31 (zebra fish), and PCB (cod) were 1,256, 0.15; 1,072, 0.037; 5,188, 0.081; and 1,132, 0.0091, respectively. Compared to the values given for k_1 and k_2 in Table 3, it can be seen that k_1 and k_2 determined by the different methods correspond well, and it is therefore concluded that the estimation of the rate constants on the basis of the uptake phase is valid. This is, however, only true when the uptake phase shows a significant curvature. In addition, since in some of the experiments no significant elimination in the given time period was observed, only indicative k_2 values were achieved for pyrene (zebra fish eggs), benzo[a]pyrene (zebra fish eggs), and PCB 105 (zebra fish larvae and cod larvae). The corresponding BCF_k (and BCF_L) values are therefore also only indicative. These experiments are not included in the calculated correlations relating the BCF or k_2 to K_{ow} (Table 4).

As there is no overlap of the confidence intervals, it is concluded that there are significant differences for the rate constants k_1 and k_2 for zebra fish eggs and larvae. Because egg and larval stages do not differ much in weight, the reason for the lower rate constants across the chorion of the eggs may, besides the smaller surface area of eggs compared to the larvae, be explained by the fact that embryos do not have any active circulation of body fluids near the chorion surface [3]. Low uptake and elimination rates of PAHs in eggs compared to larvae have been shown in previous investigations with salmonids [3], cod [28], and zebra fish [9]. Despite the slow rate of uptake and concomitant slow elimination rate by zebra fish eggs in this study, the indicative BCF_k estimated for eggs was comparable to the ratio of larvae. The resulting BCF in fish eggs is thus expected to be at the same level as in the larvae if steady-state conditions are reached before hatching. Al-

Table 3. Experimental conditions, toxicokinetic parameters, and bioconcentration factor (BCF) obtained on zebra fish, cod, herring, and turbot[a]

Substance/species	Temp. (°C)	$C_w \pm$ SD (μmol/L)	$k_1 \pm$ conf.[b] (L/kg dry wt per hour)	$k_2 \pm$ conf.[b] (per hour)	log BCF[c] (exp.)	log BCF_k[d]	log BCF_L[e]
Naphthalene							
Zebra fish (eggs)	27 ± 1	4.70 ± 0.43	567 (433–744)*	0.325 (0.241–0.439)*	3.26**	3.24	4.23
Zebra fish (larvae)	27 ± 1	3.03 ± 0.15	1,309 (746–2,298)*	1.044 (0.55–1.82)*	3.25**	3.10	3.87
Phenanthrene							
Zebra fish (eggs)	27 ± 1	1.41 ± 0.092	201 (165–246)*	*0.016 (0.011–0.025)**	3.96	*4.09*	*5.08*
Zebra fish (larvae)	27 ± 1	0.87 ± 0.15	1,104 (998–1,221)*	0.17 (0.15–0.19)*	3.90**	3.80	4.51
Cod (larvae)	6.5 ± 1	0.70 ± 0.15	292 (211–405)	0.020 (0.012–0.033)	4.03	4.16	5.03
Herring (larvae)	7.5 ± 1	0.84 ± 0.05	879 (658–1,175)	0.041 (0.029–0.059)	4.32**	4.33	5.15
Turbot (larvae)	15 ± 1	0.94 ± 0.07	968 (856–1,095)	0.085 (0.074–0.099)	4.05**	4.06	4.79
Pyrene							
Zebra fish (egg)	27 ± 1	0.12 ± 0.0053	230 (186–285)*	*0.0077 (0.0023–0.025)*	4.00	*4.48*	*5.46*
Zebra fish (larvae)	27 ± 1	0.13 ± 0.021	1,169 (781–1,821)*	0.022 (0.013–0.038)	4.74	4.73	5.43
Cod (larvae)	6.5 ± 1	0.23 ± 0.033	955 (798–1,143)	0.011 (0.008–0.016)	4.78	4.93	5.80
Herring (larvae)	7.5 ± 1	0.12 ± 0.021	1,380 (1,043–1,827)	0.011 (0.005–0.022)	4.99	5.11	5.93
Benzo[a]pyrene							
Zebra fish (egg)	27 ± 1	$1.98 \times 10^{-3} \pm 2.99 \times 10^{-4}$	405 (359–457)*	*0.00014 (5.8×10^{-15}–3.2×10^{6})**	4.32	*6.47*	*7.46*
Zebra fish (larvae)	27 ± 1	$1.75 \times 10^{-3} \pm 8.9 \times 10^{-5}$	2,812 (2,198–3,598)*	0.0064 (0.0037–0.011)*	5.52	5.64	6.35
PCB 31							
Zebra fish (larvae)	27 ± 1	0.0032 ± 0.0004	4,965 (4,011–6,149)	0.065 (0.051–0.085)	4.80**	4.88	5.59
Cod (larvae)	6.5 ± 1	0.0031 ± 0.0005	1,268 (1,039–1,546)	0.0088 (0.0054–0.014)	5.00	5.16	6.03
PCB 105							
Zebra fish (larvae)	27 ± 1	$4.13 \times 10^{-4} \pm 9.5 \times 10^{-5}$	3,192 (2,968–3,432)	*0.0049 (0.0040–0.0059)*	5.54	*5.82*	*6.53*
Cod (larvae)	6.5 ± 1	$5.37 \times 10^{-4} \pm 7.2 \times 10^{-5}$	1,104 (822–1,483)	*0.0016 (7.9×10^{-5}–0.031)*	5.18	*5.85*	*6.72*

[a] Values given in italics are to be regarded as indicative only; for further explanation see text. PCB = polychlorinated biphenyl.
[b] Parentheses indicate 95% confidence limits. * Indicates rate constants significantly different for, respectively, zebra fish eggs and larvae.
[c] Bioconcentration factor (in terms of dry weight) achieved at the end of the exposure period. ** Indicates tests in which steady–state conditions were reached during the exposure period.
[d] Determined from k_1/k_2 (in terms of dry weight).
[e] Bioconcentration factor normalized on lipid basis.

though steady-state conditions were not achieved during the embryonic stage in any of the experiments except with naphthalene, remarkably high BCF levels of phenanthrene, pyrene, and benzo[a]pyrene were attained (Table 3). Thus, for fish species having a long embryonic stage, such as salmon, cod, and herring, a considerable accumulation (body burden) of lipophilic chemicals in the egg stage is to be expected upon exposure, with the possibility for exerting toxic effects in an early and sensitive life stage. Therefore, it is important to include the embryonic stage in early life stage toxicity tests and in tests with highly lipophilic chemicals.

A consistent difference in the uptake and elimination rate constants between zebra fish and the marine species was observed. Differences in exposure temperature are expected to be one possible factor for the differences in the rate constants observed between the investigated species. In experiments with phenanthrene, the uptake rate and the elimination rate were higher for zebra fish (27°C) than for herring (7.5°C), 1.3 and 4.1 times, respectively. The reason that k_2 increased more than k_1 may be due to an additional increase of biotransformation. In a study by Jimenez et al. [29], in which juvenile bluegill sunfish (*Lepomis macrochirus*) were exposed to benzo[a]pyrene, it was indicated that the biotransformation of benzo[a]pyrene is much slower at colder temperatures. Although the biotransformation of PAHs by yolk sac larvae is expected to be insignificant, it may become more important at higher temperatures. Because k_1 seems less temperature dependent than k_2, a higher BCF for readily biotransformed xenobiotics is expected to occur in cold water species. Comparison of BCF_k obtained for PCB 31 and the PAHs studied in zebra fish and in marine species showed a 1.1- to 3.3-times increase in BCF for the marine species. Although the rate constants determined for PCB 105 are only indicative, both k_1 and k_2 were lowered by a factor of three when the temperature was lowered from 27°C (zebra fish) to 6.5°C (cod). Thus, for chemicals not readily metabolized, a change in temperature may be expected to influence the uptake and depuration rate constants and may thereby influence the resulting BCF equally. This has also been suggested by Jimenez et al. [29].

The capability of fish early life stages of metabolizing xenobiotics is only poorly investigated. Microsomal aryl hydrocarbon hydroxylase (AHH) is indicative of the presence of hepatic P-450 systems and thus of the capability of metabolizing xenobiotics. Binder and Stegeman [5] found that, at different embryonic stages of killifish assayed before hatching, AHH remained uniformly low. However, within 24 h of hatching, AHH-specific activity increased about ninefold. Goksøyr et al. [6] compared the induction of P450 in eggs and larvae of cod exposed to crude oils and found that the induction process was dose dependent and restricted until the time of hatching. After hatching, P-450 activity gradually increased. Although the larvae may have a metabolic capability, as previously shown by the possible temperature influence on k_2, the metabolic capability in larvae is still far from the capability in juvenile/adult stages. Comparing BCFs for PAHs obtained in larvae with literature values for juvenile/adult stages (Table 5), the BCFs obtained for pyrene and benzo[a]pyrene in larvae are substantially higher, by a factor of 23 to 43. Comparison of bioaccumulation of low metabolizable compounds such as PCBs with that of PAHs may give a good indication whether

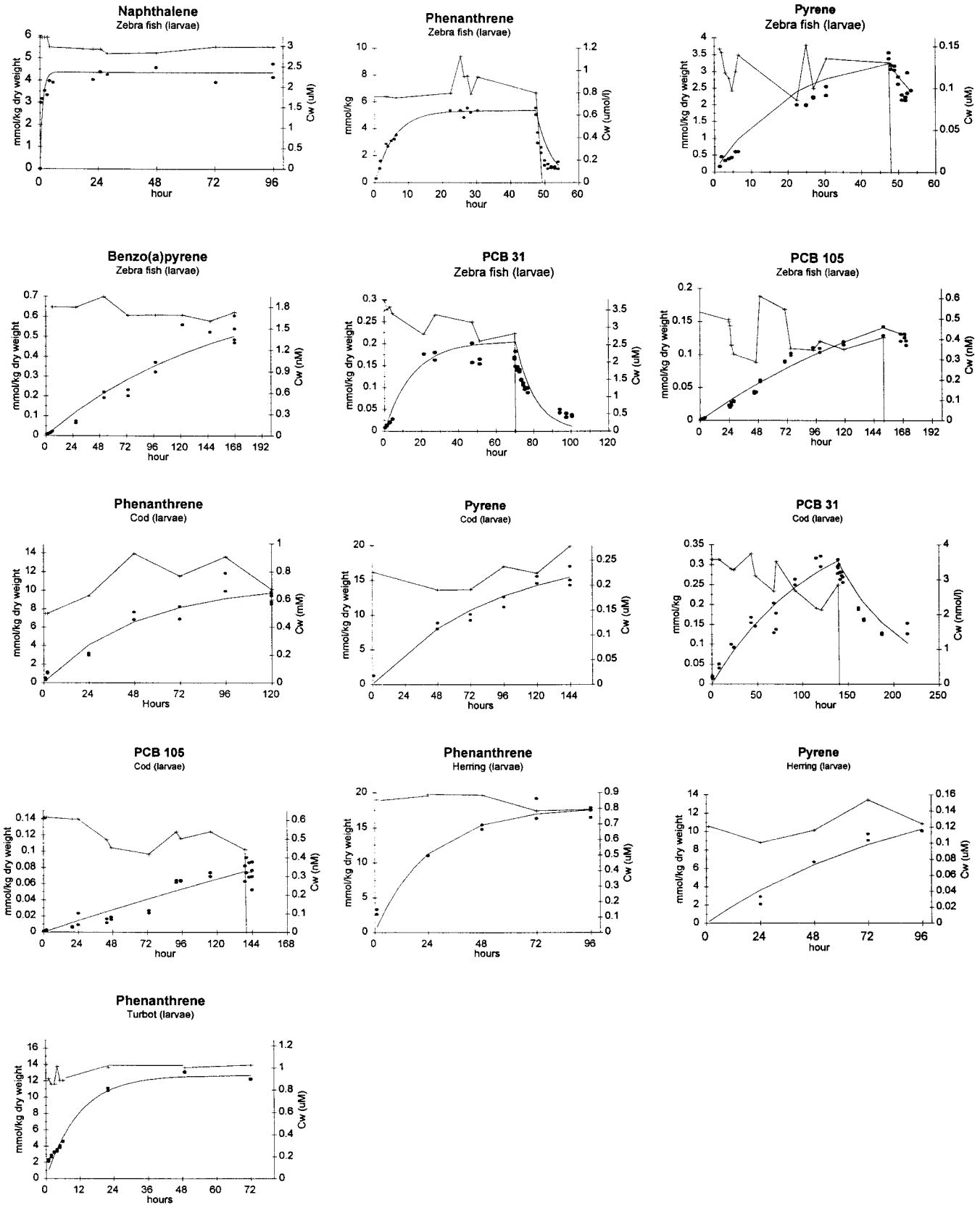

Fig. 1. Accumulation of polycyclic aromatic hydrocarbons and polychlorinated biphenyls investigated. Solid circles (●) represent the experimental determined and lines (——) the expected values based on the model calculations. The 24-h mean measured water concentrations during the exposure periods are represented by (+).

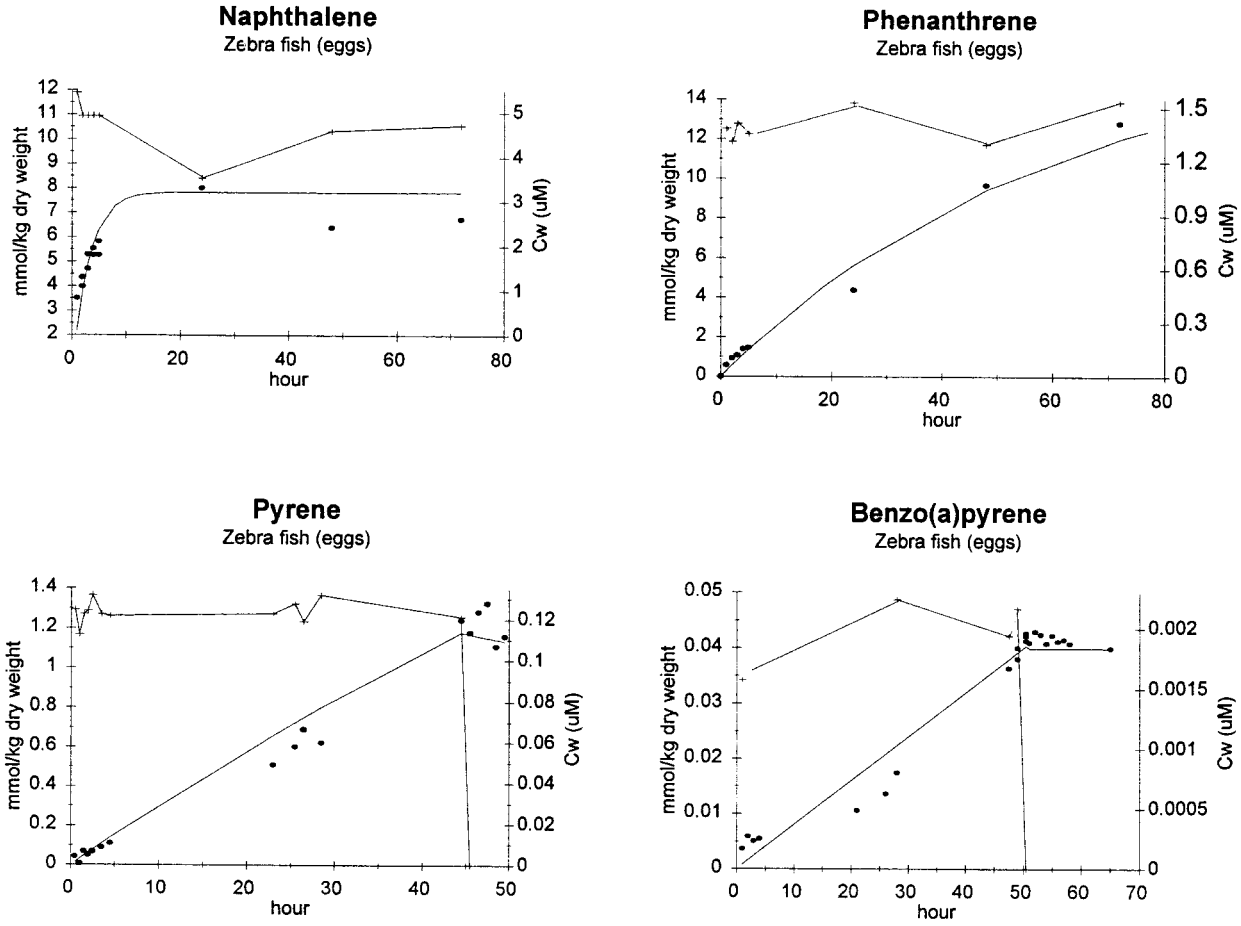

Fig. 1. Continued

PAHs have been accumulated and then metabolized. Polychlorinated biphenyls may be metabolized in fish as well, but are metabolized at a slower rate than are PAHs [30]. The BCFs obtained on PCBs were about the same in the larvae as those reported on juveniles (Table 5). The BCFs obtained for PAHs and PCBs with similar K_{ow}, i.e., pyrene and PCB 31 (log K_{ow} 5.18 and 5.67, respectively) and benzo[a]pyrene and PCB 105 (log K_{ow} 6.5 and 6.4, respectively), are within the same order of magnitude (Table 3). This indicates that the PAHs investigated are not or are only insignificantly metabolized in the investigated larvae stages.

As previously suggested for hydrophobic compounds, e.g., [31], the uptake rate constant is mainly a function of the size of the organism, whereas the elimination rate constant is mainly a function of the size of the organism, the lipid content of the organism, the biotransformation process of the organism,

Table 4. Regression equations for estimating BCF_{WW}, BCF_L, k_1, and k_2 from log K_{ow}[a]

Equation form	—[b]	—[c]	n	r^2	Life stage	Reference
log BCF_{ww} = a + b log K_{ow}	−0.46 ± 0.46	0.86 ± 0.09	11	0.91	Larvae	This study
	−0.7	0.85	55	0.90	Juvenile/adults	[46]
	−0.23	0.76	84	0.91	Juvenile/adults	[47]
	−0.4	0.79	16	0.93	Juvenile/adults	[38[b]]
	−1.32	1	51	0.95	Juvenile/adults	[8[b]]
log BCF_L = a + b log K_{ow}	0.64 ± 0.29	0.92 ± 0.06	23	0.91	Larvae and juveniles	This study, [39]
	0.61	0.89	18	0.90	Juvenile/adults	[40]
	0.65 ± 0.97	0.91 ± 0.29	20	0.61	Juvenile/adults	[32]
log k_1[c] = a + b log K_{ow}	3.14 ± 0.45	0.16 ± 0.08	6	0.48	Larvae	This study
	0.147	1.98	14	0.74	Juvenile/adults	[22]
log $k_2(d^{-1})$ = a + b log K_{ow}	3.25 ± 0.82	−0.66 ± 0.16	11	0.65	Larvae	This study
	1.47	−0.41	14	0.95	Juvenile/adults	[22]
	1.69 ± 0.52	−0.53 ± 0.07	31	0.83	Juvenile/adults	[32]
log BCF_L = a + b log $k_2(d^{-1})$	5.24 ± 0.09	−1.05 ± 0.13	11	0.86	Larvae	This study

[a] BCF_{ww} = wet weight-normalized bioconcentration factor; BCF_L = lipid-normalized bioconcentration factor; k_1 = first-order uptake rate constant; k_2 = first-order elimination rate constant; K_{ow} = octanol/water partition coefficient.
[b] Most frequently encountered relationships.
[c] Liter per kilogram wet weight per day.

Table 5. BCF_k (in terms of wet weight) obtained with polycyclic aromatic hydrocarbons in larvae compared to literature values on juvenile/adult stages[a]

Substance	Species (this study)	log BCF_k (this study)	log BCF (literature data)	Species (literature data)	Reference
Naphthalene	Zebra fish	2.46	2.50	Bluegill	48
			2.63	Fathead minnow	47
Phenanthrene	Zebra fish	3.10	3.42	Fathead minnow	46
	Cod	3.46			
	Herring	3.62			
	Turbot	3.35			
Pyrene	Zebra fish	4.03	2.66	Goldfish	49
	Cod	4.23	3.7	Guppy	50
	Herring	4.29			
Benzo[a]pyrene	Zebra fish	4.94	3.42	Bluegill	48
			3.69	Bluegill	51
PCB 31	Zebra fish	4.18	4.66	Zebra fish	34
	Cod	4.46	4.63	Gold fish	52
			3.64	Atlantic salmon	53
PCB 105	Zebra fish	5.12	5.47[b]	Zebra fish	34
	Cod	5.15			

[a] BCF_k = bioconcentration factor calculated from ratio of first-order uptake rate constant to first-order elimination rate constant; PCB = polychlorinated biphenyl.
[b] No values could be obtained on PCB 105; the BCF given here is for PCB 101 (log K_{ow} = 6.38).

and the lipophilicity of the test compound. In Figure 2A, the relationship between log K_{ow} and the rate constants k_1 and k_2 obtained on larvae of the different species is shown together with regression lines between K_{ow} and k_2 that had previously been reported [22,32] on juvenile/adult fish. Only a moderate increase in k_1 with increasing log K_{ow} was seen. Earlier studies showed that k_1 increased with K_{ow} for organic chemicals having a log K_{ow} <3, that k_1 was independent of K_{ow} for organic chemicals with log K_{ow} between 3 and 6, and that k_1 decreased with K_{ow} for organic chemicals with a log K_{ow} >6. It was also found that k_2 was independent of K_{ow} for organic chemicals with log K_{ow} <3 and decreased with increasing K_{ow} [33]. The mean k_1 obtained on zebra fish larvae (temperature 27 ± 1°C) in this study for substances with a log K_{ow} range from 3.4 to 6.4 was found to be 485 ± 307 (1 kg wet weight per hour). In a study performed by Fox et al. [34], juvenile zebra fish (temperature 22 ± 0.2°C) with an average weight of 243 mg were exposed to different PCB congeners having a log K_{ow} range from 5 to 8. In this study, k_1 was shown to increase up to log $K_{ow} \leq 7.4$. The mean k_1 value for PCBs in the log K_{ow} range 5 to 7.4 was found to be 185 ± 51 (1 kg wet weight per hour). These results thus support previous assumptions that, since larger fish have a relatively lower gill surface to weight ratio, a lower mean uptake rate constant is to be expected [31,35] and is further supported by the fact that the surface area of the gills to volume ratio steadily declines from the early larval stage throughout the development of fish [36]. An allometric relationship between k_1 and weight has previously been described by Sijm et al. [37]. The present data obtained on k_1 are approximately 20 times higher than expected when based on this relationship. Because the relationship is developed on the basis of perfused gills of juvenile fish, the reason for the deviation may probably be explained by the higher gill surface to weight ratio of the larvae and a relatively higher dermal uptake, which for small organisms is also expected to be an important uptake route.

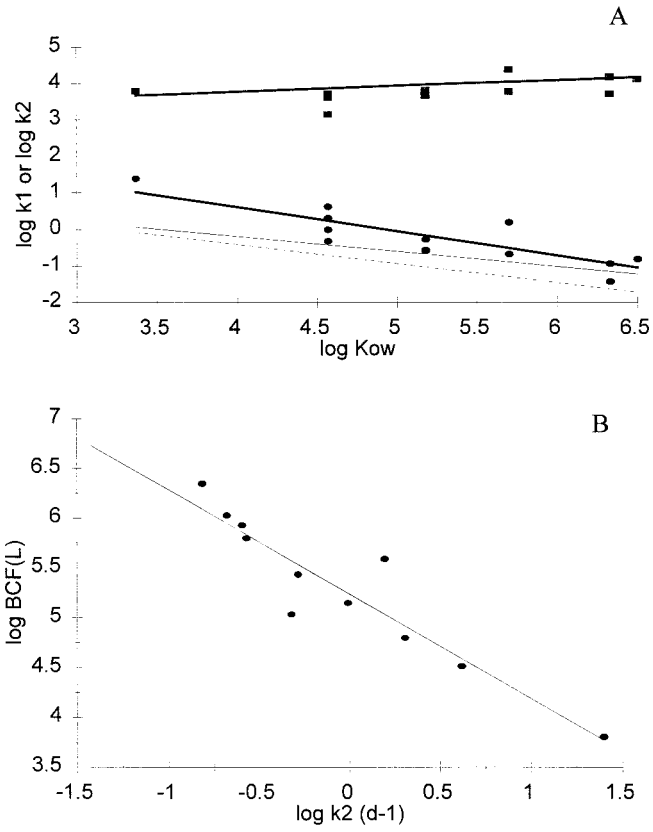

Fig. 2. (**A**) Correlation between log k_1 (liters per kilogram wet weight per day) − K_{ow} (■) and log k_2 (per day) − K_{ow} (●), respectively, compared to correlations described for juvenile/adult fish; (– – –) [22] and (——) [32]. (**B**) Correlation between lipid-normalized bioconcentration factor and k_2 (per day) obtained on larvae (●) of all species investigated. For regression equation, see Table 4.

A negative correlation between k_2 and log K_{ow} was observed in this study, whereas k_1 was more or less independent of log K_{ow} (Fig. 2A and Table 4). As seen from the figure, the regression line for larvae is above the regression line described by Spacie and Hamelink [22] and Gobas et al. [32], indicating that steady state will be reached sooner in larval than in juvenile/adult stages. A highly significant correlation between

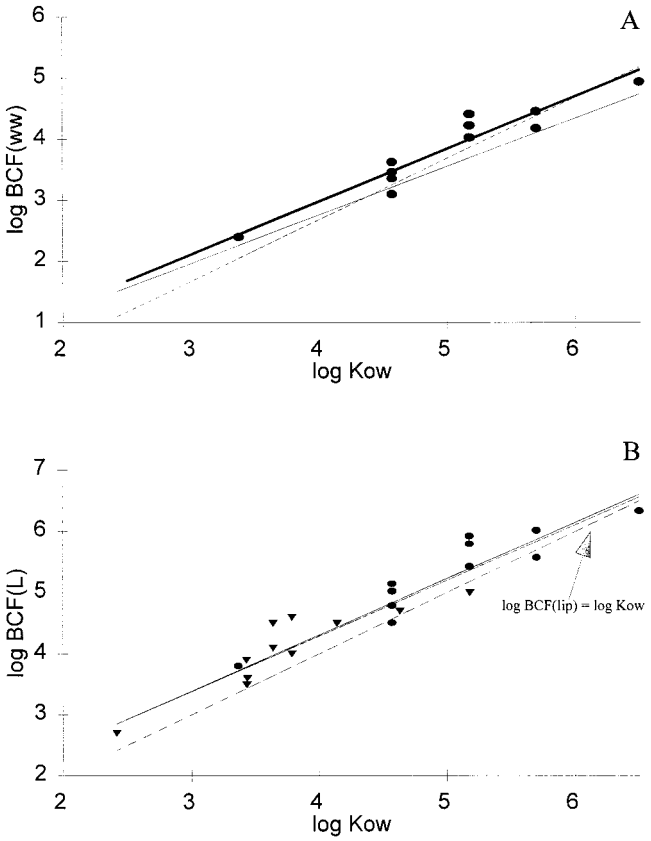

Fig. 3. (**A**) Correlation between log wet weight-normalized bioconcentration factor-log K_{ow} (●) obtained on larvae compared to most frequently used correlations on juvenile/adult fish; (·····) [8] and (——) [38]. For regression equations, see Table 4. (**B**) Correlation between log lipid-normalized bioconcentration factor (BCF_L)-log K_{ow} (●). ▼ indicates BCF_L on juvenile fish obtained by Sijm et al. [39]. The regression equations for BCF_L-log K_{ow} given in Table 4 are indicated on the figure.

BCF_L and k_2 was also found (Fig. 2B and Table 4), which is in agreement with earlier observations of juvenile fish [1].

For estimating the bioconcentration potential of chemicals, different regression equations between BCF and log K_{ow} have been elaborated for juvenile/adult fish (Table 4). Figure 3A shows a comparison between the most frequently encountered relationships [8,38] and the BCF_k values, in terms of wet weight, obtained in this study. The correlation obtained for larvae is placed above other reported correlations for juvenile/adult fish. The higher BCF values obtained for larvae, compared to BCF values for juvenile/adult fish, can be explained by the relatively higher lipid content in larvae compared to juvenile fish. In this study, the measured lipid content of zebra fish larvae at the end of the yolk sac stage was 19.6% of dry weight, whereas the mean lipid content of juvenile zebra fish has been reported to be 11.2% of dry weight [34]. The bioconcentration of lipophilic substances in early life stages of fish and fatty fish (e.g., eel and salmon) may thus be underestimated when the regression equations most frequently encountered are used for estimating the bioconcentration potential of lipophilic chemicals and no correction of the lipid content in the organism studied is made. The data points in Figure 3B show the BCF_L for fish larvae from the present study together with data points obtained by Sijm et al. [39] on juvenile guppies and fathead minnows exposed to halogenated benzenes. By combining all the data, the following correlation (including confidence limits) is obtained: $BCF_L = 0.64(0.04 - 1.23) + 0.92(0.78 - 1.06)\log K_{ow}$. As seen on the figure, this correlation corresponds well with those found in other studies (see Table 4). From Figure 3B, it can be seen that the correlation obtained is above the line of equality (log BCF = log K_{ow}), indicating that octanol is a poor model for fish lipids. This is in agreement with the conclusions of Sijm et al. [39]. Octanol is generally accepted as an adequate surrogate for fish lipids [1,40]. If, however, the correlation between BCF_L and K_{ow} achieved in this study is used, BCF for a chemical with a log K_{ow} of 3 will be underestimated by a factor of 2.6 compared to the BCF obtained by use of log BCF = log K_{ow}.

The present study explicitly indicates that it is most likely the differences in bioconcentration kinetics that make early life stages of fish more sensitive than juvenile/adult stages. The reasons for the higher sensitivity are (1) activities of xenobiotic metabolizing enzymes are higher in juvenile/adult life stages than in the early life stages. This implies that, for chemicals readily metabolized, a higher BCF and thus a higher body burden will be achieved in early life stages. (2) Higher body burdens will be achieved due to the relatively higher lipid content in early life stages. Xenobiotics are primarily accumulated in the yolk sac lipids, which may act as a toxicant sink during the embryonic and early larval stage as previously discussed by van Leeuwen et al. [4]. During the development of the larvae, xenobiotics sequestered in the yolk are transported to sensitive organs in which either the toxic action or metabolism to toxic reactive intermediates may occur. (3) Due to the higher gill surface (and surface in general) to weight ratio in larvae, time to steady state and thus to a ''toxic dose equilibrium'' is reached sooner in early life stages than in juvenile/adult stages.

CONCLUSION

A considerable uptake of lipophilic chemicals in eggs is to be expected, especially for fish species having a long embryonic stage, such as salmon, cod, and herring. As biotransformation of xenobiotics in embryonic and larval stages in this study was indicated to be insignificant compared to juvenile/adult stages, higher body burdens of readily biotransformed chemicals may be reached in early life stages of fish. Since there was little difference between weight and lipid content between the investigated species, one of the main reasons for the variability in BCFs between marine species (cold water species) and freshwater species (warm water species) was considered to be caused by differences in exposure temperatures and thereby in the degree of biotransformation. Due to the smaller size of the larvae and thus an increased total surface of the membranes per unit fish weight, steady-state conditions were reached sooner in early life stages compared to juvenile/adult stages. For many substances, toxic dose equilibrium may not be reached in toxicity tests of juvenile stages. For most substances, equilibrium will, however, be reached in early life stage tests (duration 3–30 d posthatching), resulting in a lower effect concentration. Lipid-normalized bioconcentration factors were linearly related to K_{ow}, but BCF_L was in general higher than K_{ow}, indicating that octanol is not a suitable surrogate for fish lipids. Due to a generally higher lipid content in early life stages of fish and a lower metabolic rate of readily metabolized chemicals in these stages, an elevated body burden will be achieved. Differences in bioconcentration kinetics are thus considered to be the main reason for the higher sen-

sitivity, with respect to the external effect concentration, generally obtained for early life stages of fish.

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