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Uptake and elimination of aromatic hydrocarbons and a chlorinated biphenyl in eggs and larvae of cod *Gadus morhua*

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ABSTRACT: Artificially fertilized eggs and newly hatched larvae of cod *Gadus morhua* L. were exposed to ^{14}C labelled naphthalene, phenanthrene, benzo(a)pyrene and 2, 4, 5, 2', 4', 5'-hexachlorobiphenyl (PCB) for 24 h and thereafter transferred to clean seawater. Radioactivity in eggs and larvae was measured at different times during exposure and after transfer to clean seawater. Maximum accumulation was found with phenanthrene, whereas naphthalene accumulated only slightly in both eggs and larvae. Naphthalene was, on the other hand, rapidly eliminated in contrast to the slow elimination of the other more lipophilic components. Most of the radioactivity accumulated in eggs was transferred to the larvae upon hatching. These findings show that cod eggs and larvae accumulate lipophilic xenobiotics from seawater and that the components will be stored for long time even after eggs and larvae are out of the polluted area.

INTRODUCTION

Marine fish resources are of great economic and nutritional value. Increasing concern is, however, expressed by fishermen and consumers that oil pollution and other worldwide distributed pollutants, like polychlorinated biphenyls (PCB), could be a threat to this renewable food resource. Xenobiotics may affect fish eggs and larvae and result in decreasing recruitment.

In Norway, promising offshore oil fields are situated in the vicinity of spawning grounds of cod *Gadus morhua* L. The most sensitive periods in the life cycle of marine fishes are the embryonic and larval stages (e.g. Struhsaker, 1977; Lønning, 1977; Kühnhold et al., 1979; Leung and Bulkley, 1979; Linden et al., 1980). Sublethal effects at low levels (40 to 60 $\mu g\ l^{-1}$) of the water-soluble fraction of crude oil have been demonstrated in larval cod (Tilseth et al., 1981; Solberg et al., 1982a, b). In an oil spill situation, the hydrocarbon concentration in the upper water layer is quite variable. High concentrations may occur under a drifting oil slick, but as the slick drifts away from a given water mass the concentrations decrease due to mixing and dilution. Thus, we may expect drifting fish eggs and larvae to be exposed to relatively high concentrations of hydrocarbons for relatively short periods. In such situations, the rates of accumulation and elimination of the pollutants will be important factors determining their effects. The present study was performed in order to determine the accumulation and elimination in cod eggs and larvae of some selected hydrocarbons (naphthalene (Nph), phenanthrene (Phe), benzo(a)pyrene (BaP)), as well as a PCB compound (2, 4, 5, 2', 4', 5'-hexachlorobiphenyl, IUPAC no. 153, Ballschmiter and Zell, 1980).

MATERIALS AND METHODS

Biological material

Eggs were stripped from ripe ovaries of coastal cod *Gadus morhua* L. caught north of Bergen, western Norway, in March 1982. The eggs were artificially fertilized in the laboratory and gently washed in clean seawater (34 ‰ S) for 2 h. Dead eggs sank to the bottom and were discarded. After 8 h, 10 ml aliquots of eggs were transferred to 10 black 10 l plastic aquaria with white bottoms. Antibiotics were administered according to Shelbourne (1963), and 2500 IE Mycostatin l^{-1} was also added. These doses were administered only once. The aquaria were placed in a waterbath at 4.5 °C. During incubation, filtered air (0.2 µm Millipore filter) was gently bubbled through the aquaria (Tilseth et al., 1981).

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Dosing and sample preparation

Each of the compounds (Table 1) was dissolved in 100 µl of ethanol and mixed with 250 ml UV-sterilized and filtered seawater. Of this, 100 ml was transferred to a 400 ml glass beaker and placed in a waterbath. Experiments were performed with eggs, yolk-sac larvae (1 d old) and 9 d old larvae, respectively. The yolk is absorbed approximately 8 d after hatching (Ellertsen et al., 1980). Eggs or larvae (n = 300) were gently transferred to each of the experimental beakers. The beakers were aired with filtered air, after which they were sealed with Parafilm and kept in the dark. Then 1 d exposed eggs or larvae were transferred to 1500 ml clean, aerated seawater (34 ‰ S, 4.5 °C). Samples of seawater (2 × 1 ml) and of eggs or larvae (20 of each per sample) were analysed at various times (Table 3). Eggs and larvae were rinsed by successive transfer to 3 vials containing clean seawater to remove radioactivity which adhered to the surface. After addition of Soluene −350 and Dimilume −30 (Packard Instrument) the samples were analysed with a Packard 300 CD scintillation counter.

Table 1. Specific activities and molecular weights (Mw) of the ^{14}C-labelled xenobiotic compounds used for experiments

Compound	Spec. act. (MBq mmol^{-1})	Mw
1(4, 5, 8)-^{14}C Naphthalene (Nph)	185 (Amersham)	130
9-^{14}C Phenanthrene (Phe)	714 (Amersham)	179
7, 10-^{14}C Benzo(a)pyrene (BaP)	803 (Amersham)	253
^{14}C 2, 4, 5, 2′, 4′, 5′-Hexachlorobiphenyl (PCB)	766 (New England Nuclear)	361

RESULTS AND DISCUSSION

Results from radioactivity analyses are given in Tables 2 and 3. The concentrations of radioactivity in the seawater differed for the various compounds (Table 2). The initial exposure concentrations for BaP (5 µg l^{-1}) and PCB (25 µg l^{-1}) are above the solubility limits for these components in seawater. However, the components were dissolved in ethanol and thereafter mixed into the seawater thus giving a higher water concentration. Replicates of water samples showed that the concentrations of the components were distributed homogeneously. There were losses in activity during the 24 h exposure which were considerably greater than the uptakes in the eggs and larvae. No attempt was made to identify these sources of loss in activity. The accumulation of radioactivity in eggs and larvae (Table 3) is given relative to the initial concentration in 1 µl of the contaminated seawater. The volume of 1 egg or larva is approximately 3 µl (Tilseth, unpubl.). Fig. 1 illustrates uptake and elimination of radioactivity in eggs and yolk-sac larvae expressed as percent of the maximum value obtained.

Uptake of radioactivity was higher with Phe than with the other compounds for both eggs and larvae (Table 3). Lowest uptake in eggs was found with the PCB compound. This corresponds to the higher molecular weight of the PCB as compared to the other compounds (Table 1). The relatively low uptake of the more lipophilic compounds (PCB, BaP) by eggs reflects probably the low lipid content of cod eggs (1 to 2 %; Riis-Vestergaard, pers. comm.).

The high degree of chlorination of PCB compounds may affect their uptake through the egg case (chorion). Hydrocarbons probably enter the chorion through

Table 2. Concentrations of radioactivity in seawater at start and end of 24 h exposure in experiments with cod eggs and larvae. Values given as disintegrations min^{-1} ml^{-1} (dpm ml^{-1}), with µg l^{-1} in parentheses

Compounds	Eggs		Larvae	
	Start	End	Start	End
Naphthalene	1146 (14)	999 (12)	1759 (21)	1375 (16)
Phenanthrene	7924 (33)	4018 (17)	3607 (15)	1981 (8)
			3311 (14)*	1852 (8)*
Benzo(a)pyrene	996 (5)	411 (2)	1459 (8)	784 (4)
2, 4, 5, 2′, 4′, 5′-Hexachlorobiphenyl	3378 (25)	862 (6)	1219 (9)	498 (4)

* Experiment with 9 d old larvae; all other values are for experiments with yolk-sac larvae

Table 3. Radioactivity in cod eggs and larvae during and after exposure to naphthalene, phenanthrene, benzo(a)pyrene, and 2, 4, 5, 2', 4', 5'-hexachlorobiphenyl. Results are expressed as ratio of (dpm egg^{-1} or $larva^{-1}$)/(dpm μl^{-1} seawater at start of exposure)

Experiment		Exposure period (d)		Time after transfer to clean seawater (d)									
Stage	Compound	0.1	1	1	2	4	7	bh*	ah**	10	12	15	20
Eggs	Nph	15	18	1	–	1	0	0	2				
	Phe	38	108	129	–	79	43	15	9				
	BaP	7	22	29	–	22	27	33	25				
	PCB	4	9	11	–	13	12	12	12				
Yolk-sac larvae	Nph	22	23	1	1	1	0			0		0	–
	Phe	103	223	150	114	92	74			39		37	21
	BaP	10	80	76	61	59	43			31		24	17
	PCB	12	93	131	123	147	185			115		108	–
9 d old larvae	Phe	–	427	195	142	88	69				46	25	–

* 0.5 to 1 d before hatching
** 0.5 to 1 d after hatching

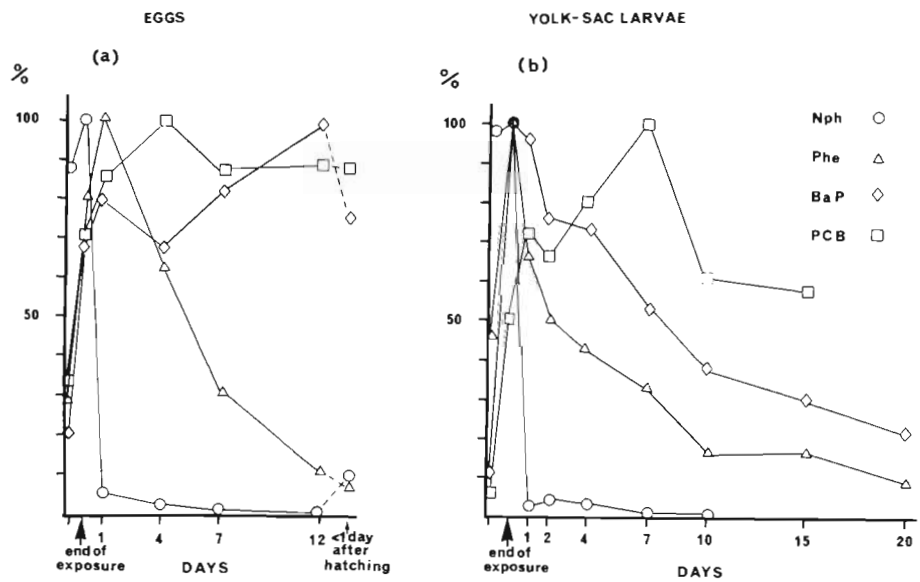

Fig. 1. *Gadus morhua*. Uptake and elimination of radioactivity from ^{14}C-labelled naphthalene, phenanthrene, benzo(a)pyrene and 2, 4, 5, 2', 4', 5'-hexachlorobiphenyl in eggs (a) and yolk-sac larvae (b). Results expressed as percentages of maximum contents of radioactivity for each compund. Arrows: end of 24 h exposure; numbers 1 to 20: days in clean seawater

pores, like water. A compound bound to macromolecules in the water may not be able to penetrate the pores in the chorion or the vitelline membrane. During the 24 h exposure, the larvae accumulated slightly more radioactivity from Nph and much more from Phe, BaP and PCB than did the eggs (Table 3).

In some instances the radioactivity was higher early in the depuration period than at the end of exposure, particularly with the PCB (Fig. 1). This is artificial and reflects variability among the individuals constituting a sample.

The rapid uptake of Nph in cod eggs is in accordance with results of Kühnhold and Busch (1978) and Sharp et al. (1979). Kühnhold and Busch (1978) found higher accumulation of Nph than of BaP in salmon eggs *(Salmo salar)* after 12 h exposure. With longer incubations, however, the accumulation of BaP gradually exceeded that of Nph. Thus after 7 d the accumulation factor was 60 % higher for BaP than for Nph. Sharp et al. (1979) reported that the uptake rate of chrysene in eggs of mummichog *Fundulus heteroclitus* was only 10 % of the uptake of Nph after 2 h exposure time.

Samples of chorion and yolk of cod eggs exposed to ^{14}C-labelled phenanthrene revealed that most of the radioactivity was associated with the yolk, and not with the chorion (Solbakken, unpubl.). This agrees with Kühnhold and Busch (1978), who found that following exposure of salmon eggs to Nph and BaP, most of the radioactivity was associated with the vitelline fluid and the yolk. Hose et al. (1982) reported that BaP after 1 d of exposure accumulated primarily in the yolk-sac of sand sole eggs *(Psettichthys melanostichus)*.

The elimination differed for different xenobiotic

components (Fig. 1). Nph was rapidly eliminated from eggs and larvae while Phe was more slowly eliminated. There was no apparent elimination of BaP and PCB from eggs, and high concentrations of radioactivity remained 12 d after transfer to clean seawater. Radioactivity from all components was transferred to larvae upon hatching (Fig. 1a). The high content of radioactivity in newly hatched larvae is further evidence that most of the radioactivity in eggs prior to hatching was associated with the yolk or embryo.

Nph is more water soluble than the other components; this may explain its rapid uptake by eggs and larvae. Rapid elimination of Nph from eggs and larvae can also be explained in terms of the low lipid/water partition coefficient and the high aqueous solubility of Nph. Although Nph has a high affinity for lipids, its lipid/water partition coefficient favours rapid release to water when the Nph concentration in the medium is reduced (Neff, 1979).

As for the eggs, there was no apparent elimination of PCB from yolk-sac larvae (Fig. 1b). In contrast, there was a clear elimination of BaP-derived radioactivity. This may indicate higher activity of enzymes capable of metabolizing aromatic hydrocarbons in yolk-sac larvae than in eggs. Thus, Sharp et al. (1979) found no metabolism of Nph and chrysene in mummichog embryos. The elimination of Nph and Phe from the eggs in the present study, may be a result of water transport across the chorion, in spite of low water exchange across the cellular membrane of cod eggs as compared to other biological membranes (Riis-Vestergaard, pers. comm.). The results reported by Sharp et al. (1979) indicate decreasing permeability of the embryonic cellular membrane to Nph during development. The cellular membrane is thought to represent the main barrier to diffusion of water and solutes between the medium and the embryonic tissues (Potts and Eddy, 1973). Such a decrease in permeability would presumably not have affected the elimination of Nph-derived compounds from the eggs in the present study, since most of the radioactivity was eliminated during the first 24 h. For Phe, BaP and PCB, a change in the permeability can have influenced their elimination and this may partly explain the high levels of radioactivity remaining during the depuration period in these studies.

The fates of Nph, Phe, BaP and PCB in cod larvae are in general agreement with results obtained for the same compounds in experiments with adult flounders *Platichthys flesus* (Solbakken et al., 1983). Also in flounder the highest accumulation was found with Phe, whereas Nph was most efficiently eliminated. Only a slight elimination of PCB derived radioactivity occurred during 70 d of depuration.

The 9 d old larvae which had absorbed their yolk-sacs accumulated approximately twice as much Phe as the yolk-sac larvae, but the elimination was more efficient (Table 3). This shows that the more advanced physiological development resulted in a higher uptake and indicates a higher enzymatic capacity for metabolizing aromatic hydrocarbons. In newly hatched larvae the mouth is not opened, preventing intragastric uptake of contaminated seawater.

We did not attempt to study the effects of the aromatic components on eggs and larvae. However, the survival of yolk-sac larvae in experimental beakers give some indication as to the toxicity. Thus only 3 larvae were alive 15 d after transfer to clean seawater in the PCB study, whereas more than 20 survived in each of the other experiments.

Eggs and larvae from the same parent fish as in the present study have been used to study behavioral and physiological effects of low levels of the water-soluble fraction and a fraction with boiling point > 150 °C of Ekofisk crude oil (Solberg et al., 1982a, b). These results show that cod larvae exposed to oil contaminated seawater exhibited retarded growth, decreased oxygen consumption rate and reduced ability to capture prey. These clear effects at relatively low oil concentrations may be related to our present findings of low elimination rates of aromatic hydrocarbons from the larvae.

According to our results aromatic hydrocarbons in the environment can easily be accumulated in eggs and larvae of cod, the degree of uptake being dependent on the molecular weight and/or lipophilic characteristics of the xenobiotics. The chorion appears to serve as a barrier for molecules such as PCB's, probably because the hydrocarbons are chlorinated and/or bound to macromolecules or particles in the water. Eggs and young larvae of cod are planktonic and their distribution is patchy and dependent on the hydrographic conditions (Ellertsen et al., 1981). It is likely that fish eggs and larvae will come in contact with relatively high concentrations of xenobiotic components for a short time only. Our results show that even if the exposure time is short, lipophilic xenobiotic components will accumulate and follow eggs and larvae during their development. The high uptake rates and low elimination rates of lipophilic xenobiotics in eggs and larvae may explain the increased toxicity of pollutants in the early part of the cod's life.

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