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DISTRIBUTION AND METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) INJECTED INTO EGGS OF CHICKEN (*GALLUS DOMESTICUS*) AND COMMON EIDER DUCK (*SOMATERIA MOLLISSIMA*)

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Abstract—The present study focuses on the uptake, distribution, and metabolism of 16 polycyclic aromatic hydrocarbons (PAHs) injected into eggs of chicken (*Gallus domesticus*) and common eider (*Somateria mollissima*). Of the total dose of 0.2 ppm, injected on day 4 of incubation, 94% was metabolized within the chicken egg by day 18. The gallbladder showed the highest PAH concentrations of the analyzed organs/structures (gallbladder, liver, kidney, adipose tissue) of the chick and eider embryos. The proportion of the total PAH content present in these organs/structures was higher in the eider embryo (40%) than in the chick embryo (16%). Chick embryos, eider embryos, and wild juvenile eiders had similar PAH concentrations and PAH profiles. The characteristics of this "PAH pool" seemed to be largely unrelated to the relative concentrations of the PAHs to which the birds were exposed, the age of the organisms, and the routes of exposure. The largest studied PAH molecule, coronene, was not taken up from the yolk by the embryo as efficiently as the other PAHs, but once taken up it was metabolized as readily as the others. Basal aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) activities of chick and eider embryos were of similar magnitude.

Keywords—Polycyclic aromatic hydrocarbons *Somateria mollissima* *Gallus domesticus* Metabolism

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are well-known and widely distributed environmental contaminants of which many have toxic, mutagenic, and carcinogenic properties [1–4]. PAHs are formed upon incomplete combustion of organic material such as fossil fuels, which are also the major sources in most urban areas. PAHs are transported throughout the atmospheric environment adsorbed to particles or present in the vapor phase. The accumulation-mode particles (submicron) are of greatest importance, especially in air at remote locations. In the near-source environment, coarse-mode particles also contribute a major part of the PAH load [5,6]. The long-range transport of fine particles can result in "background loads" of PAHs in air at remote locations that are about one-fourth of the loads found in densely populated areas [7]. Crude oil and petroleum products also contain PAHs, but environmental contamination resulting from discharges of these products is usually of a more local character.

Avian exposure to PAHs probably occurs primarily via oral intake. The respiratory intake of atmospheric PAHs by the wild eider duck in remote locations of the Baltic proper was found to be insignificant [8]. Birds generally display high oxidative P-450 enzyme activities, for example, aryl hydrocarbon hydroxylase (AHH) activity [9,10]. The biotransformation capacity of the birds results in low PAH levels in the tissues regardless of the PAH concentrations in their food and despite the high octanol/water partition coefficients (K_{ow}) of the PAH compounds. In a previous study it was shown that the relative PAH concentrations in some organs/structures of the eider duck were, on a dry-weight basis, gallbladder > adipose tissue – liver, and on a lipid-weight basis, gallbladder > liver > adipose tissue [8].

In the present study we describe the distribution, metabolism, and embryo uptake rates of 16 PAHs injected into eggs of chicken (*Gallus domesticus*) and common eider duck (*Somateria mollissima*). These two species were chosen to compare a species widely used in laboratory tests with a wild species. We also have previous data on the embryotoxicity of various PAHs in the two species [1,2].

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Contents of each of the 16 PAHs were also determined in juvenile eider ducks (whole body) collected in their natural breeding habitat. Basal aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) activities (i.e., activities of untreated embryos) in liver and kidney of the chick and eider embryos are also presented.

EXPERIMENT

Sample preparation

A mixture of 16 PAHs, listed in Table 1, was injected into 26 eggs of chicken and 14 eggs of common eider. The PAHs were purchased from Aldrich Chemie (Steinheim, Germany) and Radiant Dyes Chemie (Wermelskirchen, Germany). The proportion of each compound in the mixture was approximately the same (6.25% ± 0.45%). Hens' eggs (white leghorn, shaver) were obtained from Linköpings Kontrollhönseri, Linköping, Sweden. Eider eggs and juvenile eider ducks (approximately four months old) were collected in the open coastal areas of the northern Baltic proper in the spring and autumn, respectively. The eider eggs picked from the nests were all newly laid. All eggs were incubated at 60% relative humidity and 37.5 to 38.0°C, and they were turned every 6 h.

The technique used for egg injection has been described in detail [11,12]. The chicken eggs and eider eggs were preincubated for 4 and 5 d, respectively, before injecting the PAHs, which were dissolved in an emulsion of peanut oil, lecithin, and water. The volumes injected into the yolks of the eggs were 0.1 ml for the chicken eggs and 0.2 ml for the eider eggs. A dose corresponding to 0.2 μg of the PAH mixture per gram egg (wet weight, including shell) was administered to the eggs.

Five of the chicken eggs were frozen immediately after injection (which was made on day 4 of incubation) to determine the amounts of injected PAHs recovered after the chemical workup procedures. The other 21 chicken eggs were incubated for 18 d, whereas the eider eggs (injected and controls) were incubated for 24 d. At the end of the experiment, five chicken eggs were frozen whole. All other eggs were opened, and the embryos were either frozen whole or dissected and the organs frozen separately. All samples were then kept frozen until workup and analysis. The different kinds of analyzed samples are listed in a table later in this paper; two bile samples from eider embryos (treated and control) were also analyzed, but are not listed.

Table 1. PAH amounts in chicken and eider eggs injected with 0.2 ppm of PAH mixture on day 4 and 5 of incubation, respectively; treated chicken eggs from day 18 of incubation; chick and eider embryos from the treated eggs incubated 18 and 24 d, respectively; and juvenile eider duck

PAH		Chicken egg day 4[a] (ng/egg)	Chicken egg day 18 (ng/egg)	Chick embryo (ng/embryo)	Eider egg day 5[b] (ng/egg)	Eider embryo (ng/embryo)	Juvenile eider[c] (ng/duck)
Phe	Phenanthrene	610	50	32	1,300	55	2,200
Ant	Anthracene	400	5.1	N.D.	830	8.7	140
1-mePhe	1-Methylphenanthrene	500	21	N.D.	1,100	7.0	550
Flu	Fluoranthene	610	40	13	1,300	20	1,300
Pyr	Pyrene	540	57	16	1,100	19	770
2-mePyr	2-Methylpyrene	440	8.1	N.D.	920	N.D.	N.D.
BghiF	Benzo[*ghi*]fluoranthene	540	11	1.3	1,100	2.4	100
BaA	Benz[*a*]anthracene	530	17	0.7	1,100	3.6	110
Chr/Tri	Chrysene/triphenylene	640	28	1.8	1,300	11	390
BkF	Benzo[*k*]fluoranthene	510	7.5	0.9	1,100	2.3	55
BeP	Benzo[*e*]pyrene	540	7.7	0.8	1,100	2.0	82
BaP	Benzo[*a*]pyrene	550	8.1	0.4	1,100	1.0	47
Per	Perylene	520	3.7	N.D.	1,100	N.D.	N.D.
Ind	Indeno[1,2,3-*cd*]pyrene	560	16	N.D.	1,200	N.D.	77
BghiP	Benzo[*ghi*]perylene	550	26	2.9	1,100	5.3	120
Cor	Coronene	520	250	13	1,100	20	63
Sum		8,560	550	83	17,800	160	5,930

N.D. = not detected.

[a]Day 4 of incubation = day of injection for chicken eggs.

[b]The content of eider eggs immediately after injection on day 5 of incubation, calculated according to the content analyzed in the chicken eggs on day 4.

[c]Whole-body (without feathers, bones, head, and feet) homogenate of juvenile eiders collected in their natural breeding habitat, northern Baltic proper.

Chemical workup and analysis

Pooled samples of five chicken eggs from day 4 and five from day 18 were separately homogenized without the shells in a mixer. The same homogenization procedure was applied to pooled samples of eight chick embryos and six eider embryos. All organ/structure samples (liver, kidney, gallbladder, and adipose tissue) were pooled samples containing organs/structures from eight embryos (i.e., eight livers = one pooled sample). The analysis of juvenile eider was made on a pooled whole-body homogenate (without the head, feathers, bones, feet, and the intestinal contents) from five ducks. Subsamples of each pooled homogenate were collected for extraction.

To extract all PAHs, the day 4 chicken egg sample and the two eider bile samples were each shaken for 24 h in toluene. All other samples were placed in preextracted cellulose thimbles and Soxhlet extracted with toluene for 24 h. The Soxhlet apparatus was equipped with a Dean–Stark trap for collecting water. All samples were spiked with internal standards (2-methylanthracene, D_{12}-perylene, picene, and dibenzo[*a*,*i*]pyrene), which were added before extraction. The volume of toluene in all samples was reduced after extraction, and after adding 2 M ethanolic (95%) potassium hydroxide (KOH), the solution was refluxed for 2.5 h. After the mixture had cooled, it was centrifuged, and the water phase was removed and washed with toluene. The combined toluene extracts were washed twice with water and once with saturated sodium chloride (NaCl), and first water phase removed was washed again with fresh toluene.

After evaporation of the neutral toluene phase, the residue was subjected to dimethylformamide (DMF) cleanup extraction in two steps (modified version of the cleanup procedure described by Grimmer and Böhnke [13]). In the first step, 4 ml of cyclohexane (C-6) and 4 ml of DMF/H_2O (9:1) were added to the residue, and the solution was shaken and centrifuged. The DMF phase was then removed and 2 ml of new DMF/H_2O was added to the C-6. The solution was then shaken again and centrifuged, and the DMF phase was removed. Six milliliters of water and 4 ml of C-6 were added to the combined DMF phases, after which the solution was shaken and centrifuged, and the C-6 phase was removed. The DMF/H_2O was then repeatedly extracted with 4 ml of C-6. The C-6 phases were combined and the volume reduced. Thereafter the residues were cleaned up on a 100- × 10-mm column packed with 10% deactivated silica, Merck (Darmstadt, Germany) 63-200 μm. *n*-Hexane was used as mobile phase, and the PAHs were collected after the first 10 ml, which contained the alkanes, had eluted.

All samples of organs/structures were analyzed on a GC-MS with electron impact by monitoring the molecular ions recorded at full scan. A Hewlett Packard (Avondale, PA) 3790 GC with a SE-54 capillary column and a JEOL (JOEL Ltd., Tokyo, Japan) JMS-D300 MS with a Finnigan Incos (Finnigan MAT, San Jose CA) data system were used. The extracts from embryos of chicken and eider and the chicken eggs from days 4 and 18 were analyzed on a GC-MS system consisting of a Hewlett Packard 5890 Series II GC with an HP1 capillary column (methyl silicone) and a Hewlett Packard 5971A mass selective detector. The detector was operating in electron impact mode, and the molecular ions of the compounds were monitored at full scan.

Two samples of a PAH standard mixture, consisting of known amounts of all PAHs analyzed in the samples, including the four internal standards, were extracted, saponified, and further cleaned up in the same manner as the two series of samples (i.e., chicken eggs from day 4 and bile comprised the first series; liver, kidney, gallbladder, adipose tissue, whole embryos, and whole eider body comprised the second series). These standard mixtures were then used to establish the response factors of all compounds relative to the internal standard compounds on the GC-MS systems. Before GC injection of the PAH standard mixtures, D_{12}-chrysene was added for recovery estimations. The recoveries of all compounds (including the internal standards) in the PAH standard mixtures were 85 to 95%.

For the two series of samples, procedure blanks were also processed to ensure that no impurities were introduced during sample workup. All solvents were of glass-distilled quality.

The amounts of each of the individual PAH compounds in the samples were determined as the ratio between the area of the molecular ion of the compound and that of the internal standard multiplied with amount of internal standard added and the relative response factor determined (as described above). Before GC injection of the samples, D_{12}-chrysene was added for recovery estimations. The recoveries of the internal standards in the samples were 60 to 110%.

Lipid weights of all samples were determined on subsamples that were shaken for 4 h in a solution of *n*-hexane/acetone (1:1), the organic phase being washed with a 1% NaCl solution. After centrifugation, the water was removed and the *n*-hexane/ac-

etone evaporated under a stream of nitrogen. The dry residue was then weighed. This method for lipid weight determinations is a modification of the method described by Jensen et al. [14].

AHH assay

Kidneys and livers were homogenized in ice-cold, 0.15 M Tris-HCl buffer (pH 7.2) in a Potter-Elvehjem glass/Teflon® homogenizer. AHH activity was determined essentially as described by Nebert and Gelboin [15]. The reaction mixture (total volume 1.0 ml, pH 7.0) contained 60 μmol Tris-HCl, 0.36 μmol NADPH, 0.39 μmol NADH, 3.0 μmol $MgCl_2$, 0.60 mg bovine serum albumin, and 0.1 ml tissue homogenate. The amount of kidney and liver tissue in the reaction mixture ranged from 5.0 to 21.2 mg and from 0.6 to 1.2 mg, respectively. The reactions were started by adding 80 nmol benzo[*a*]pyrene in 40 μl of methanol. After 10 min at 37°C, the reactions were stopped by adding 1.0 ml ice-cold acetone. Following the addition of 3.25 ml of hexane, benzo[*a*]pyrene and its metabolites were extracted into the organic layer by rotating the tubes for 10 min. After centrifugation, 1.0 ml of the organic phase and 1.0 ml of 1 M NaOH were mixed on a vortex mixer for 1 min. The tubes were centrifuged, and the fluorescence of the polar metabolites in the aqueous phase was immediately determined (excitation wavelength 396 nm, emission wavelength 522 nm). Quinine sulfate in 0.1 N H_2SO_4, previously calibrated against 3-hydroxybenzo[*a*]pyrene, was used as standard. The values were corrected for a recovery of 55%. One unit of AHH activity is defined as the amount of enzyme that, in 1 min, catalyzes the formation of enough products to cause a level of fluorescence equivalent to that caused by 1 pmol 3-hydroxybenzo[*a*]pyrene.

RESULTS AND DISCUSSION

PAH distribution

The amounts of PAHs found in chicken eggs on day 4 corresponded to approximately 80% of the theoretically injected amounts (Table 1). The discrepancy between the observed and expected values could have been due to error in the injection volume measurements or to the degradation and/or vaporization of certain PAHs in the mixture during the preparation of the solutions for injection. The recovery of anthracene, which is very sensitive to photooxidation, was lowest (62%). The discrepancy could not, however, be a result of biotransformation of the PAHs because the eggs were frozen immediately after injection. In the following discussion, however, the amounts of PAHs found in the chicken eggs on day 4 are considered to reflect actually injected amounts, and the amounts in the eider eggs have been corrected according to the results from the chicken eggs. The total amount of the 16 PAHs (PAH_{16}) was 8,560 ng per egg for chicken and 17,800 ng per egg for eider (Table 1).

The chicken eggs from day 18 (approximately 3 d before hatching) were found to contain 550 ng PAH_{16} per egg, which corresponds to approximately 6% of the injected amount. Thus by this time, the embryos had metabolized approximately 94% of the PAH_{16} injected into the eggs. The chick embryo itself contained approximately 1% of the injected amount and the eider embryo approximately 0.9%. It was not possible to determine how much of the total injected amount had been metabolized by the eider embryo (on day 24) because no whole eider eggs were analyzed. A comparison of the basal enzyme activities (Table 2) indicates, however, a similar capability of the two species to metabolize PAHs. Basal AHH activities in the liver and kidney of eider showed greater variation than those in chicken, which is not surprising since wild animal species show greater genetic variation than domestic breeds/strains of experimental animals.

Differences in within-embryo PAH distributions between the two species are indicated when comparing organs/structures in terms of the proportion of the embryo PAH_{16} content that they contain. The amount of PAH_{16} of the analyzed organs/structures (i.e., the kidney, liver, and gallbladder with its bile) of the eider represented approximately 40% of the embryo PAH_{16} content, which was 160 ng per embryo. The corresponding figure for the chick embryos was approximately 16% (83 ng PAH_{16} per embryo). This difference was due mostly to a much higher content in the gallbladder of the eider embryo (approximately 21% of the em-

Table 2. Basal aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) activities for chick and eider embryos

Species	Age (days)	Organ	AHH activity,[a] mean ± SD (units/mg min)
Chick embryo	11	Liver	17.9 ± 2.4
	18	Liver	18.3 ± 3.4
	18	Kidney	1.3 ± 0.4
Eider embryo	14	Liver	31.2 ± 9.3
	24	Liver	38.0 ± 16.8
	24	Kidney	2.7 ± 0.9

[a] $n = 8$.

bryo PAH_{16} content) compared to the chick embryo gallbladder (approximately 5% of the embryo PAH_{16} content). The difference might also be the result of dissimilarities in lipid distribution within eider and chick embryos. The whole eider embryo had a lipid concentration of 7.3% (on a wet-weight basis, see Table 3), and about 14% of the total lipid material was found in the liver and kidney. By contrast, the chick embryo had a lipid concentration of 3.3%, and only about 7% of the total lipid material was associated with the liver and kidney. Thus, the somewhat higher lipid concentration in the eider organs might result in higher PAH content in these organs. The lipid contents of the gallbladders of both eider and chick embryos were <0.2% of total lipid content of the embryos. The contribution of the gallbladder itself (without bile) was very small (0.6% of embryo PAH_{16} content) compared to that of the bile in the eider embryos (20% of embryo PAH_{16} content). In the chick embryo the liver contained the highest content of PAHs, followed by the gallbladder (with the bile content). (The bag and bile of the chick embryo gallbladder were not analyzed separately.) The PAH_{16} amounts in the kidney and the gallbladder (without bile) in treated eider embryos did not differ from those of the controls. The only difference that was indicated between the treated eider embryos and the controls was the PAH_{16} contents of the bile and liver, which were higher in the treated embryos. In the bile and liver of the treated eider embryos, 32 ng PAH_{16} per gallbladder and 21 ng PAH_{16} per liver were found, respectively, and for the control embryos the gallbladder contained an amount of 1.7 ng PAH_{16} (no detectable amounts were found in the liver of the controls).

The concentrations of PAH_{16} (nanograms per gram wet weight) of the different organs/structures and adipose tissue samples are presented in Table 4. The highest concentrations in the treated eider and chick embryos were found in the gallbladder, including the bile, whereas lower concentrations were found in the liver, kidney, and adipose tissue. In eider embryo controls, the highest concentrations were also found in the gallbladder and were lower in adipose tissue and kidney.

Table 3. Dry material and lipid contents (fresh weight) of chicken egg and embryo, eider embryo, and juvenile eider duck

	Dry material (% of wet weight)	Lipids (% of wet weight)
Chicken egg, day 4	24	6.9
Chicken egg, day 18	25	5.7
Chick embryo	18	3.3
Eider embryo	23	7.3
Juvenile eider duck[a]	23	2.5

[a]Whole-body homogenate of five eiders (without feathers, bones, head, and feet) collected in their natural breeding habitat, northern Baltic proper.

Biotransformation—comparisons of chemical profiles and PAH concentrations between chick and eider embryos from treated eggs and juvenile eiders

The PAH profiles (relative proportions of the various PAHs) of the chicken and eider organs/structures were similar to those of the whole chick embryo and whole eider embryo, respectively (see Table 1 and Fig. 1 for the embryos). Phenanthrene, fluoranthene, pyrene, chrysene/triphenylene, benzo[*ghi*]perylene, and coronene are the compounds that were found in the highest concentrations in almost all samples. We did not detect coronene in the kidney of either chicken or eider. The major difference between the gallbladder of the eider and chick embryos was that the eider

Table 4. PAH_{16} concentrations of all analyzed samples

Sample	Concentration (ng/g wet weight)
Chicken	
Adipose tissue	6.9
Gallbladder, bag and bile	240
Kidney	7.3
Liver	17
Embryo	4.0
Egg, day 4	190
Egg, day 18	15
Eider	
Adipose tissue	16
Gallbladder, bag and bile	490
Kidney	18
Liver	24
Embryo	4.1
Control	
Adipose tissue	11
Gallbladder, bag and bile	46
Kidney	13
Liver	N.D.
Juvenile eider duck[a]	4.5

[a]Whole-body homogenate (without feathers, bones, head, and feet) of five eiders collected in their natural breeding habitat, northern Baltic proper.
N.D. = not detected.

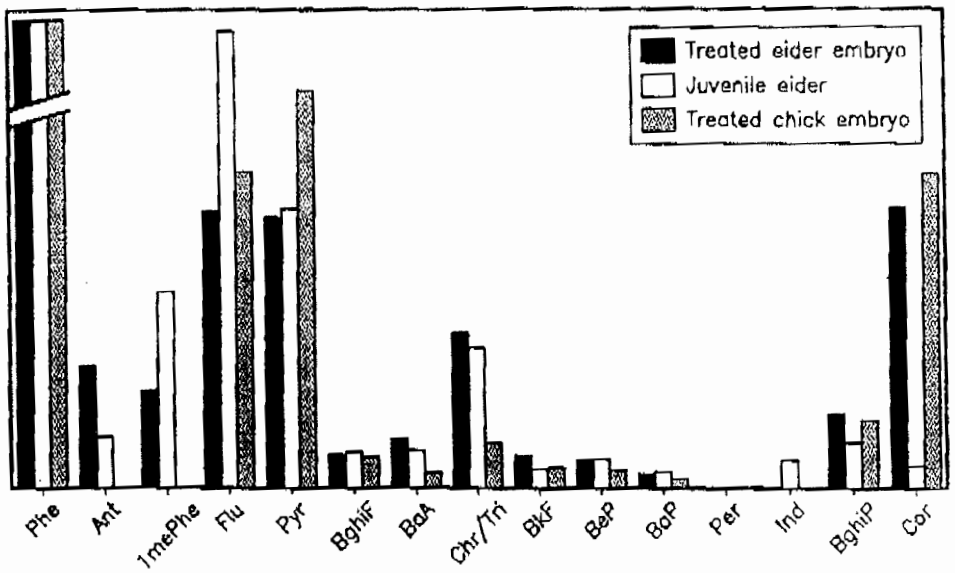

Fig. 1. Relative proportions of the various PAHs (chemical profile) in the eider and chick embryos from treated eggs and in untreated juvenile eiders. (The phenanthrene bar should be multiplied by 1.6.)

gallbladder had higher contents of benz[*a*]anthracene and chrysene/triphenylene than the chicken gallbladder. Concentrations of these two compounds in the gallbladder of the eider embryo were higher than those of any other compound except phenanthrene.

The PAH profile of the pooled sample of whole-body wild juvenile eider duck was similar to that of the eider embryo (Fig. 1). Because the concentration of phenanthrene was higher than the concentration of the other PAH compounds in all three types of samples shown in Figure 1, it was given a relative-content value of 1.0 in the figure. Although the chick embryo PAH profile also shows some resemblance to the eider embryo and juvenile eider profiles, more compounds in the chick embryo were below the detection limit.

Juvenile eiders and the embryos were alike not only in terms of their PAH profiles but also in terms of their PAH_{16} concentrations. On a wet-weight basis, the PAH_{16} concentration of the juvenile eider sample was 4.5 ng/g, which is comparable to the concentrations of the eider embryo (4.1 ng/g) and the chick embryo (4.0 ng/g) (Table 4).

This similarity in profiles, together with the similarities in PAH_{16} concentrations, indicates that a PAH pool with a specific composition became established and was not available for further biotransformation. In other words, for the embryos of the two species and for the juvenile eider, biotransformation seemed to result in similar whole-body PAH concentrations and similar PAH profiles. A similar pool was also found by Sijm and Opperhuizen [16] for dichlorodibenzodioxin in fish in which a certain amount of the compound was not subjected to either metabolic or physical-chemical elimination, which was suggested to be due to a strong binding to a receptor. The PAH mixture to which the eider and chick embryos were exposed had much more uniform concentrations of the various compounds than that to which the juvenile eiders were exposed. Wild juvenile eiders in the northern Baltic proper feed almost exclusively on blue mussels (*Mytilus edulis*), which have a different PAH profile from that of the artificial PAH mixture used for injection [8]. The profile of the pool in the present study thus appeared to be unrelated to the composition of the PAHs (except with regard to coronene, further discussed in the next section) to which the organisms had been exposed, that is, the PAHs in the injection mixture and the mussel meat.

Broman et al. [8] estimated that the juvenile eider ducks were exposed to approximately 44 μg PAH_{16} per day via mussel consumption. Using this figure and the body weight of the juvenile eider (1,600 g whole-body wet weight), we estimated PAH exposure in nanograms per day and gram body weight. For the eider embryo (approximately 39 g wet weight per embryo) and chick embryo (ap-

proximately 21 g wet weight per embryo), it was also possible to estimate the PAH_{16} exposure on a daily basis by assuming that the injected amount was taken up evenly from the yolk during the period between injection and the 24th and 18th days of incubation for the eider and chick embryos, respectively. According to the calculations, daily PAH exposure rates for the eider embryo (24 ng/g/d) and chick embryo (30 ng/g/d) were similar to the exposure rate for juvenile eider (27 ng/g/d). These estimations are however, very rough, as several factors were not considered; for example, embryo weights do not remain constant during the incubation period. Furthermore, the efficiency with which the wild eider takes up PAHs from the mussel meat is probably not 100%, as was assumed in the calculation. Thus, the exposure rates of the embryos were probably higher than that of the juvenile eider duck.

Uptake efficiency

The greatest difference in chemical profiles between the embryos (both species) and the juvenile eider concerned the relative contribution of coronene, which was found to be lower in the juvenile eider duck. This might simply be due to the fact that the coronene content of mussel meat is very low [8]. The observed differences in the relative contribution of coronene would also have been expected if juvenile eiders were less efficient than the embryos at taking up this large molecule. As mentioned earlier, juvenile eiders take up PAHs present in food through the intestinal tract, whereas PAHs in the eggs are probably taken up from the yolk by the yolk-sac membrane and transported to the embryo via the yolk-sac vessels. Perhaps due to its large size, coronene has more difficulty penetrating membranes in the intestinal tract of the juvenile eider than the yolk-sac membrane of the embryos. It has been suggested [17,18] that the efficiency with which molecules are taken up via biological membranes should decrease at a critical steric molecular size of the compound in question. When the minimal cross-section exceeds 0.95 nm, uptake via biological membranes is reduced [17,18]. Miller et al. [19] reported a cross-section of 0.934 nm for coronene, which is close to 0.95 nm and could indicate that the uptake efficiency is somewhat reduced.

Of the total PAH material injected into the chicken eggs, >90% was metabolized (by day 18 of incubation), except for coronene (53%). In the chicken eggs from day 18, a smaller amount of the injected PAHs was still outside the embryo, indicating that it was not yet taken up by the embryo. Although the embryo may excrete some unmetabolized PAHs, which may be deposited in the allantois, the amount excreted is considered to have been negligible. The portion that was taken up by the embryo was determined by subtracting the amount of PAHs outside the embryo on day 18 from the total amount in the chicken egg on day 4, which allows us to calculate the proportion of the total amount of PAHs taken up by the embryo that was metabolized. According to these calculations, 95% of all PAHs, including coronene, was metabolized. Thus, it may be that coronene is not taken up by the embryo as efficiently as the other PAHs, but once it has been taken up it is metabolized as readily as the other PAHs.

The divergent uptake behavior of coronene might also be a result of higher yolk/plasma partitioning for coronene than for the other PAHs. If the solubility of coronene in the blood is lower than that of the other PAHs, its rate of uptake from the yolk to the blood should also be lower. For instance, Hawker and Connell [20] have shown that an increase in the partition coefficient between octanol and water (K_{ow}) is accompanied by an increase in the time needed for equilibrium to be established. For instance, uptake from water by fish of compounds with a log K_{ow} of 6 theoretically require approximately 0.5 year before equilibrium is established, whereas for compounds with a log K_{ow} of 10, a period of about 250 years is needed [20]. Thus coronene, which has the highest log K_{ow} (approximately 7.6–8.0 [5]) of the studied PAHs, probably has the slowest rate of uptake. The log K_{ow} values of the other PAHs in this study are between approximately 4.6 and 7.6 (Phe-Cor) [21].

For compounds that are constantly removed from the circulatory system of the embryos, for example, through lipid storage or biotransformation, equilibrium can never be reached. Consequently, because all PAHs in this study probably are readily biotransformed, they may be taken up from the yolk at a higher rate than would be expected if they were nondegradable. A tetrachlorobiphenyl (log K_{ow} of approximately 5.7–5.8 [22,23]) injected into the yolk was recovered in high concentrations in the yolk at the end of incubation [24]. One reason for this could be that PCB, taken up from the yolk to the blood, are not removed from the system as quickly as the PAHs, which are more readily biotransformed, which means that PCB concentrations in the blood are in a state closer to equilibrium than are PAH concentrations, thereby resulting in a lower continuous uptake of PCBs. This is, however, only plausible if the PCBs are not

continuously removed through lipid storage at the same rates as they are taken up from the yolk.

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