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Embryotoxicity of Polycyclic Aromatic Hydrocarbons (PAHs) in Three Domestic Avian Species, and of PAHs and Coplanar Polychlorinated Biphenyls (PCBs) in the Common Eider

Björn Brunström*

Department of Zoophysiology, Uppsala University, Box 560, S-751 22 Uppsala, Sweden

Dag Broman

Department of Zoology, University of Stockholm, S-106 91 Stockholm, Sweden

& Carina Näf

Department of Analytical Chemistry, University of Stockholm, S-106 91 Stockholm, Sweden

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ABSTRACT

The embryotoxicity of an artificial mixture of 18 polycyclic aromatic hydrocarbons (PAHs) was tested by injection into the yolk sacs of eggs of four avian species: chicken Gallus domesticus, *turkey* Meleagris gallopavo, *domestic duck* Anas platyrhynchos *and common eider* Somateria mollissima. *A dose of 2·0 mg kg egg^{-1} of the PAH mixture increased the mortality among the embryos of all four species. In the domestic duck, but not in the three other species, there was a significantly increased embryonic mortality at a dose of 0·2 mg kg^{-1} of this mixture. All 18 individual compounds in the mixture were tested for embryotoxicity in the chicken. The compound most toxic to chick embryos was benzo[k]fluoranthene. This substance also proved to be highly embryotoxic in the three other species. Previous studies have*

* To whom correspondence should be addressed.

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shown coplanar polychlorinated biphenyls (PCBs) to be much more embryotoxic in the chicken than in other avian species studied. In accordance with this, eider duck embryos proved to be considerably less sensitive to 3,3',4,4'-tetrachlorobiphenyl and 3,3',4,4',5-pentachlorobiphenyl than previously found for chick embryos. For PAHs, however, chick embryos did not have a higher sensitivity than the other species tested.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants. Some of them, e.g. benzo[*a*]pyrene (B[*a*]P), are well-known mutagens and carcinogens. The ultimate carcinogenic metabolites of many PAHs have been identified as the bay-region diol-epoxides (Levin *et al.*, 1982). Among the PAHs are compounds which bind to the Ah receptor and which induce certain cytochrome P-450-dependent enzyme activities, e.g. aryl hydrocarbon hydroxylase (AHH) activity (Bigelow & Nebert, 1982; Piskorska-Pliszczynska *et al.*, 1986). PAHs are formed when organic material is incompletely combusted, fossil fuel combustion being the major source in urban areas. Thus, atmospheric transport constitutes the most important input of PAHs to most ecosystems. Birds are exposed to PAHs directly through the respiratory uptake of atmospheric PAHs in the particulate phase or the vapour phase, or indirectly through the consumption of PAH-contaminated food. A recent study (Broman *et al.*, 1990) on the common eider *Somateria mollissima* suggests that the latter input route strongly predominates. In an organic extract derived from airborne particulate matter, the fraction containing PAHs was found to be highly embryotoxic to chick embryos (Matsumoto & Kashimoto, 1986). Persistent lipophilic substances, such as polychlorinated biphenyls (PCBs), are excreted into the eggs by laying birds. However, biotransformation by the adult birds probably makes this route of excretion less prominent for PAHs.

PAHs are also present in crude oils and refined petroleum products. The overall environmental contamination with PAHs from these products is of less importance than that with the combustion-related PAHs. However, seabirds are frequently exposed to oil spills, and several studies on avian species have dealt with the embryotoxic effects of different types of oils. For example, Albers and Szaro (1978), Hoffman (1978) and White *et al.* (1979) have shown that experimental contamination of eggs of various avian species with crude or refined oils leads to increased embryonic mortality. The aromatic hydrocarbons seem to be the most embryotoxic and teratogenic components of the oils (Hoffman & Gay, 1981; Ellenton, 1982; Walters *et al.*, 1987).

In the study described here, we tested the embryotoxicity of a mixture of 18 PAHs in the chicken *Gallus domesticus*, the turkey *Meleagris gallopavo*, the domestic duck *Anas platyrhynchos* and the common eider *Somateria mollissima*. In addition, the toxicities of all 18 individual PAHs in the mixture were tested in chick embryos. Benzo[*k*]fluoranthene, which was found to be the most toxic in chick embryos, was also tested in the other species. In egg injection experiments, chick embryos have proved to be considerably more sensitive than embryos of other avian species, including turkey and domestic duck, to a coplanar chlorobiphenyl, 3,3′,4,4′-tetrachlorobiphenyl (TCB) (Brunström, 1988; Brunström & Lund, 1988). Because information about the sensitivity of the embryonic eider duck to coplanar chlorobiphenyls is lacking, the embryotoxicities of TCB and 3,3′,4,4′,5-pentachlorobiphenyl were tested in the eider duck in the present study.

MATERIALS AND METHODS

The 18 PAHs tested are all commercially available and were purchased from Aldrich-Chemie, Steinheim, Germany and Radiant Dyes Chemie, Wermelskirchen, Germany. The composition of the mixture is given in Table 2. The amount of each substance in the mixture was similar (4·6–6·5% of total, w/w).

Hens' eggs (White Leghorn, Shaver) were obtained from Linköpings Kontrollhönseri, Linköping, Sweden, while turkey eggs (BUT, Triple 5) were from Kronfågel AB, Degerberga, Sweden. Eggs from the domestic duck (Swedish duck) were purchased from ESF-produkter, Estuna, Sweden. Newly laid eider duck eggs were collected from nests in the archipelago of Stockholm, in the northern Baltic proper. The eggs were incubated at 60% relative humidity and 37·5–38·0°C, and they were turned every 6 h. The duck eggs were sprayed with water once daily.

The substances, dissolved in an emulsion of peanut oil, lecithin and water, were injected into the yolks of the eggs using the technique previously described (Brunström & Örberg, 1982; Brunström & Darnerud, 1983). The hens eggs were preincubated for 4 days prior to injection, and the volume injected was 0·1 ml. The eggs from the other species used have longer incubation periods and are larger than hens' eggs; they were, therefore, preincubated for 5 days and the volumes injected were 0·15 or 0·2 ml (eider).

The toxicity of the PAH mixture was tested at a concentration of 0·2 and 2·0 mg kg^{-1} in eggs of all four species. Chicken eggs were injected with 2·0 mg kg^{-1} of all the individual components in the mixture (0·5 mg kg^{-1} of coronene due to its low solubility). In the case of benzo[*k*]fluoranthene and

indeno[1,2,3-*cd*]pyrene lower doses than 2·0 mg kg^{-1} were also given. The embryotoxicity of benzo[*k*]fluoranthene was also tested in the three other species. Single doses of TCB (1·0 mg kg egg^{-1}) and 3,3′,4,4′,5-pentachlorobiphenyl (0·1 mg kg egg^{-1}) were injected into eider eggs for comparison with previous data on the toxicity of coplanar PCBs in the three other species.

Following injection, the eggs were candled every 2 to 3 days, and eggs containing dead embryos were opened in order to make a gross examination of the embryos. At the end of the experiment, on day 18 of incubation for chick embryos and day 24 of incubation for the other species, the mortality rates were noted and all embryos were grossly examined for external macroscopic malformations and oedema. Thereafter, the embryos were dissected and macroscopic hepatic lesions and pericardial oedemas were noted.

Frequencies of mortality were compared statistically with the corresponding control value using the Chi-square test of heterogeneity or, when the smallest expected frequency was less than 5, the Fisher exact probability test.

RESULTS

In Table 1 the mortality rates after treatment with 0·2 and 2·0 mg kg egg^{-1} of the PAH mixture are shown for the four species studied. At a dose of 2·0 mg kg^{-1}, mortality was significantly increased in all four species. The most pronounced effect was found in the domestic duck, in which all embryos died at this dose, whereas in chick embryos the mortality rate was 40%. At the lower dose tested, 0·2 mg kg^{-1}, a significant effect on survival was observed in the domestic duck, but not in the other species. Several of the chick embryos surviving a dose of 2·0 mg kg^{-1} exhibited abnormalities, including degenerative hepatic lesions (6/12), pericardial edema (5/12), microphthalmia (2/12) and subcutaneous oedema (1/12). Severe edema (subcutaneous and/or pericardial) was noted in three embryos which had died between days 16 and 18 of incubation. One of these embryos also had a shortened upper beak. In the turkey embryos, three cases of microphthalmia (in two dead embryos and one living one) and two cases of shortened upper beak (in dead embryos) were noted at a dose of 2·0 mg kg^{-1}. In the eider and domestic duck only occasional abnormalities were noted.

Toxicities in chick embryos for each of the 18 individual compounds in the PAH mixture are shown in Table 2. The most toxic of the PAHs in the mixture was benzo[*k*]fluoranthene, followed by indeno[1,2,3-*cd*]pyrene. These compounds severely affected the survival of the embryos at a dose of 2·0 mg kg^{-1}, while benzo[*a*]pyrene also significantly reduced survival at this

TABLE 1

Mortality in Embryos of the Chicken, Turkey, Domestic Duck and Common Eider Treated with a Mixture of 18 Polycyclic Aromatic Hydrocarbons. The Mixture was Injected into the Yolks of Chicken Eggs Preincubated for 4 Days, and Mortality was Measured on Day 18. For the Other Species the Corresponding Periods were 5 and 24 Days, Respectively

Species	*Dose* ($mg\ kg\ egg^{-1}$)	*Embryonic mortality*	
		Number of dead embryos/ Total number	*%*
Chicken	0	1/20	5
	0·2	1/20	5
	2·0	8/20	40**
Turkey	0	4/18	22
	0·2	4/18	22
	2·0	15/18	83***
Domestic duck	0	0/22	0
	0·2	7/22	32**
	2·0	22/22	100***
Eider	0	0/17	0
	0·2	3/17	18
	2·0	16/17	94***

** Significantly different from the corresponding control value (Fisher exact probability test, $P < 0{\cdot}01$).

*** Significantly different from the corresponding control value (χ^2-test, one-tailed, $P < 0{\cdot}001$).

dose. Many of the embryos treated with these more toxic components exhibited abnormalities similar to those noted in embryos exposed to the mixture. Although no significant increase in embryonic mortality was found in embryos treated with benz[*a*]anthracene or chrysene, more than 50% of these embryos had some form of lesion.

Also in the turkey, domestic duck and eider, benzo[*k*]fluoranthene was more toxic than the complete PAH mixture (Table 3). Three cases of microphthalmia and shortened upper beak (in two dead embryos and one living one) were observed in the turkey embryos exposed to $0{\cdot}2\ mg\ kg^{-1}$ of benzo[*k*]fluoranthene. No such malformations were noted in the control embryos of any species or in the benzo[*k*]fluoranthene-treated ducks and eiders.

At the doses of TCB and 3,3′,4,4′,5-pentachlorobiphenyl administered to the eider eggs, no significant increase in embryo mortality was detected (Table 4).

TABLE 2

Mortality in Chick Embryos Treated with Each of the 18 Polycyclic Aromatic Hydrocarbons Constituting the Artificial Mixture. The Relative Concentration, by Weight, of Each Substance in the Mixture is Given in Parentheses. The Compounds were Injected into the Yolks of Eggs Preincubated for 4 Days, and Mortality was Measured 2 Weeks Later (day 18)

Compound	*Dose* (*mg kg egg*$^{-1}$)	*Embryonic mortality*	
		Number of dead embryos/ Total number	*%*
Fluorene (5·4%)	2·0	1/20	5
Phenanthrene (5·7%)	2·0	0/20	0
Anthracene (5·3%)	2·0	0/20	0
1-Methylphenanthrene (5·2%)	2·0	0/20	0
Fluoranthene (5·6%)	2·0	4/20	20
Pyrene (5·6%)	2·0	0/20	0
2-Methylpyrene (4·6%)	2·0	1/20	5
1-Methylpyrene (5·4%)	2·0	0/20	0
Benzo[*ghi*]fluoranthene (5·4%)	2·0	3/20	15
Benz[*a*]anthracene (5·5%)	2·0	2/20	10
Chrysene (5·5%)	2·0	3/20	15
Benzo[*k*]fluoranthene (5·7%)	2·0	20/20	100***
	0·2	5/20	25
	0·05	0/20	0
Benzo[*e*]pyrene (5·8%)	2·0	0/20	0
Benzo[*a*]pyrene (5·6%)	2·0	6/20	30*
Perylene (5·3%)	2·0	0/20	0
Indeno[1,2,3-*cd*]pyrene (6·0%)	2·0	17/20	85***
	0·5	2/20	10
Benzo[*ghi*]perylene (5·8%)	2·0	1/20	5
Coronene (6·5%)	0·5[a]	0/20	0

Several experiments were performed, and in each experiment control eggs were injected with the vehicle. In all experiments, mortality in the controls was 0/20 or 1/20.

[a] The solubility of coronene in the injection medium was too low to permit a dose of 2·0 mg kg^{-1}.

* Significantly different from the corresponding control value (Fisher exact probability test, $P < 0{\cdot}05$).

*** Significantly different from the corresponding control value (χ^2-test, $P < 0{\cdot}001$).

TABLE 3

Mortality in Embryos of the Chicken, Turkey, Domestic Duck and Common Eider Treated with Benzo[*k*]fluoranthene. The Substance was Injected into the Yolks after 4 Days (Chicken) or 5 Days Preincubation of the Eggs, and Mortality was Measured on Day 18 (Chicken) or Day 24

Species	*Dose* ($mg\ kg\ egg^{-1}$)	*Embryonic mortality*	
		Number of dead embryos/ Total number	*%*
Chicken	0	1/20	5
	0·05	0/20	0
	0·2	5/20	25
	2·0	20/20	100***
Turkey	0	2/20	10
	0·2	13/20	65***
	1·0	20/20	100***
Domestic duck	0	0/20	0
	0·2	20/20	100***
	2·0	20/20	100***
Eider	0	2/18	11
	0·05	2/18	11
	0·2	8/18	44*
	1·0	16/18	89***

* Significantly different from the corresponding control value (χ^2-test, one tailed, $P < 0{\cdot}05$).
*** Significantly different from the corresponding control value (χ^2-test, one tailed, $P < 0{\cdot}001$).

TABLE 4

Mortality in Embryos of the Common Eider Treated with 3,3′,4,4′-Tetrachlorobiphenyl or 3,3′,4,4′,5-Pentachlorobiphenyl. The Substances were Injected into the Yolks after 5 Days Preincubation of the Eggs, and Mortality was Measured on Day 24

Treatment	*Dose* ($mg\ kg^{-1}$)	*Embryonic mortality*	
		Number of dead embryos/ Total number	*%*
Vehicle	0	4/20	20
3,3′,4,4′-tetrachlorobiphenyl	1·0	4/20	20[a]
3,3′,4,4′,5-pentachlorobiphenyl	0·1	7/20	35[a]

[a] Not significantly different from the control value ($P > 0{\cdot}05$).

DISCUSSION

The artificial mixture of 18 PAHs tested appears to be approximately potent in causing embryolethality and malformations in chick embryos as the previously tested technical PCB preparation Aroclor 1248 (Brunström & Örberg, 1982). The various PAHs in the artificial mixture seem to differ substantially in toxicity to avian embryos. There are also great differences in toxicity to chick embryos between the individual chlorobiphenyls in technical PCB mixtures. Certain non-*ortho*-chlorinated chlorobiphenyls are the most toxic PCB congeners in chick embryos (Brunström & Andersson, 1988; Brunström, 1989), as well as in mammalian species (Safe, 1984). They are, in their coplanar conformations, approximate isostereomers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and are ligands to an intracellular receptor protein, designated the Ah receptor. The toxicity of TCDD and its congeners is considered to be mediated, at least partly, via binding to the Ah receptor (Poland & Knutson, 1982). The individual compounds in the artificial PAH mixture which caused embryolethality and/or a high frequency of lesions at a dose of 2·0 mg kg^{-1} (benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene) all are known to bind to the rat or mouse Ah receptor (Bigelow & Nebert, 1982; Toftgård *et al.*, 1985; Piskorska-Pliszczynska *et al.*, 1986), and the compound with highest affinity for the receptor (benzo[*k*]fluoranthene) was the most toxic to the embryos. Some of the other substances in the mixture also bind to the receptor, although less avidly than the five most toxic compounds. This indicates that a similarity exists between the mechanism for the toxicity of the most potent PAHs and that of coplanar chlorobiphenyls in chick embryos. A common mode of action is further supported by the similarity in abnormalities, including liver lesions, oedema, shortened beak and microphthalmia, occurring in chick embryos treated with coplanar PCBs (Brunström & Darnerud, 1983; Brunström, 1988) and, as found in the present study, with the PAH mixture and its most toxic components.

The turkey has previously been shown to be less sensitive than the chicken to the embryotoxicity of coplanar PCBs, and the domestic duck is even less sensitive than the turkey (Brunström, 1988; Brunström & Lund, 1988; Brunström, 1989). A low sensitivity to these PCBs was also found for the eider in the present study. However, embryos from these species seem to be at least as sensitive to the PAHs studied as chick embryos. In all four species, benzo[*k*]fluoranthene was more toxic than the complete PAH mixture. If it is assumed that binding to the Ah receptor is involved in the mechanism by which benzo[*k*]fluoranthene causes toxicity, then it becomes difficult to explain why the turkey, domestic duck and eider all are relatively insensitive,

compared with the chicken, to Ah receptor ligands such as the coplanar PCBs. The presence of the Ah receptor was demonstrated in 7-day-old chick embryos, while it was not detected in 9-day-old turkey embryo liver (Brunström & Lund, 1988). To the best of our knowledge there is no information about the Ah receptor in domestic duck or eider.

PAHs are more easily metabolized than the halogenated aromatic hydrocarbons, and the bulk of biotransformation leads to less toxic metabolites which are conjugated and excreted. As intermediates in the metabolic transformation, however, highly reactive arene oxides are formed (Jerina & Daly, 1974; Conney, 1982). Chick embryos are able to metabolize benzo[*a*]pyrene at an early stage of development (Hamilton *et al.*, 1983; Brunström, 1986). However, the basal benzo[*a*]pyrene hydroxylase activity in early turkey embryos seems to be higher than that of chick embryos at a comparable developmental stage (Brunström & Lund, 1988). It is thus improbable that a higher rate of biotransformation in chick embryos, compared with embryos from the other species studied, compensated for a higher sensitivity of the chick embryos to the PAHs injected in the present experiment.

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