EFFECT OF POLYCYCLIC AROMATIC HYDROCARBONS ON HEPATIC EROD INDUCTION IN EXPERIMENTALLY DOSED AND ENVIRONMENTALLY EXPOSED SHREWS (*CROCIDURA RUSSULA / SOREX ARANEUS*).

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INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are wide spread environmental contaminants, in majority considered to originate from incomplete combustion of organic material at high temperature. Important sources for emission of PAHs into the environment are industry (e.g. oil refinery, cokes production, anode production for aluminum industry), timber preservation, combustion/heating, and traffic. PAHs have been subject of extended study due to the carcinogenic properties of some of these compounds and their potential hazard to man. However, little is known about the fate of PAHs in terrestrial ecosystems and their effects on wildlife. This paper focusses on the potential hazards of PAHs in the higher levels of the foodchain in the terrestrial ecosystem. As a model for carnivorous terrestrial predators we study the common shrew (*Sorex araneus*) and white toothed shrew (*Crocidura russula*).

A number of studies with mammals show that they efficiently metabolize PAHs. As a consequence the parent compounds are detected only at relatively low levels in species higher in the foodchain. However, exposure levels can be considerable and it is questioned if these exposures can result in adverse effects in wildlife species. Recent studies by van Brummelen (1995) showed that PAH concentrations in earthworm (*Lumbricus rubellus*), which is a major food item for shrews, along a transect near a blast furnace plant ranged from 20 ng/g (background) to 92 ng/g fat weight (near blast furnace). At present we study the effect of these varying concentrations in the food items on the common shrew along this transect. In addition to this field survey, we study the effect of experimental exposure to Benzo[α]pyrene (BαP) in a related species, the white toothed shrew. In a number of studies as reviewed earlier (Bosveld and van den Berg 1994) the induction of mixed function oxidase (MFO) enzymes has been proven a biomarker for exposures and effects of halogenated aromatic hydrocarbons. PAHs have been shown to induce EROD activity as well (Brunström et al 1991). Our present efforts focus on effects of PAHs on hepatic MFO enzymes in relation to neurotoxicity, immunotoxicity and effects on reproductive capacity in shrews and the potential of this effect as a biomarker for PAH toxicity in terrestrial wildlife species. In this paper we report on the effects on the induction of EROD. Other effects are currently under investigation and will be reported later.

MATERIALS AND METHODS

Experimental dosing study

The experimental dosing study was conducted with three groups of three female common shrews. The animals were housed individually in polyethylene boxes (60x40x27 cm) with an open top. Drinking water was supplied *ad libitum* and food (Felix catfood hearth/liver) was supplied on a fixed bases: Daily at 4 pm 2 g of contaminated food and at 11 pm, when all the contaminated food was consumed, 15 g of normal food. The contaminated food contained 50 μg BαP/g in the low dosed group and 500 μg BαP/g in the high dosed group. The controls received at 4 pm food mixed with the carrier only (50 μl peanut oil/g). The dosing resulted in a daily intake of 0, 100, and 1000 μg BαP per day (approximately 0, 10, and 100 mg BαP/kg bodyweight) in the control, low dosed and high dosed group respectively. After 9 days the experiment was terminated.

Field survey

The fieldwork for the survey was conducted in februaary 1996. During 4 nights longworth lifetraps were placed at three different locations at increasing distances from a blast furnace plant (0.4 km, 1.2 km, and 4.5 km for location 1,2 and 3 respectively). The same locations were selected as by van Brummelen (1995), who measured PAH concentrations in soil and soilevertebrates (earthworms and isopods) and found significantly decreasing concentrations with increasing distance from the plant. At each location 60 lifetraps were placed. All traps were examined for catches early in the evening, after midnight and early in the morning. During the whole period 4, 3 and 6 specimen of *sorex araneus* were trapped at the locations 1, 2 and 3 respectively. Trapped animals were transported to a fieldlaboratory where they were sacrificed.

Dissection and hepatic EROD measurement

The shrews were narcotisized using diethylether, blood was taken by hearth puncture, the liver was dissected, immediately frozen and stored at -80°C prior to microsome preparation. The thyroid, spleen, kidneys, ovaria and brains were dissected weighed and stored for later histopathalogical analysis. Microsomal preparation from livertissue was performed basically according to the methods as described before (Bosveld et al 1995). Approximately 0.5 g of liver was homogenized in a 10 ml glas-teflon homogenizer. The homogenate was centrifuged in a Beckman L-60 ultracentrifuge at 15.000 *g* during 12 min. The resulting supernatant was centrifuged twice at 150.000 *g* during 70 min at 4°C. Microsomal EROD activities were assayed in 24-wells plates from Costar (Costar Europe Ltd, The Netherlands). Incubation mixtures were 25 μl 7-ethoxyresorufin 50 μM, 375 μl TRIS, 50 μl BSA 10 g/ml and 25 μl microsomal suspension. The enzymatic metabolism was started by adding 25 μl NADPH. After exactly 10 min. the reaction was stopped by adding 1 ml icecold methanol. The formation of resorufin was measured fluorimetrically (ex/em = 530/590) on a Cytofluor II from PerSeptive Biosystems. Rhodamin (Sigma) was used as a standard. Microsomal protein content was measured with the fluorescamine assay, basically as described by Lorenzen and Kennedy (1993). 100 μl fluorescamine (0.3 g/l in aceton) was added to each well and fluorescence (ex/em = 360/460) was measured after 5 min. BSA (purety 99% from Sigma) was used as a standard.

RESULTS

Experimental dosing study

Average bodyweight gain (BWG) during the experimental period was positive in the control and low dosed group, but negative in the high dosed group. The liver somatic index (LSI= ratio liverweight/bodyweight) was increased in both dosegroups. However, for none of these effects significant differences were detected (table 1). Hepatic EROD activity was significantly 2 times induced in both the low dosed and high dosed group respectively, compared to the controls (table. 1).

table 1. Body and liver weight and hepatic EROD activity in white toothed shrew (*Crocidura russula*) exposed to various concentrations of BαP in the food (n=3). BWG = bodyweight gain during experimental period. LSI = liver somatic index.

Dose group	initial bodyweight (g)	terminal bodyweight (g)	BWG (g)	terminal liverweight (mg)	LSI (%)	EROD (pmol/min.mg)
control	9.8±1.0	10.1±1.6	0.34±0.73	531±29	53±6	670±116
0.1 mg BαP/day	8.6±0.8	9.2±1.0	0.53±0.17	537±79	58±3	1328±83
1.0 mg BαP/day	8.9±0.4	8.1±1.1	-0.87±0.79	462±73	57±2	1265±204

Field survey

The average bodyweight differed among the locations and was highest at the location nearest to the blast furnace plant (7.3±0.7 g). No significant differences among the locations were found for liverweight, LSI or EROD activity.

Table 2. Terminal body and liver weight and hepatic EROD activity in common shrews (*Sorex araneus*) from various locations with increasing distance to the blast furnure plant. Shown are averages and standard deviations (n=3).

Location	distance to plant (km)	bodyweight (g)	Liverweight (mg)	LSI (%)	EROD (pmol/min.mg)
1 (n=4)	0.4	7.3±0.7	548±101	76±14	158±6
2 (n=3)	1.2	6.3±0.5	475±49	76±12	113±94
3 (n=7)	4.5	6.3±0.1	542±100	86±15	180±62

DISCUSSION AND CONCLUSIONS

As shown in our experimental dosing study, EROD activity is induced 200% in both the low and high dosed group (BαP doses equivalent to 10 and 100 mg/kg bodyweight per day respectively) when compared to the controls. This result suggests that the maximum induction is achieved at BαP doses equivalent to 10 mg/kg/day. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) resulted in an >800% induction of EROD activity following a dose of 10 µg/kg.day during 7 days (unpublished data). These differences between the maximum inducing activity of BαP and TCDD may be explained in part by the rapid metabolism of BαP compared to TCDD. However, internal doses of BαP and its metabolites have to be measured to validate this hypothesis. In addition, it is suggested that low binding capacities of BαP to the Ah receptor and successive interactions of the receptor-ligand complex with the DNA may be due to the relatively low maximum inducing potency of this compound. The results from our field survey show that EROD activity in environmentally exposed shrews is not related to the distance from a blast furnace plant as a source of PAHs. It is suggested that the present levels of PAHs in the food for shrews near the blast furnace plant and as analyzed by van Brummelen (1995) are not important as EROD inducing agents. However, this conclusion is based on a limited number of specimen analyzed and further sudies are needed to validate this conclusion.

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