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DDE-Induced microsomal mixed-function oxidases in the puffin (*Fratercula arctica*)

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Hepatic microsomal mixed-function oxidases of different avian species respond to organochlorine insecticides in various ways. Davison and Sell [1] found, for example, that DDT pretreatment decreased aniline hydroxylase activity in chicken liver microsomes, but increased this activity in duck liver microsomes. Although a considerable volume of work has been done concerning enzyme induction of hepatic mixed-function oxidases in birds [2–5], it has been largely confined to domesticated species. The sole exception is a study on the American kestrel (*Falco sparverius*) [6]. In view of the observed species-to-species variation it is desirable that studies designed to elucidate possible environmental effects be carried out on the species concerned rather than extrapolating from others. This report is part of a series of studies undertaken to evaluate the effect of toxicants on a variety of physiological mechanisms in seabirds [7]. In this note the effects of DDE, a metabolite of DDT that occurs as a widespread environmental contaminant, on some xenobiotic-metabolizing enzyme activities of microsomes prepared from puffin liver are reported. The puffin is a fish-eating pelagic species of the auk family for which large declines in population have recently been noted on both sides of the North Atlantic [8, 9]. This report describes the response of puffin hepatic microsomal aniline hydroxylase and benzphetamine demethylase activities to oral dosing of puffins with DDE.

Immature puffins (approximately 40-days-old) were dosed orally by intubation with DDE dissolved in corn oil (experimental birds) or with corn oil alone (control birds) for 16–21 days before sacrifice. The DDE administered was selected to approximate 50 ppm in the diet, based on a total daily intake of 120 g fish per day. (Thus, each treated bird received approximately 6 mg daily). Birds were maintained in artificial burrows made of tile until the pre-fledging starvation phase had started when they were brought into the laboratory. This pre-fledging starvation phase is a normal physiological change that occurs a few days before fledging and is not influenced by the presence of food. This lack of feeding caused mobilization of DDE previously stored in the fat and this release was enough to cause toxic manifestations in the treated birds.

After decapitation of puffins, an aliquot of liver, minus the gall bladder, was immediately removed and frozen. Liver was assayed for mixed-function oxidase activity within 48 hr of sacrifice. Use of this procedure has not resulted in significant loss of mixed-function oxidase activity in any of the mammalian or marine species we have tested.

Prior to microsome preparation, the liver aliquots were placed in ice-cold 0.15 M KCl adjusted to pH 7.5 with HEPES-NaOH buffer and were allowed to thaw slowly. All subsequent steps were carried out at 0–4°C. The livers were minced with scissors and homogenized in a glass Pot-

Table 1. Effect of DDE administration on the hepatic microsomal mixed-function oxidase system of the Puffin and on tissue residues.

	Puffin		
	Control	DDE-fed[1]	P[2]
Yield Microsomal Protein (mg/g liver)	22.2 ± 2.2 (4)[3]	24.2 ± 1.0 (5)	N.S.
Aniline Hydroxylase (nmoles/min/mg protein)	0.19 ± 0.05 (4)	0.70 ± 0.10 (5)	< 0.05
Benzphetamine Demethylase (nmoles/min/mg protein)	1.05 ± 0.19 (4)	5.46 ± 0.45 (5)	< 0.05
Residual DDE in liver (ppm dry weight)	4.9 ± 1.8 (3)	2037 ± 586 (4)	< 0.05

[1] Puffins dosed orally with DDE in corn oil for 16–21 consecutive days (amount selected to approximate 50 ppm in diet). Controls received only corn oil.
[2] Statistical comparisons made using the nonparametric Mann-Whitney U test [14].
[3] Mean ± S.E.M. (*n*).

ter–Elvejhem homogenizer having a motor driven teflon pestle. Four passes of the pestle (from top to bottom of the homogenizer) were used. The final homogenate was diluted to 33 1/3 percent (w/v) by addition of buffered ice-cold 0.15 M KCl. Cell debris, nuclei and mitochondria were removed by centrifugation at 10,000 *g* for 20 min. The 10,000 *g* supernatant was carefully aspirated into a syringe so that the fat layer was left behind. Microsomes were isolated from the 10,000 *g* supernatant by sedimentation at 177,700 *g* for 40 min (Beckman 60 Ti rotor, 50,000 rpm). The resulting pellet was washed by resuspension in buffered 0.15 M KCl and resedimentation at 177,700 g for 20 min. The washed microsomal pellets were resuspended in KCl to a final protein concentration of 10–20 mg/ml. The Lowry procedure was used for protein measurement [10].

Aniline hydroxylase activity was measured by determination of the *p*-aminophenol formed [11] and *d*-benzphetamine demethylase activity by determination of the formaldehyde produced using a modified Nash procedure [12]. Incubation mixtures (2.5 ml) used to measure aniline hydroxylase activities contained 3.75 mg microsomal protein, 0.1 M HEPES buffer, (pH 7.6), 8 mM aniline HCl and an NADPH-generating system consisting of 1.0 mM NADPH, 5 mM $MgSO_4$, 5 mM glucose 6-phosphate and 2 units glucose 6-phosphate dehydrogenase. Those used to determine benzphetamine demethylase activity (final vol. 2.5 ml) varied only in that *d*-benzphetamine HCl (3.0 mM) replaced aniline HCl and 5 mM semicarbazide HCl was included in the incubation mixture. Reactions were initiated by the addition of substrate in both cases.

Incubations were carried out at 37° for 15 min in air in a Dubnoff metabolic shaker whose shaking rate was 100 rpm. Analysis of DDE residue levels in liver was carried out as previously described [13].

As shown in Table 1, hepatic microsomal aniline hydroxylase and benzphetamine demethylase activities in the immature puffin were significantly increased (2.7- and 5.2-fold, respectively) by animal pretreatment with DDE. The DDE treatment, at our dosage levels, did not cause significant increases in liver weight nor in the yield of microsomal protein on a per gram basis (Table 1).

In conclusion, we have demonstrated that the xenobiotic-metabolizing enzyme system of puffin liver is readily induced by DDE treatment. To our knowledge, no previous studies of this type have been carried out with any species of sea bird. Unfortunately, the mobilization of DDE and tissue wasting caused by the pre-fledging starvation makes it impossible to compare the degree of induction with that which has been found in other avian species.

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