We conclude that there is no evidence to suggest that the thinning of egg-shells by DDT or DDE in ducks is due to the inhibition of carbonic anhydrase.

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1974 #23

EFFECTS OF DDE ON PLASMA OSMOREGULATION AND NASAL GLAND Na-K-ATPASE IN THE BLACK GUILLEMOT *Cephus grylle* AND THE COMMON PUFFIN *Fratercula arctica*

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To assess the possibility that organochlorine pesticide disruption of osmoregulation is responsible for recent large kills of young sea birds (e.g., shearwaters and puffins), we previously studied the effect of DDE feeding (10 - 250 ppm) on plasma osmoregulation and nasal gland function in mallard and white Pekin ducks (both *Anas platyrhynchos*) and found that neither of these closely related strains was sensitive enough to demonstrate conclusive DDE effects at environmental levels (Miller, et al., Bull. MDIBL, 13: 77, 1973; Fed. Proc. 33: 220, 1974). Since the genus *Anas* are not strictly marine we speculated that, due to species differences, increased sensitivity might be found in true pelagic species, members of the genus *Alcidae*. The present study was undertaken to determine the effect of DDE feeding on osmoregulation and nasal salt gland Na-K-ATPase in two alcids, the black guillemot, *Cephus grylle*, and the common puffin, *Fratercula arctica*.

Sixteen black guillemots approximately 25 days old were collected from nests on Old Man Island off Cutler, Maine and transported to the Laboratory

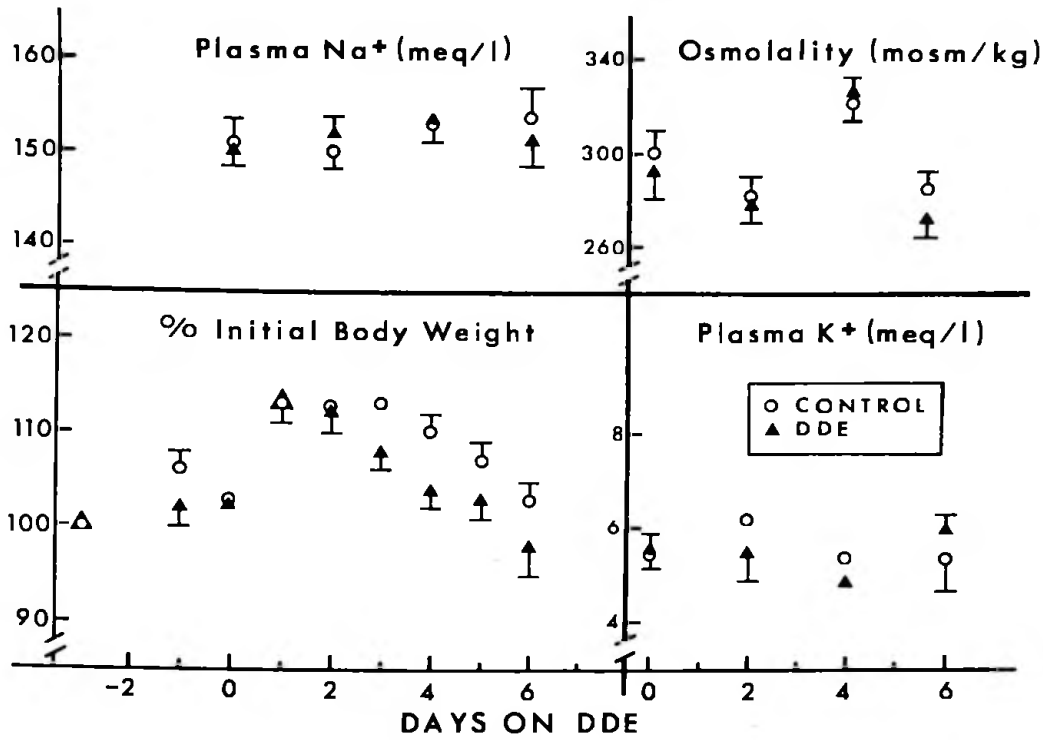

Figure 1: Effect of oral DDE dosing on body weight and plasma composition in paired groups of guillemots. Experimental birds were given daily doses of DDE calculated to approximate 50 ppm (days 0 - 4) and 100 ppm (days 5, 6) of the diet. Day zero values are prior to the start of DDE dosing. Each point represents the mean value derived from eight birds; when large enough, variability is indicated by SE bars.

where they were maintained in metal cages in a partially darkened, air-conditioned room (approximate temperature 16° C), and fed live killifish. Birds were weighed daily and blood samples taken from a wing vein every other day. After five days birds were assigned to control or experimental treatment (eight birds each) and twice daily thereafter each bird received an oral dose of DDE dissolved in corn oil (experimental group) or corn oil alone (controls). Initially the amount of DDE fed was selected to approximate 50 ppm in the diet, a level which equals or exceeds the maximal DDE

concentration found in the environment. After three days of dosing the DDE level was raised to 100 ppm. Although both experimental and control guillemots initially gained weight, after dosing began they stopped eating and began to lose weight (Figure 1). We suspect additional handling during corn oil feeding may have been a factor. After six days of dosing one control bird died and others (both control and DDE fed) exhibited signs of weakness. We therefore terminated the experiment.

Common puffins approximately 14 days old were collected in mid-July on Great Island, Newfoundland and flown to Eastern Egg Rock in Muscongus Bay where they were housed in individual tile burrows and fed frozen smelts. On August 7 the first blood samples were taken and birds were assigned to experimental (50 ppm DDE) or control treatments (5 birds to each treatment). Birds were maintained on Egg Rock and dosing in corn oil began as described for guillemots. One week later additional blood samples were taken and two control birds that had started fasting were brought back to the Laboratory (Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine). At MDIBL puffins were maintained as described above for guillemots except that they were given sea water to drink. On August 22 all eight remaining birds were removed to MDIBL. At this time three additional birds were fasting; within one week the remaining puffins had stopped eating. After only five to seven days of fasting two experimental -- DDE-fed -- birds died and two other experimentals were near death. In contrast controls exhibited few signs of weakness when they were arbitrarily sacrificed after six to eleven days of fasting.

At the time of sacrifice guillemots and puffins were decapitated and the nasal glands removed, weighed, homogenized, and freeze-dried for Na-K-ATPase determination. Tissue samples were also set aside for DDE residue

analysis (data not yet available).

Although DDE feeding tended to increase body weight loss in both guillemots (Figure 1) and puffins (data not shown), it had no effect on plasma osmolalities or electrolyte concentrations in either species (Figures 1 and 2). Even though DDE was clearly toxic to the fasting puffin (see above), plasma sodium concentrations in two moribund birds were not abnormally high (154, 156 mEq/l). Thus it is unlikely that these puffins were dying as a result of osmoregulatory failure.

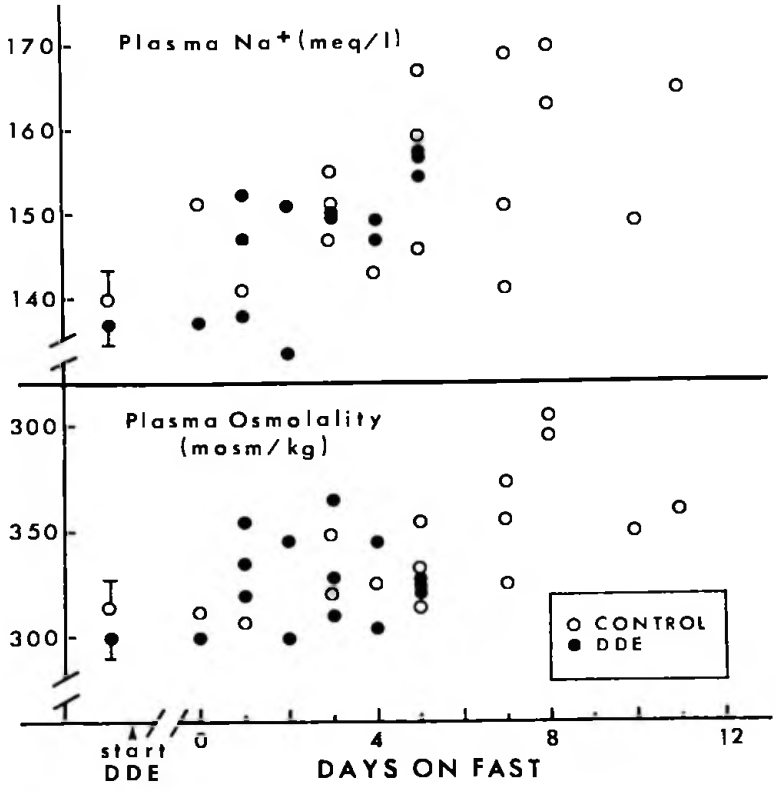

Figure 2: Effect of oral DDE dosing (approximately 50 ppm of pre-fasting diet) on plasma osmolalities and sodium concentrations in fasting puffins. DDE dosing began approximately 2 weeks before birds started fasting. Each point represents a single determination from one bird except for the initial points, which represent the mean ± SE for the experimentals and controls (5 in each group) before dosing began.

TABLE 1

EFFECTS OF DIETARY DDE (50 - 100 ppm) ON NASAL GLAND WEIGHT AND Na-K-ATPase IN GUILLEMOTS AND PUFFINS*

	Control	DDE Fed	P
Guillemot			
Enzyme Specific Activity (µmoles P_i/mg protein/hr)	49.9±6.8(5)	34.0±4.2(5)	>.05
Gland Weight (mg)	75.3±7.4(8)	71.2±5.2(8)	>.05
Total Gland Activity† (µmoles P_i/nasal gland/hr)	183.0±22.0(5)	125.0±23.0(5)	>.05
Puffin			
Enzyme Specific Activity (µmoles P_i/mg protein/hr)	45.8±1.5(5)	26.8±1.7(5)	<.01
Gland Weight (mg)	151.0±17.0(5)	239.0±22.0(5)	<.02
Total Gland Activity† (µmoles P_i/nasal gland/hr)	419.0±83.0(5)	324.0±71.0(5)	>.05

*Values given are for one of the pair of glands. Data expressed as Mean ± SE (n), where n is the number of birds.
†Calculated from specific activity, gland weight, and approximate protein content of gland. DDE feeding did not affect nasal gland protein content.

Guillemot Na-K-ATPase activity was not significantly affected by DDE treatment (Table 1). In contrast puffin enzyme specific activity (per mg gland protein) was reduced in experimental birds. Total gland activity however was not affected by DDE feeding due to compensatory hypertrophy of nasal gland tissue (Table 1). In terms of the comparative pharmacology of the nasal gland Na-K-ATPase the discovery of an *in vivo* inhibitory effect of

DDE in the puffin and the absence of an in vivo effect in the duck (Miller, et al., Bull. MDIBL, 13: 77, 1973) correlates well with the higher sensitivity of the puffin ATPase to DDE added in vitro (Miller and Kinter, unpublished observations). A similar comparison cannot be made for the guillemot enzyme due to the short DDE dosing period.

In conclusion DDE feeding does not affect plasma osmoregulation or nasal gland function in fasting, immature puffins and guillemots. These findings for two pelagic species, one of which (the puffin) is declining in population (Flegg, Bird Study, 19: 7, 1972) provide strong evidence against the osmoregulatory failure hypothesis. Our results in the puffin however do suggest that organochlorine toxicity, at a site other than the nasal gland, cannot be ruled out as a possible contributing factor in seabird kills.

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POSSIBLE ENZYMATIC BASIS OF DDE-INDUCED EGGSHELL THINNING IN THE WHITE PEKIN DUCK, *Anas platyrhynchos*

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Although the occurrence of DDE-induced eggshell thinning is well documented in many avian species, the biochemical mechanism of action remains unclear. Since there is considerable evidence (see review by Cooke) suggest-