

Effects of Di-n-butyl and Di-2-ethylhexyl Phthalate on the Eggs of Ring Doves

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Introduction

The phthalic esters are used in large amounts as plasticizers, the annual production in the United States being about 850 million pounds (U.S. TARIFF COMMISSION REPORT 1972). Recently there have been indications that appreciable quantities may be present in the environment (MORRIS 1970, HITES and BIEMANN 1972, MAYER et al. 1972). MAYER and co-workers found di-n-butyl phthalate (DNBP) and di-2-ethylhexyl phthalate (DEHP) in many samples of fish from various areas of North America. The values for DNBP ranged from trace to 0.5 ppm, while those for DEHP ranged from 0.14 to 3.0 ppm. The values for phthalates were frequently in excess of those of polychlorinated biphenyls. In this paper the effects of a diet of 10 ppm DNBP and 10 ppm DEHP on eggshell thickness, breaking strength, permeability, and shell structure of the eggs of Ring Doves (*Streptopelia risoria*) are described.

Materials and Methods

Pairs of Ring Doves were maintained in individual cages as previously described (PEAKALL 1970). The length and breadth of each egg were measured. During the experiment the eggs were opened and the shells washed, dried overnight at 45°C, and weighed. The eggshell index (weight in mg divided by the product of the length times breadth, in mm) could then be calculated (RATCLIFFE 1967). This index was considered a more sensitive measure of thickness since the thickness of the eggs is only 0.10 mm. Ashed weights (1000°C, overnight) were also measured.

Permeability studies were made by maintaining eggs in a desiccator and weighing at intervals. Details have been given previously (PEAKALL et al. 1973). The breaking strength of the eggs was determined using an Instron Universal Testing machine (SCOTT et al. 1971). Cross sections of eggshells were examined using an AMOR-900 scanning electron microscope.

Results and Discussion

The most significant findings were that eggshell thickness was decreased and permeability to water increased by a diet containing 10 ppm di-n-butyl phthalate (Table 1). In contrast, no significant effect was found with di-2-ethylhexyl phthalate. The rate of water loss

increased by 23% whereas the decrease in eggshell thickness was only 10%. It was noted that small dents occurred rather frequently in eggs from birds treated with DNBP. While damaged eggs were excluded from those used for the calculation of water loss and permeability, micro-damage to the eggshell may have been the cause of increased water loss. The experiments on the breaking strength of the egg were unsatisfactory, due to the fact that the low values (0.5-1.0 lb) are at the low end of the range of the Instron Testing machine used. Damage to the surface, rather than hairline cracks, was observed and breaking strength correlated poorly with shell index. Structurally, the main differences appear to be the presence of more fibrous materials in the eggshell (Figure 1) compared to control eggs. The size and distribution of pores appear to be unaffected despite the considerable increase in permeability. The effect of DNBP thus has considerable differences from DDE where decreased numbers of pores are observed (PEAKALL et al. 1973), permeability is decreased, and cracking is more common than denting.

In view of the interest in, and controversy over, eggshell thinning that has been noted in several species in Europe (RATCLIFFE 1970) and North America (ANDERSON and HICKEY 1972), a question arises: Has DNBP contributed to eggshell thinning in the field? Analysis of the egg contents from doves on DNBP, employing the exact procedure used for the analysis of organochlorines, shows a complex pattern of which only one peak refers to DNBP itself. The identification of these unknown peaks has not been made. This pattern has not been found in the birds-of-prey analysis in this laboratory.

Metabolic studies reported suggest that phthalates are rapidly metabolized in mammals (SCHULZ and RUBIN 1973) and more slowly in fish (STALLING et al. 1973). While the data is meager, it does suggest that cold-blooded creatures metabolize phthalates much more slowly than do warm-blooded ones and thus the avian species at greatest risk would be the fish eaters. The studies reported here show that eggshell thickness recovers rapidly when the birds are placed on a clean diet. This is in marked contrast to the organochlorines where the clearance rate is slow and even a year on clean food has only a small effect (LINCER, unpublished data). The evidence suggests that only in the circumstances where DNBP is continually available in the food could this compound be of importance in eggshell thinning.

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TABLE 1
Effect of di-n-butyl and di-2-ethylhexyl phthalates on eggs of Ring Doves

Diet	Eggshell thickness index	Ashed weight (mg)	Rate of water loss (mg/hr)	Surface area (cm ²)	Permeability*
Control	0.749 ± 0.020 (48)	231.6 ± 15.7 (48)	3.96 ± 0.15 (24)	20.0 ± 0.6 (24)	0.0801 ± 0.0119 (24)
10 ppm DNBP	0.674 ± 0.027 (36)**	205.6 ± 24.0 (36)**	4.86 ± 0.22 (24)***	20.4 ± 0.5 (24)	0.1019 ± 0.0178 (24)***
10 ppm DEHP	0.729 ± 0.040 (24)	227.0 ± 17.8 (24)	4.03 ± 0.17 (24)	19.6 ± 0.4 (24)	0.0834 ± 0.0134 (24)
10 ppm DNBP, then clean food	0.721 ± 0.053 (24)	225.3 ± 23.6 (24)	—	—	—

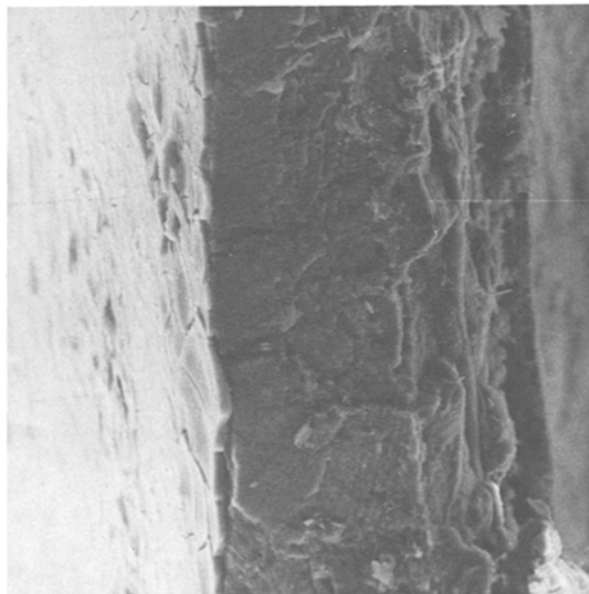
All results are means, standard deviations, and sample size

* (mg H₂O day⁻¹ cm⁻² torr⁻¹ H₂O)

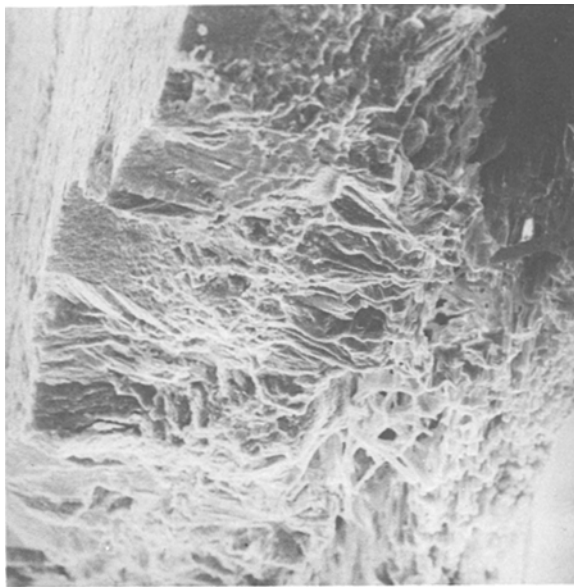
**Difference significant at 0.01

***Difference significant at 0.05

FIGURE 1 Scanning electron micrograph of cross section of eggshell.
(Magnification X520)



(A) Control.



(B) 10 ppm DNBP in diet.

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