# Effect of One Polychlorinated Biphenyl on Size and Activity of the Gull Thyroid

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## Introduction

In September and October, 1969, the bodies of over 12,000 seabirds, mostly guillemots (Uria aalge), were washed ashore on the coasts of the Irish Sea. Analyses of thelivers showed relatively large residues of polychlorinated biphenyls (PCB's) (1). The presence of these residues and their possible involvement in the mortality led to the British Nature Conservancy undertaking further research into the sub-lethal and lethal effects of these materials on seabirds. A commercial PCB product, Aroclor 1254, was chosen as the test material as the pattern of peaks produced on gas liquid chromatograms resembled that obtained from extracts of some of the guillemot livers (2). The lesser black-backed gull (Larus fuscus) was used as one of the test organisms because it is easy to obtain, easy to keep in captivity and is in the same taxonomic order as the auks (Charadriiformes). The present paper reports the initial part of the investigation, that of the effect on the thyroid. This gland was examined as previous work showed marked sub-lethal effects of other organochlorines (the insecticides, pp'-DDT, pp'-DDE, dieldrin) on thyroid size and activity (3, 4, 5) with significant changes in the resulting metabolic rate of the bird (4, 6).

## Experimental procedure

Twenty five wild-taken (2-4 weeks) lesser black-backed gulls were reared in pen conditions until 10-12 weeks old. They were then divided into five groups of five (4 groups of 3 males + 2 females, and one, subsequently given the highest dose rate, of 4 males + 1 female) and maintained in outside pens at a mean air temperature of 12.5°C. Fresh water was provided for drinking and pools of reconstituted sea water for bathing. The food consisted of sprats (Clupea sprattus). One group formed a control (mean weight : 788 ± 29 g) and four groups (775 ± 16 g) were dosed daily with 50, 100, 200 and 400 mg/kg/ Aroclor 1254 dissolved in cod liver oil at concentrations of 15, 25, 50 and 50% w/w, respectively. The mean daily oil consumption in the four dosed groups was 226, 235, 147 and 312 mg. The controls received 300 mg/bird/day of cod liver oil and all 25 also received 100 mg of wheat-germ oil daily. These oil doses were force fed in gelatin capsules for 8 weeks. No deaths occurred and the birds were killed with ether and dissected. The thyroids were removed, weighed individually and fixed in buffered formol saline. Longitudinal sections were then cut, as near as possible to the centre of the glands, stained with haematoxylin and eosin and examined visually. The weights of the left and right thyroids of the same individual differed considerably (mean difference, 22%; max., 72%) and in the following analyses the left and right thyroids are treated individually.



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In order to make a quantitative examination of any changes in colloid area, a typical portion, constituting a mean of 5.5% of the total thyroid area and touching the edge of each thyroid, was drawn on thick paper using a microscope and camera lucida (diameter of drawing 26.5 cm). The follicles containing colloid were then counted and the colloid areas cut out and weighed separately to the remainder of the field. As the sum of the two weights is equal to the known microscope field area (580, 880 $\mu^2$), the percentage and thus actual area of colloid can be calculated. Similarly, using objectives of lower power, the area of the whole thyroid across the centre was calculated.

## Results

Examination of the thyroid weights showed that dosing with PCB caused an increase in the number of heavy thyroids (i.e. over 29 mg) ($\chi^2$ = 4.8828; 1 d.f.; P < 5%) and the mean individual thyroid weight was significantly (t = 2.0636; 48 d.f.; P < 5%) increased by 32.0% from 30.51 ± 1.81 mg in controls to 40.28 ± 2.31 mg in PCB-dosed birds. There was also an increase in mean cross-sectional area from 10.13 ± 0.87 $mm^2$ to 11.53 ± 0.45 $mm^2$. There was no significant increase in weight with dose rate and although the largest thyroids found at each dose rate increased in weight progressively (43.9, 52.1, 68.9, 56.7 and 85.7 mg at control, 50, 100, 200 and 400 mg/kg/day respectively), the mean weight and area were, in fact, greatest at the two lowest dose rates (41.53 ± 2.56 mg & 12.23 ± 0.56 $mm^2$ at combined 50 + 100 mg/kg/day).

Visual examination of thyroid sections from control birds showed follicles with cubical epithelium and normal colloid quantities reasonably evenly distributed throughout the gland. Unlike birds fed DDT, where the thyroids showed almost complete colloid loss and hyperplasia (3), those of birds dosed with PCB showed apparently larger follicles which were well filled with colloid. At high doses the follicles appeared to be more compressed into a polyhedral pattern, with flattened epithelia and fewer interfollicular spaces.

Analysis of the colloid areas showed that the area of colloid per microscope field of 580,880 $\mu^2$ increased with the dose rate from 280,410 ± 15,530 $\mu^2$ in controls to 329,560 ± 14,800 $\mu^2$ at 400 mg/kg/day; an increase of 17.5%. In all dosed birds the overall increase was 8.8% to 304,970 ± 6,350 $\mu^2$. Regression analysis showed the positive correlation with dose rate to be significant (r = 0.3793; 48 d.f.; P<1%; equation: y = 283,050 + 113.38x where y = colloid area per microscope field in $\mu^2$ and x = dose rate in mg/kg/day). Accompanying the increased colloid area per field was a 4.7% reduction in the number of follicles per field from 183 to 174. Taking the two together, there was an overall increase of 23.9% in colloid



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area per follicle from 1,605 ± 169 $\mu^2$ in the controls to 1,988 ± 169 $\mu^2$ in the dosed birds (2,099 ± 223 $\mu^2$ at 400 mg/kg/day). Similarly, extrapolating to calculate the colloid area of the whole section, the increase in colloid area per microscope field together with the increased thyroid cross section caused a total increase of colloid area from 4.99 ± 0.67 $mm^2$ in the controls to 6.73 ± 0.92 $mm^2$ in birds dosed at 400 mg/kg/day (34.8% increase) and a mean of 6.13 ± 0.33 $mm^2$ in all PCB dosed birds (22.9% increase).

Separate analyses for males and females showed that the thyroids of dosed females contained more colloid (mean 316,310 $\mu^2$ per field) than those of dosed males (298,870 $\mu^2$) and suggests they may be more affected. They were, however, lighter in weight (females: 35.58 mg; males 42.81 mg) though about the same size (females: 11.43 $mm^2$; males: 11.59 $mm^2$). Correlation of the weight of individual thyroids of controls and dosed birds and their cross sectional areas (Table 1) showed that as the thyroid volume increases by 3.7 times (with areas 8 to 18 $mm^2$) the weight increases by only 1.7 to 3.0 times (i.e. density decreases with increased size).

TABLE 1

The equations relating cross sectional area in $mm^2$ (x) and weight in mg (y) of individual thyroids of controls and birds dosed at 50 to 400 mg/kg/day PCB. Also shown are the calculated weights of thyroids with areas of 8 and 18 $mm^2$

| Dose rate (mg/kg/day) | Equation and significance of relationship | Calculated weights (mg) of thyroids with areas of: 8 $mm^2$ | 18 $mm^2$ |
|---|---|---|---|
| Control | $y = 1.803x + 12.251$ ($P < 1\%$) | 26.7 | 44.7 |
| 50 | $y = 2.361x + 11.036$ ($P < 1\%$) | 29.9 | 53.5 |
| 100 | $y = 2.940x + 7.183$ ($P > 5\%$) | 30.7 | 60.1 |
| 200 | $y = 3.583x - 1.191$ ($P > 5\%$) | 27.5 | 63.3 |
| 400 | $y = 5.043x - 15.284$ ($P < 0.1\%$) | 25.1 | 75.5 |
| All dosed birds | $y = 3.782x - 3.330$ ($P < 0.1\%$) | 26.9 | 64.7 |



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Regression also showed that the colloid area per microscope field is positively correlated ($P < 0.1\%$) with the area of thyroid cross section (i.e. they increase together). Thus the comparatively low weight of dosed female thyroids and the reduced density of the larger thyroids is due to the comparatively low density of the colloid itself. The density of the thyroid, however, does not remain stable at different dose rates and the slope of the regression line relating weight and area increases progressively with the dose rate (Table 1). At 400 mg/kg/day the slope was significantly different from that of the control ($t = 3.4540$; 16 d.f.; $P < 1\%$). Although the small (8 $mm^2$) thyroids of all dose rates have similar densities to those of the controls and are presumably unaffected, the larger thyroids, once affected and increased in size, show a progressive increase in density with dose rate, despite the increasing colloid content. This could be due to the structural changes already noted and perhaps changes in colloid density.

## Discussion

This histological picture of increased gland weight and size with increased follicle size and colloid content is similar to that of the simple goitre found in mammals (7) and birds (8) which results from deficient intake or utilisation of iodine. Such goitres can be produced by chemicals, and thiocyanate is known to act by interfering with the iodide trap in the thyroid (9). PCBs may act in this way or by reducing the quantity of thyroid stimulating hormone (TSH) from the pituitary, which produces a similar histological picture (9). The thyroid tissue resulting from PCB administration is in a state of involution and suggests that there is a minimal depletion of secretion and possibly hypothyroidism. Some support for this suggestion is given by the mean heart weights of the experimental birds. These decreased significantly ($P < 2\%$) with increasing dose rate (6.83, 6.29, 6.17, 5.93 and 5.56 g at control, 50, 100, 200 and 400 mg/kg/day, respectively). A similar reduction in heart weight of pigeons dosed with pp'-DDT was correlated with reduced pulse rate and amplitude and other features of hypothyroidism (6). Thus, although the histological appearance of the thyroids of PCB and DDT-dosed birds is very different and the above suggested mechanism of action of PCB differs from that suggested for DDT-type compounds (6, 10) the resulting reduced metabolic rate may be very similar with these two persistent environmental contaminants. However, the lowest dose rate used in the present experiment (50 mg/kg/day PCB) was very much higher than that found to produce thyroid effects with DDT (3 mg/kg/day) (4, 6) and the reaction of the birds to the degree of reduction of circulating thyroxine produced by these doses of PCB has yet to be evaluated.

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