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Reduction in the Testosterone Levels by PCBs and DDE in Dall's Porpoises of Northwestern North Pacific

AN. SUBRAMANIAN*, S. TANABE*§, R. TATSUKAWA*, S. SAITO† and N. MIYAZAKI‡
**Department of Environment Conservation, Ehime University, Matsuyama 790, Japan*
†Departments of Medicine and Laboratory of Medicine and Clinical Laboratory Technicians School, The University of Tokushima, Tokushima 770, Japan
‡Department of Zoology, National Science Museum, Tokyo, Japan
§To whom communications should be sent

The increasing residue levels of PCBs and DDE in the blubber of *dalli*-type Dall's porpoises were found to have a negative effect on the testosterone levels in blood. Decrease in the levels of testosterone was statistically significant with increase in DDE concentrations. The results obtained suggest that the present levels of environmental contamination by persistent organochlorines can cause an imbalance of sex hormones and subsequent reproductive abnormalities in wild. The other hormone measured, aldosterone, which has no sexual function, was independent of the effects of both PCBs and DDE.

In natural biological systems, the conspicuous toxicological effects of persistent organochlorines such as PCBs, DDTs and related compounds seem to be prominently observed in pinnepeds (Delong *et al.*, 1973; Helle *et al.*, 1976a, 1976b; Reijnders, 1980) and birds (Weimeyer *et al.*, 1984; Peakell & Kiff, 1979; Newton *et al.*, 1982) especially causing reproductive abnormalities. Tanabe *et al.* (in press) suggested that small cetaceans and birds have lower activities of drug-metabolizing enzyme systems and so are vulnerable to reproductive toxicities of persistent organochlorines. A recent study on Dall's porpoises, *Phocoenoides dalli* collected from the northwestern North Pacific showed varying levels of PCBs and DDE in their blubber (Subramanian *et al.*, 1986).

To find out the possible effect of organochlorines in these animals we measured the levels of testosterone and aldosterone in their blood. In adult male Dall's porpoises, higher levels of xenobiotics seem to have a significant effect on the testosterone levels. This is the first report to demonstrate a significant correlation between the organochlorine concentrations and sex hormone levels in the bodies of wild animals.

Material and Methods

Twelve male *dalli*-type Dall's porpoises caught in the northwestern North Pacific within an area covering 37–48°N latitude and 151–175°E longitude in the R.V. *Hoyomaru* (No. 53 cruise) during May–June 1984 were used in this study. All the specimens studied here had a left testis weight of more than 40g (Table 1) and hence can be considered to have attained sexual maturity (Kasuya & Jones, 1984; Kasuya & Shiraga, 1985). Subcutaneous blubber samples and blood from heart were collected immediately after the animals were caught. PCBs and DDE in the blubber samples were analysed by the alkaline-alcohol digestion method of Wakimoto *et al.* (1971). Required amounts of the samples were digested in KOH–C_2H_5OH. The PCBs and *p,p'*-DDE (*p,p'*-DDT is transformed to *p,p'*-DDE during alkaline-alcohol digestion) thus brought into ethanol were transferred to hexane. After silica-gel and 5% fuming H_2SO_4 clean-up, PCBs and DDE were measured in Shimadzu GC-9A splitless technique glass capillary gas chromatograph equipped with ^{63}Ni electron capture detector. Testosterone and aldosterone levels in blood were measured by radioimmuno assay. The ages of the specimens were determined by counting the growth layers in the cementum of the teeth. All the specimens used in this study were collected about one or two months before the parturition season of this species (Kasuya, 1978). As all the specimens were collected in May–June, that is well before the yearly mating period (middle August–late October), the sex hormone levels of the specimens can be expected to be lower. The effects of other parameters, not related to sexual activities, on levels of sex hormones during this period may be more apparent than in the sexually active period.

Results and Discussion

Biological data, biometry and the values of PCBs and DDE and hormone levels are given in Table 1. Comparison of the testosterone levels in blood and the PCB and DDE levels in the blubber of the specimens clearly shows that with increase in the concentrations of these xenobiotics, testosterone levels decrease (Fig. 1). In these two chemicals, possible effect of DDE on the male sex hormone level is significant ($r=-0.66$:$p<0.05$). Even though the depression in the levels of testosterone with increase in PCB concentrations is not statistically significant the variations are apparent as seen in Fig. 1.

Organochlorines have been found to cause microsomal enzyme induction, accelerating hydroxylation of body steroids such as estrogens (Reijnders, 1986) and also to have an effect on the normal reproductive activities of many experimental animals, such as advancement of vaginal opening in rats (Gellert *et al.,* 1972) and inducement of abnormal changes in male embryos of gull eggs by development of an ovarian-like tissue on left testis (Fry & Toone, 1981). Haake *et al.* (1987) noticed the induction of the formation of testosterone hydroxylases by the administration of various pesticides to rats.

Results obtained by the treatment with DDT in Leghorn cockerels could be easily duplicated by the injection of an estrogen (Burlington & Lindemann, 1950). Such an estrogenic effect of other organochlorine compounds like methoxychlor (Bitman & Cecil, 1970) and Kepone (Eroschenko & Mousa, 1979) have been demonstrated in experimental animals.

Even though such a drastic estrogenic action of PCBs and DDE could not be shown clearly, a significant decrease in the levels of testosterone with increasing organochlorine levels is an obvious fact to show that these ubiquitous pollutants are affecting the normal reproductive hormone levels in wild animals. In fact, the other hormone measured in this study, aldosterone, which has the function of controlling the sodium levels in blood and has no sexual function was not affected by either of the chemicals (Fig. 2).

TABLE 1
Concentrations of PCBs and DDE in blubber and levels of aldosterone and testosterone in blood of *Dalli*-type Dall's porpoises *Phocoenoides dalli* collected from northwestern North Pacific during May–June 1984.

No.	Body length (cm)	Body weight (kg)	Gonad wt. (g) L	R	Levels of hormone aldo ($pg\,l^{-1}$)	testo ($ng\,l^{-1}$)	Concentration ($\mu g\,g^{-1}$ wet wt) PCBs	DDE
1	212	156	101	110	67	1.18	17.8	16.3
2	185	124	178	190	123	4.12	6.97	10.3
3	197	123	140	165	180	4.92	5.62	7.61
4	199	123	57	56	16.5	6.24	7.44	11.6
5	180	101	70	64	190	2.72	8.00	10.9
6	210	149	130	120	180	3.92	14.9	14.0
7	190	120	67	57	88	3.36	6.49	10.1
8	196	155	120	68	195	2.60	11.7	16.5
9	188	–	130	135	100	6.40	6.67	8.16
10	194	119	145	140	170	4.68	7.08	10.1
11	176	129	115	128	32	10.6	5.69	7.87
12	181	114	75	84	1500	9.4	9.91	8.67

Recently Reijnders (1986) reported the reproductive failure of common seal, *Phoca vitulina* in the wild due to the consumption of PCB-polluted fish in the Dutch Wadden Sea, but he did not notice any circumannual variation in the levels of circulating sex hormones in the experiments conducted on the females of these animals fed with PCB contaminated fish and also on the American mink, *Mustella vison* fed with commercial PCB spiked diet. Irrespective of the precise mechanisms involved, the plausible effect of PCBs and DDE in reducing the testosterone level in Dall's porpoise may be an indication of the causative effects of PCBs and DDE in affecting the normal sexual functions of wild animals. From the present results it seems that PCBs and DDE affect only the male sex hormones whereas the reproductive abnormalities observed in females of various marine mammals (Helle *et al.*, 1976b; Reijnders, 1986) may be via different mechanism or such a reduction in male sex hormone by PCBs and DDE may be species-specific in Dall's porpoise.

At present, we are not aware of the extent of damage done by the existing levels of xenobiotics on the reproductive capabilities of Dall's porpoise. But small cetaceans have specific features of metabolism (Saito *et al.*, 1986) and especially the Dall's porpoises have very weak metabolic enzyme systems for dealing with such chemicals (Tanabe *et al.*, in press). Bush *et al.* (1986) reported the reduction of sperm motility with increasing PCB concentration in humans, indicating the possible causative effect of PCB congeners themselves or the effect of a more potent series of associated compounds. With all these facts added together it can be presumed that a situation may arise where the level of exposure of Dall's porpoise, other animals and probably also man, to those compounds is high enough to elicit a severe estrogenic effect.

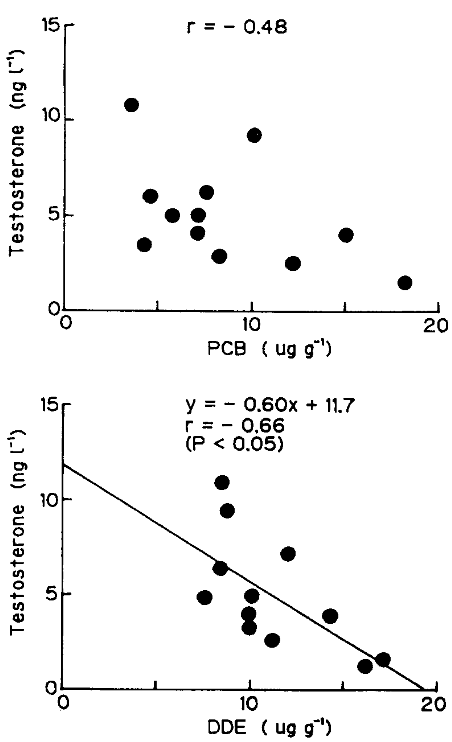

Fig. 1 Relationship between the concentrations of PCBs and DDE in blubber and levels of testosterone in blood of adult male *Dalli*-type Dall's porpoises collected from northwestern North Pacific.

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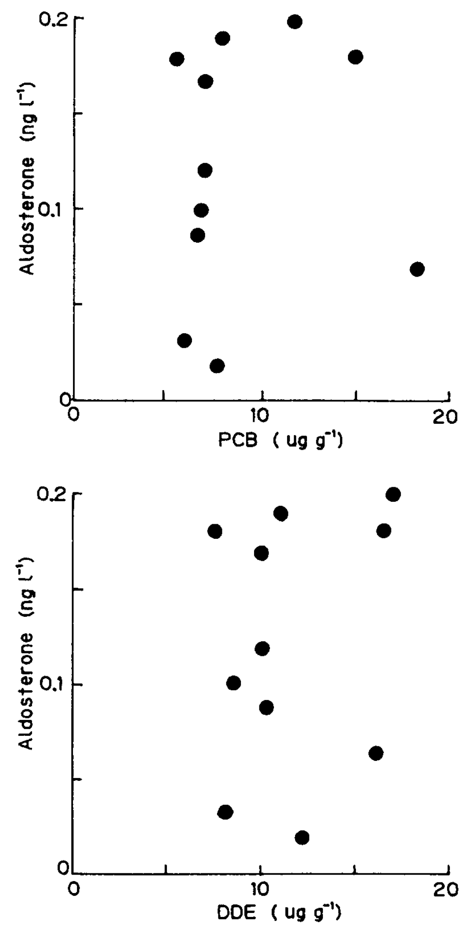

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Estimation of Meiobenthic Nematode Diversity by Non-specialists

COLIN G. MOORE, SCOT MATHIESON, DAVID J. L. MILLS and BRIAN J. BETT
Department of Biological Sciences, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX, UK

A methodology is presented for the estimation of nematode diversity using Cairns' technique of sequential comparisons. The accuracy and precision of the method are examined and a test presented for the comparison of diversity estimates.

In recent years, several authors have recognized the potential for the employment of measures of nematode community structure in pollution monitoring programmes (e.g. Ferris & Ferris, 1979; Platt & Warwick, 1980; Lambshead, 1986). In common with most taxa, nematode diversity tends to decrease with pollution (Heip *et al.*, 1985), although this is not invariably the case (e.g. Tietjen, 1977; Hodda & Nicholas, 1986).

Platt *et al.* (1984) have drawn attention to the advantages of using Simpson's index for the measurement of nematode diversity in pollution studies. One benefit is that it can be estimated by employing a slight modification of the sequential comparison index of Cairns *et al.* (1968). This avoids the necessity to identify species or even to sort animals into distinct taxa.

Cairns *et al.* (1968) suggested estimating diversity by a process which involved arranging the individuals from a sample into a random sequence and recording the number of runs; a run is defined as a maximal sequence of consecutive specimens of the same species. Thus specimen 1 is compared to specimen 2, then specimen 2 with specimen 3 etc.

For a sample of N specimens, Cairns linked estimator is defined as

$$C = \frac{\text{No. runs} - 1}{N}$$

(Patil & Taillie, 1976). C provides an unbiased estimator of Simpson's Index, $1-\delta$, whereas δ is the probability that two specimens chosen randomly from the community belong to the same species, i.e.

$$\delta = \sum_{i=1}^{\sigma} \pi_i^2$$

where π_i is the proportion of the i th species in the community, comprising a total of σ species. For a sample, Simpson (1949) has shown that

$$D = \frac{\sum_{i=1}^{s} n_i(n_i - 1)}{N(N-1)}$$

is an unbiased estimator of δ, where n_i is the number of specimens of the i th species in a sample of s species.

A simple method for determination of nematode diversity, which obviates the requirement for specialist taxonomic knowledge, would facilitate assessment of the utility of nematode community structure in the context of pollution studies, and may itself contribute to a protocol for monitoring benthic communities. This