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Loss of Organochlorine Compounds in the Tissues of a Decomposing Stranded Dolphin

Assumpció Borrell and Alex Aguilar

Department of Animal Biology (Vertebrates), Faculty of Biology, University of Barcelona, 08071 Barcelona, Spain

A large number of organochlorine pollution surveys in cetaceans have been traditionally carried out on samples from stranded individuals, which are found in variable states of freshness and from which the cause of death can often not be ascertained. The validity and representativeness of these surveys has been questioned for at least three reasons (Aguilar 1985; 1987).

First, some diseases or morbid states affect the normal physiological processes and may lead to abnormal rates of metabolization or excretion of pollutants. Second, many stranded specimens have suffered handicapped physical faculties over a period of time before deceasing, for which reason their fat reserves have been mobilized to a variable extent. No information is available about organochlorine dynamics during fat mobilization in cetaceans, but it is highly unlikely that pollutants remain at stable concentrations, especially in fat-rich tissues. Finally, tissue specimens from a stranded animal are hardly ever collected immediately after the cetacean's death, but carcasses usually remain on the beaches under different conditions of conservation over a variable period of time before being sampled. During this outdoor exposure, pollutants may be affected by a number of physical and biological a gents: direct exposure to the sun, high temperatures, wind, bacterial activity, etc. It is known that these agents may produce the concentration, volatilization, or chemical transformation of organochlorines (Barker et al 1965; Jefferies and Walker 1966; Crosby 1969; Spencer 1975; Brown et al, 1987) thus altering the concentration and composition of the pollutants originally present in the tissues.

This study addresses the latter problem. The pattern of variation in organochlorine pollutants (p,p'-DDE, o,p'-DDT, p,p'-DDT, p,p'-TDE and PCBs) in the tissues of a fresh dolphin carcass left under outdoor conditions for 55 days is examined at different time intervals, and the importance for common field-work situations is discussed.

Send reprint requests to Assumpció Borrell at the above address.

MATERIALS AND METHODS

For this study, we used a fresh, 201 cm, 78 Kg, female striped dolphin (Stenella coeruleoalba) stranded in Gavá (N.E. coast of Spain). The necropsy did not reveal significant pathologies or parasitic infections.

Blubber and muscle samples (3 replicates from each) were collected from the mid-dorsal region of the trunk a few hours after death and immediately analysed for organochlorines. Parallel to this, the rest of the dorsal region of the trunk was left unpreserved out of doors. At time intervals of 6, 13, 21, 29, 41 and 55 days after the capture date, 3 further replicates of blubber and muscle were collected and analysed. Over this period of time, air temperatures ranged from 5 to 20°C (mean: 14°C), air humidity ranged from 31 to 97 % (mean: 76.89 %) and 81 % of the days were overcast.

For the organochlorine analysis, the three replicates of muscle (each of approximately 3-4 g) and blubber (each of approximately 1 g) were weighed and homogenized with 50 g of anhydrous sodium sulphate and the lipidic phase extracted with n-hexane in a Soxhlet apparatus for 4 hours (about 40 cycles). The resulting extract was brought to 40 ml, from which an aliquote of 10 ml was used for the lipid calculation, which was carried out by evaporation at 60°C under an air stream and later gravimetry.

A portion of the extract corresponding to 30 mg of lipids was concentrated to 3 ml and treated with concentrated sulphuric acid at room temperature for the lipid clean-up following the method of Murphy (1972). This process does not alter the speciation of DDT and PCB compounds and yields a lipid-free organic phase which, after concentration to 1 ml and addition of internal standards, is ready for the chromatographic run.

For the analysis, we used a Perkin-Elmer Sigma 3B gas chromatograph with an electron capture detector and a Perkin Elmer Sigma 15 data computer station. The chromatograph was equipped with a fused-silica capillary column of 60 m length, 0.25 mm internal diameter, and a stationary phase SPB-1 with a film thickness of 0.25 μm. For the chromatographic runs, temperatures were programmed as follows: Injection at 40°C. Oven steady for the first 2 min. and then an increase from 40°to 160°C at a rate of 25 C/min. Oven maintained at steady temperature for 1 min. and then an increase from 160 to 250°C at a rate of 2°C/min. From this point on until the end of the analytical run, the column remained isothermal at a temperature of 250°C. Nitrogen, at a fluxe of 1 ml/min., was used as gas carrier. Under these conditions, an aliquote of the purified extract was injected in splitless, the valve being opened 1.5 min later. Injector temperature was kept isothermal at 250°C.

Heptachlor was used as internal standard to calibrate fluctuations in the operational conditions. The identity of the DDT group

compounds was confirmed by an alkali conversion to their respective olefins and re-analysis by GLC. PCBs were identified and quantified by their peak characteristics and retention times in relation to a 1:1 standard mixture of Aroclors 1254 and 1260 and confirmed by their resistance to the chemical derivations detailed above.

RESULTS AND DISCUSSION

The lipid content of muscle fluctuated widely across the period of study without following any definite trend. These fluctuations were very likely caused by variation in the water content of the tissue, which was probably affected by the weather conditions (environmental temperature, relative air humidity, etc) on the day of sample collection or over the days immediately prior to them, and to the conservation state of the tissue.

The lipid content of blubber, on the contrary, progressively decreased with time ($p<0.01$). The reasons for such a decrease are not totally ascertained, but leaking and volatilization of the lipid fraction of the tissue was observed to occur, especially in periods when the carcass remained under direct exposure to the sun. This finding is especially relevant because organochlorine compounds are highly lipophilic and it is known that their residue levels in any given tissue are in most cases proportional to the richness in neutral lipids of this tissue, especially triglicerydes and non esterified free fatty acids (Aguilar, 1985).

When expressed on a fresh weight basis, the organochlorine residue levels of the blubber fluctuated strictly parallel to the changes in the lipid content of the sample. Therefore, quantification of pollutant concentrations on a fresh weight basis may be strongly affected by the particular environmental exposure conditions of the stranded carcass and should be considered as flatly unreliable. Pollutant concentrations expressed on the basis of the quantity of extractable lipids in the tissue remained more stable and are t hus considered more reliable. For this reason, in

Table 1. Variation in organochlorine compound concentrations (expressed on ppm on an extractable lipid basis) in the tissues of a decomposing dolphin.

Compound	Blubber		Muscle	
	Day 1	Day 55	Day 1	Day 55
tDDT (except p,p'-TDE)	19.8	7.7	10.4	6.3
p,p'- TDE	2.2	3.4	2.1	2.8
PCB	68.1	42.1	67.2	39.0

subsequent sections of this paper we shall only use this latter means of calculating organochlorine concentrations.
Figure 1 shows the variation in the residue levels of the different organochlorine compounds found in the blubber and muscle of the dolphin during the period of study. In both tissues there is a highly significant decrease ($p<0.001$) in the concentrations of p,p'- DDE, o,p'- DDT, p,p'- DDT and PCB with time. On the contrary, the concentrations of p,p'- TDE significantly increased in the blubber ($p<0.001$) but showed no significant trend in the muscle. The change in pollutant concentrations appeared to occur progressively during outdoor exposure and not to be dependent on particular weather conditions on the days on which samples were collected. As can be seen in table 1, at the end of the 55 day experiment, total DDT levels (excluding p,p'- TDE) had been reduced to only 39 % of their initial levels in the blubber, and to 60.5 % in the muscle. Corresponding reductions for PCB were to 61.8 % in blubber and to 58 % in the muscle. In the same period, p,p'- TDE increased to 154.5 % of its initial tissue concentration in the blubber and to 133.3 % in the muscle.

While most of the previous research into organochlorine sampling techniques has focused on the effectiveness of the different preservatives (formalin, dry ice, phenoxyethanol, etc.) in keeping tissue pollutant levels constant, rather than on the changes in concentrations which may be expected to happen in unpreserved tissues (French and Jefferies, 1971; Stickel et al 1984; Wiemeyer et al 1984), two papers contain information in this respect.

In the first one, Stickel et al (1984) analysed brain, muscle, liver, and egg content in one avian species and compared the organochlorine concentrations of subsamples preserved frozen and in formalin to those of subsamples left in a refrigerator at 7 C for 21 days, when mold appeared in the tissues. Overall, the three sets of samples differed little from each other in the concentration of chemicals or lipid content, indicating essentially no variation in concentrations in the unpreserved (or badly preserved) replicate. These results lead Stickel et al (1984) to conclude that the spoiling of samples which may occur in the field would not result in misleading analytical results.

However, the preservation conditions tested by Stickel et al (1984) are very different from those in our study, and are not representative of the conditions prevalent in a carcass left outdoors in most common field situations. Volatilization is a major pathway for loss of organochlorines, and the volatilization rate of a compound is directly related to its vapour pressure, which in turn is highly influenced by thermal gradients and air movement around the tissue. The decrease in pollutant concentrations observed in the dolphin in the present experiment was very likely due to the volatilization of organochlorine compounds produced by the combination of the increased temperature of the carcass left to direct exposure by the sun and the effect of wind, a situation which would not occur in a refrigerator. Also, the substantial variation in tissue lipid content observed

was the consequence of changes in the hydration of the tissues, undoubtedly associated with shifting weather conditions, again a source of variation inexistent in a closed environment. For these reasons, it is felt that the results achieved by Stickel et al are not representative of the changes which the carcass of a stranded cetacean would undergo in most fieldwork situations.

In a second case, Olsson et al (1974) analysed two samples of seal blubber before and after 5 months' exposure outdoors, and their results showed a decrease in concentrations of 12.5% for DDT and 25% for PCB in the surface strata of the blubber (table 1 in Olsson et al's paper). The conditions set out for that experiment are much more similar to those of the present one and also to those prevalent in natural conditions. The results of the two studies are coincidental in denoting a fall in organochlorine tissue concentrations, although the decline seems to have been much greater in the present case. Olsson et al do not specify mean temperature or weather conditions during the 5 months when the samples remained stored, for which reason it is impossible to decide upon which factors are responsible for the difference between the two experiments. However, ambient temperature, a factor known to increase pollutant volatilization in laboratory conditions, was probably higher in our experiment than in that of Olsson et al, which was carried out in Sweden.

While it remains unclear which specific agent or agents (temperature, wind, humidity, bacterial activity) bear responsibility for the process, it becomes evident that outdoor exposure has a direct and measurable effect on the tissue concentration of pollutants, in most cases leading to a decrease in their residue levels (figure 1). Under some conditions (for example under direct exposure to the sun in warm environments) this effect may be enhanced and become important even on a short time scale.

As commented on above, the pattern of change in concentrations was not identical for all the organochlorines studied. As a consequence of this, the concentration of any given chemical relative to the others varied over the period of time surveyed.

Table 2. The variation in ratios between selected organochlorine compound concentrations in the tissues of a decomposing striped dolphin.

Ratio (x100)	Blubber		Muscle	
	Day 1	Day 55	Day 1	Day 55
p, p'-TDE/tDDT	11.02	30.84	17.31	31.75
p, p'-DDE/tDDT	46.27	54.35	48.31	54.76
tDDT/PCB	29.14	26.50	18.69	20.90

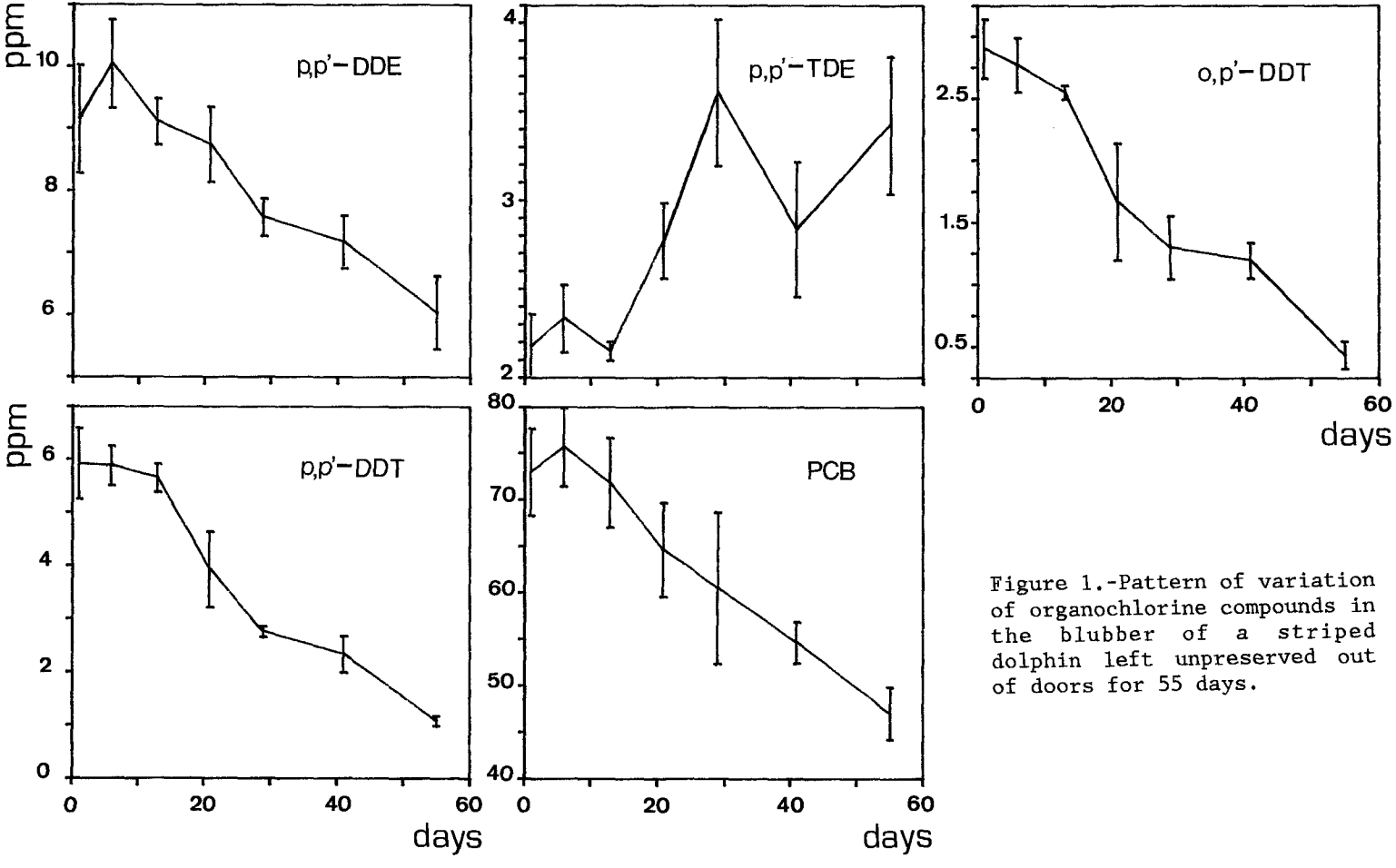

Figure 1.-Pattern of variation of organochlorine compounds in the blubber of a striped dolphin left unpreserved out of doors for 55 days.

Because p, p' - TDE increased with time instead of decreasing as the other DDT forms did, the ratio p, p' -TDE/tDDT was the one which showed the most marked change through time, almost tripling in the blubber. This increase is highly significant ($p<0.001$) both in blubber and muscle, and is totally consistent with previous available evidence of anaerobic degradation of DDT to TDE by microorganisms in decomposing tissues (Barker et al 1965; Jefferies and Walker 1966).

The p, p' - DDE/tDDT ratio also increased significantly with time in the blubber ($p<0.001$) , although the magnitude of the change was more moderate than in the preceeding case. In the muscle, this ratio also showed some increase from the beginning to the end of the experiment, but the trend was not significant. The loss of absolute quantities of p, p' - DDE from the carcass is demonstrated from data in table 1. However, it has been shown that DDT degradation also produces DDE in appreciable quantities (Ecobichon and Saschenbrecker 1967). Thus, although p. p' - DDE decreased in absolute concentrations in the tissues of the decomposing dolphin, the rate of decrease was much slower than that of DDT because of the gain in "new" DDE coming from the degradation of other DDT forms, the process resulting in a progressive increase in the relative ratio.

The tDDT/PCB ratio remained reasonably constant throughout the whole period surveyed, indicating that the rate of loss was similar for the two groups of compounds.

The concentrations of one organochlorine relative to another, especially when the chemicals involved are mother compounds and their metabolites, have been considered meaningful for understanding certain ecological processes, and are important for determining patterns of metabolization, excretion or reproductive transfer, and for establishing geographical differences and trends in pollutant loads in marine mammals (Addison et al 1984; 1986; Aguilar 1984; 1987; Massé et al 1986; Boon et al 1987; Tanabe et al 1984; Subramanian et al 1988).

The above findings clearly show that samples coming from badly preserved (or unpreserved) specimens, as stranded cetaceans typically are, are likely to produce altered ratios and should thus be considered unreliable for this type of study.

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