Polychlorinated Biphenyls and p,p′ DDE in Green Turtle Eggs from Ascension Island, South Atlantic Ocean

by

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Industrial pollutants are known to be widely distributed in global ecosystems. Polychlorinated biphenyls (PCB's), a representative of these pollutants, have been demonstrated in marine ecosystems of the North Atlantic Ocean and adjacent waters (DUSTMAN et al. 1971, JENSEN et al. 1969, ZITKO 1971) as well as California and Panama waters (RISEBROUGH et al. 1968a). In the majority of these reported occurrences the PCB-laden organisms resided in waters that presumably also contained industrial pollutants, including PCB's. Similarly, persistent pesticides are of widespread occurrence, perhaps more so than the PCB's; for example, DDT and its metabolites have been identified in terrestrial, freshwater, and marine organisms of the Western world including remote Antarctica (BREWERTON 1969, GEORGE and FREAR 1966).

One of the most isolated islands in the South Atlantic Ocean is Ascension Island (7°57'S, 14°22'W), a volcanic island of approximately 35 sq. miles located some 900 miles southwest of Liberia, Africa and 1,500 miles east of Recife, Brazil. Sea birds and turtles (especially the marine, pelagic Green Turtle, Chelonia mydas) utilize this island for breeding, perhaps feeding in nearby waters. Opportunity was afforded in March 1972 for one of us (DWJ) to visit Ascension Island, where samples of birds and turtle eggs were obtained for PCB and pesticide analyses. This was a unique opportunity to examine marine organisms from an exceptionally remote site. Furthermore, we are unaware of any previously published accounts of PCB analyses of sea turtle material. These turtles provide protein for human populations throughout the range of the species involved.

Material and Methods

Ten eggs of Chelonia mydas were taken as they were being laid into sandpit nests by adult females. The eggs came from four different females on the nights of 19-21 March 1972. Subsequently, the eggs

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were transported in sand at ambient temperatures to our laboratories where, approximately one month later, each egg was weighed and its contents removed for analysis.

Egg yolks were mixed with anhydrous sodium sulfate and extracted for six hours with petroleum ether in a Soxhlet apparatus (REICHEL and ADDY 1968). Extracts were poured into beakers and the ether was evaporated at room temperature under a hood. Beakers were weighed and residues were dissolved in hexane saturated with acetonitrile, transfered to a separatory funnel and washed with 50 ml. acetonitrile saturated with hexane. The hexane portion was washed with 3 more 50 ml portions of acetonitrile. The hexane was discarded and the combined acetonitrile washings were evaporated at room temperature. For cleanup, the residue was dissolved in hexane and placed on a column of 8% water deactivated Florisil (22 x 180 mm). The column was eluted with 200 ml of hexane:benzene, 3:1, and the eluate was concentrated to 1 ml. and analyzed by gas chromatography. A Varian 2100 gas chromatograph containing a 6' x 1/4" glass column of 6.4% OV-210/1.6% OV-17, 1:1, on Chromosorb W and a H^3 electron capture detector was used for analysis. A second glass column of 1.5% OV-17/1.95% QF1 on Gas Chrom Q of similar dimensions was used for confirmation. Other instrumental parameters were injection port 210°, column 200° and detector 215° with a N_2 flow rate of 40 ml/min.

Following gas chromatograph injections an effort was made to separate DDT and its metabolites from PCB's. The samples were eluted on Grace-Davidson grade 950 silica gel (10 x 70 mm) which had been washed with benzene and activated at 150° for three hours. Columns were eluted with 70 ml pentane and 50 ml benzene which were collected separately (SNYDER and REINERT 1971). These eluates were injected into the gas chromatograph. The benzene portions of these eluates from some samples were subjected to solvent partitioning in systems of hexane: acetonitrile and iso-octane: 80% acetone for confirmation of DDT and metabolites (BOWMAN and BEROZA 1966).

Results and Discussion

Figure 1 illustrates a gas chromatogram of an egg extract after florisil cleanup and prior to silica gel separation. The presence of multiple PCB's is evident and there are peaks at the retention times of p,p'-DDE, DDD, and DDT. Figure 2

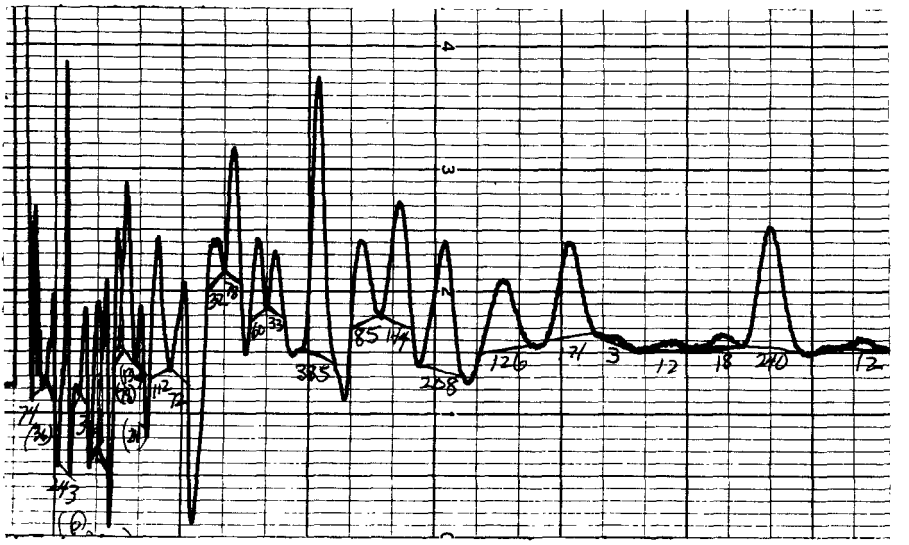

Figure 1. Gas Chromatogram of turtle egg extract #8 prior to silica gel separation showing likely presence of PCB.

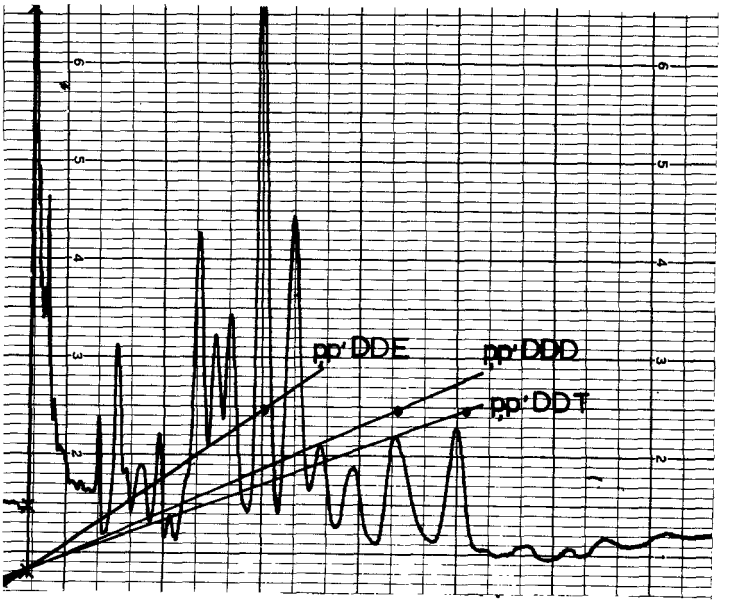

Figure 2. Gas chromatogram of turtle egg extract #9 prior to silica gel separation. Dots indicate retention times of DDT and metabolites. Dot overlay after COLLIER et al. 1971.

is a gas chromatogram from another egg extract, prior to silica gel separation, which seems to indicate presence of a larger amount of p,p'-DDE. The silica gel separation subsequently performed has been shown, by use of analytical standards, to elute PCB's (except early peaks of Aroclor 1242 and 1248) in the pentane elution with part of the p,p'-DDE. The benzene elution contains p,p'-DDD, p,p'-DDT, and early peaks from Aroclor 1242 and 1248. Figure 3 is a chromatogram resulting from the pentane elution of the silica gel column. Note that many of the early peaks (Fig. 1) were retained on the column and are absent. These peaks eluted subsequently in the benzene elution. An overlay with dots corresponding to the peaks in Aroclor 1254 indicates most of the sample is Aroclor 1254. Table 1 gives the amounts of PCB residue determined by area measurement of all peaks quantitated as Aroclor 1242, 1248 or 1254 before and following silica gel separation. The amounts of Aroclor present before silica gel separation are presented as three values, each calculated as if it were the sole Aroclor in the sample. Note that, using these figures, a less chlorinated Aroclor (i.e. 1242) gives greater residues in ppm if it is used as a standard. It was not possible to differentiate between Aroclor 1242 and 1248 by retention times in a sample in which differential metabolism of Aroclor components may have taken place, as they are represented by identical peaks which differ by concentration only. Table 1 illustrates that an error made by choosing as standard an Aroclor not identical to that in the sample would result in an error in ppm that would not exceed 3 fold. Values presented after silica gel separation are based on the standard most closely matching the sample peaks or are listed as averages of two Aroclors.

Small peaks found in almost all the benzene elutions of the egg samples corresponded in retention times to p,p'-DDD and p,p'-DDT. However, when extraction p-values were applied (BOWMAN et al. 1965) only the identification of the peak corresponding to p,p'-DDE could be considered valid in the two binary solvent systems used. The absence of DDD or DDT in the samples would make it feasible to quantitate PCB prior to any silica gel separation. In general, good agreement was found between PCB calculations before and after silica gel separation, (when DDE was not present in the sample) although the values listed after silica gel separation are somewhat reduced. An effort was made to exclude any peak area thought to be DDE in these comparisons.

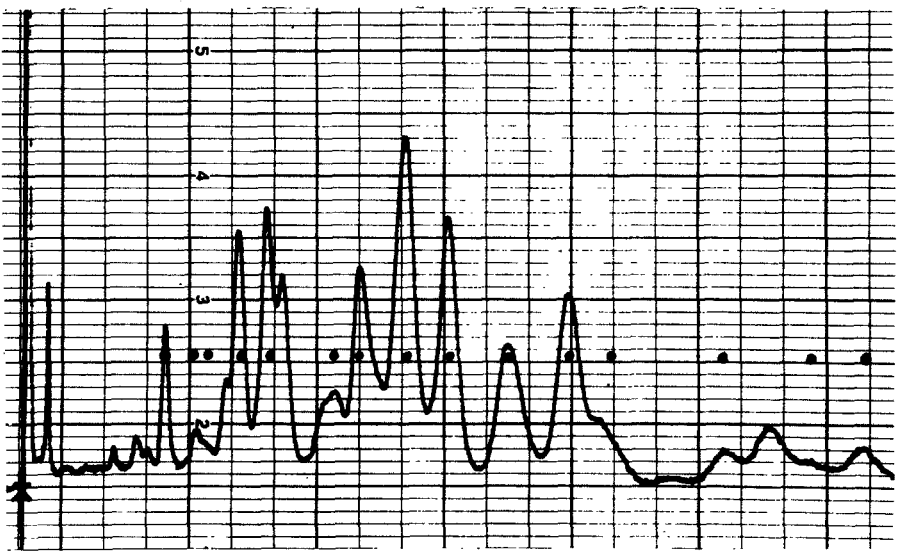

Figure 3. Gas chromatogram of pentane elution of turtle egg extract #4 with an overlay of dots corresponding to peak retention times of Aroclor 1254.

TABLE 1

Residues[a] In Turtle Eggs Collected On Ascension Island

	PCB Before Silica Gel Separation (SGS)					PCB After SGS		p,p'-DDE	
	Aroclor (Lipid)			Lipid Average	Wet	Lipid	Wet	Lipid	Wet
	1242	1248	1254						
1.	0.76[a]	0.49	0.03	0.52	0.03	0.43[b]	0.03	ND	ND
2.	2.74	2.39	1.37	2.16	0.13	1.81[b]	0.11	0.08	0.005
3.	2.53	2.28	1.35	2.39	0.03	1.79[c]	0.22	0.07	0.009
4.	0.53	0.35	0.11	0.33	0.02	0.33[c]	0.04	0.01	0.001
5.	2.96	1.93	1.25	2.05	0.14	1.61[b]	0.11	0.07	0.005
6.	ND[d]	ND	0.43	0.43	0.03	0.38[c]	0.03	ND	ND
7.	1.21	0.82	0.61	0.88	0.10	0.24[c]	0.03	0.02	0.001
8.	2.22	1.40	0.95	1.52	0.07	0.75[c]	0.05	0.05	0.003
9.	2.52	1.60	0.01	1.71	0.12	1.72[b]	0.12	0.06	0.004
10.	0.85	0.57	0.37	0.60	0.03	0.33[c]	0.02	ND	ND

a= values in ppm
b= average of 1248 and 1254
c= quantitated as 1254 only
d= none detected

Ascension Island, in addition to being geographically isolated, houses a small, generally transitory human population (ca. 2,000 in 1972). There are no streams on the island. No PCB's are produced there or released into the surrounding waters. The exceptionally small quantities of organochlorine pesticides occasionally used in a small garden atop Green Mountain on the center of the island are inconsequential.

From the extensive turtle-tagging investigations of CARR (1972), it is known that the Ascension Island Green Turtles migrate annually westward from breeding beaches on this island to principal feeding grounds off the eastern coast of Brazil. The non-breeding ranges there extended from the mouth of the Rio Do Para (1°S, 48°W) near Belem southward to Vitoria (20° 15'S 40°28'W). Inasmuch as these turtles feed upon marine plants, supplemented by some invertebrate animal food, we must conclude the female turtles laying the eggs on Ascension obtained PCB's and the DDT metabolite from organisms eaten en route to Ascension or from those in waters off the eastern coast of Brazil. None of these marine environments are in regions of high industrial pollution, but (a) the South Equatorial Current (ca. 1,000 miles wide), in which the turtles migrate, sweeps by Ascension Island from huge expanses of the South Atlantic Ocean (KOCK et al. 1969) and (b) chlorinated hydrocarbon pesticides may be air-borne for hundreds of miles (RISEBROUGH et al. 1968b). Thus, despite the remote position of Ascension Island and the pelagic feeding habits of these Green Turtles, they quite likely obtained the PCB's and p,p'-DDE from relatively unpolluted waters of the western South Atlantic Ocean.

This brief report of PCB and pesticide burdens in the turtle eggs carries no concrete information of possible effects on the organisms. PCB's can depress egg production and hatchability in ring necked pheasants (Phasianus colchicus) (DAHLGREN and LINDER 1971). In addition to the 10 Green Turtle eggs which we analyzed, several hundred more were transported to Florida for laboratory-hatching; in these there was no dramatic decrease in hatching success or occurrence of deformed hatchlings. Controlled experiments on effects of pollutant/pesticide burdens in these Green Turtles would be desirable.

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