# Organochlorines in the Seastar Acanthaster Planci

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The Indo-Pacific seastar Acanthaster planci ("Crown-of-Ihorns") has become the subject of a growing number of studies largely in response to reports of greatly increased seastar populations (CHESHER, 1969, 1970; PEARSON & ENDEAN, 1969). Among other agents, pesticides have been suggested as possibly causing the alleged population increases (FISCHER, 1969). Such a possibility seemed unlikely, but since the effects of pesticides on tropical marine fauna have never been studied, the idea warranted investigation.

### METHODS and MATERIALS

We first collected samples of <u>Acanthaster</u> gonad tissue from Guam, and Woleai and Ifalik Atolls in the Caroline Islands. These were small samples (< 1 g) originally intended for gonad index studies; but because of their uniqueness, aliquots were individually extracted and analyzed on DC-200. Data on this material appears in Table 1. In 1970-71, we collected larger samples of gonad tissue from Guam and Rota (Mariana Islands), Molokai (Hawaii), Truk and Namoluk Atolls (Caroline Islands), Eniwetok Atoll (Marshall Islands), and the Great Barrier Reef (Slasher's Reef and the Bunker Group). These extracts were analyzed on QF-1, and the data is presented in Table 2. All samples were preserved in standard Bouin's fixative or in 5% formalin in seawater.\*

Analytical techniques are as follows. The material in Table 1 was extracted with acetonitrile (BURKE et al, 1969), with the volumes and weights of chemicals and solvents reduced to 10% of the recommended amounts. Tissue samples of later collections (Table 2) were extracted with the standard volumes.

The gas chromatograph used for quantitation was a Barber Coleman 5360. Column: Pyrex 6' x 4 mm I. D., with 10% DC DC-200 (12,500 CS) on 80/90 Anakrom ABS--for material in Table 1; or 5% QF-1 on 100/120 Varaport 30--for material in Table 2. Detector: tritium source, 300 mc. Temperatures:

<sup>\*</sup> A manuscript on the recovery of insecticide residues from fixed animal tissue is in preparation.

injector 225°C, column 195°C, detector 205°C. Carrier gas: N2, approximately 120 ml/minute. Detector voltage; DC at which 0.4 ng dieldrin caused 30% FSD at 1 x 10<sup>-9</sup> AFS. A Varian 2740, equipped with a 5' x 1/8" SS column with 3% SE-30 on 100/120 Varaport 30, programmed at 8°C/minute, was used for confirmatory analysis.

The recovery was 88.7% for p,p'-DDT at 1 ppm in 0.06g of tissue--for material in Table 1. In the material in Table 2 the recovery was 87.1% for p,p'-DDT and 89.0% for dieldrin at 0.1 ppm in 12.7 g of tissue (mean of four determinations).

The earlier extracts (Table 1) were pooled, and aliquots spotted on thin layer plates coated with 0.25 aluminum oxide G. The plate was pre-washed with 50% acetone in water, and the chromatogram was developed with 1% acetone in n-heptane (LICHTENSTEIN et al, 1969). In the larger samples (Table 2), TLC analyses were done using Silicar TLC-7G (@ Mallinckrodt). The plates were prewashed, and the chromatograms developed with n-hexane. Spots were made visual with iodine vapors, scraped off, eluted with n-hexane, and aliquots of the eluates injected into the gas chromatograph.

### RESULTS

## TABLE 1

P, p'-DDT content in ppm (relative to wet weight of fixed tissue) of gonad tissue from Acanthaster planci, 1969.

	Sample Weight		p, p'-DDT	
Number	in g	rams	in ppm	
of Samples	$\frac{Mean}{}$	Range	Mean	Range
10	0.053	0.020-	1.31	0.28-
1)		0.189		3.89
3	0.079	0.041-	0.08	0.06-
		0.072		0.10
12	0.360	0.190-	1.01	0.10-
		0.653		1.24
2	0.348	0.305-	0.34	0.21-
		0.391		0.65
27			0.68	0.01-
				3.89
	of Samples  10 1) 3 12 2	Number in growth of Samples Mean  10 0.053  1) 3 0.079  12 0.360  2 0.348	Number in grams of Samples Mean Range  10 0.053 0.020- 0.189 3 0.079 0.041- 0.072 12 0.360 0.190- 0.653 2 0.348 0.305- 0.391	Number in grams in grams in grams Mean  10 0.053 0.020- 1.31 0.189 3 0.079 0.041- 0.08 0.072 12 0.360 0.190- 1.01 0.653 2 0.348 0.305- 0.34 0.391

<sup>1)</sup> Collected on August 6, 1969 by A. Wolfson

<sup>2)</sup> Collected on August 5, 1969 by A. Wolfson

<sup>3)</sup> Collected on July 28 and August 1, 1969 by A. Wolfson

<sup>4)</sup> Collected on July 18 and 21, 1969 by A. Wolfson

Samples analyzed in 1969 -- Table 1.

Quantitation of p,p'-DDT ( $R_t$  1.65 relative to dieldrin) was made difficult because of an interfering peak at  $R_t$  1.69 which remained after dehydrochlorination. P,p'-DDT quantities were estimated by measuring peak height.

Prominant peaks also occurred regularly at  $R_t$  1.17 and 1.57 (relative to dieldrin), and occasionally at  $R_t$  1.45. Peaks with similar retention times are found to be produced by certain PCBs (KOEMAN et al, 1969). The peaks at  $R_t$  1.17, 1.45, 1.57, and 1.69 did not disappear after dehydrochlorination, which suggests that they have been caused by PCBs. However, this assumption could not be confirmed because of lack of additional extracts.

Samples analyzed in 1970-71 -- Table 2.

After column chromatography, peaks produced by ether-hexane eluates which matched standard dieldrin or p,p'-DDT peaks, were interpreted to be these insecticides. There was no peak interference with the quantitation of p,p'-DDT. Dieldrin appeared as a shoulder of a usually prominant peak, and dieldrin quantities were estimated by measuring the height of the shoulder.

TLC analysis of 16 hexane eluates, combined to four samples, yielded three prominant spots with R<sub>f</sub> values of 0.94, 0.74, and 0.55 (R<sub>f</sub> of p,p'-DDT was 0.39). When analyzed by GLC, eluates of spot 0.94 yielded only one peak immediately following the solvent peak; whereas the eluates of spot 0.74 produced four peaks, none of them with retention times of peaks produced by a PCB standard--Aroclor 1254. However, two of the peaks had retention times similar to those of aldrin and p,p'-DDE. Spot 0.55 produced five larger peaks, among them one with the retention time of p,p'-DDE.

It was not possible, employing nitration (JENSEN, 1969) and repeated TLC, to separate clearly p,p'-DDE from the interfering compound contained in spots 0.55 and 0.74 which produced peaks with retention times typical for p,p'-DDE.\* Therefore, p,p'-DDE was not quantitated.

Similar problems with p,p'-DDE (and o,p'-DDT) have been encountered before (RISEBROUGH et al, 1968), when extracts of airborne dust were analyzed on QF-1 and DC-200. RISEBROUGH et al (1968), however, found no indications for the presence of PCBs.

The amounts of insecticide residue in <u>Acanthaster</u> varied from 0.04 to 3.89 ppm for p,p'-DDT, and from 0.01 to 1.04 ppm for dieldrin.

<sup>\*</sup>A number of mixtures of compounds producing "DDE peaks" are under study for their interference with insecticide residues in GLC.

TABLE 2.

P, p'-DDT and dieldrin contents in ppm (relative to wet weight of fixed tissue) of gonad tissue from <u>Acanthaster planci</u>, 1970-71.

Sampling	Number	Mean Sample	p,p'-DDT in ppm		Dieldrin in ppm	
Site	of Samples	Weight	Mean	Range	Mean	Range
GUAM 1)	4	7.02	0.75	0.16- 1.16	0, 27	0.01- 1.04
GUAM, Buoy (Merizo 2)	3, 1	8.49	0.92		0.09	- · · · - ·
GUAM, Cocos Lagoon, Me		5.93	0.69		0.11	
GUAM, west s Anae Island	ide 5	7.11	0.41	0.32- 0.97	0.06	0.02- 0.09
ROTA 1)	5	5.36	0.11	0.06- 0.15	0.02	0.01- 0.03
MOLOKAI, Hawaii 3)	1	10.37	0.04		0.02	
NAMOLUK Atoll <sup>4</sup> )	5	7.56	0.06	0.04- 0.11	0.02	0.01- 0.05
TRUK Atoll, Dublon Isla	1 nd 2)	9.49	0.09		0.02	
TRUK, reef w of Pis Islam		9.79	0.04		0.01	
TRUK, Northe Passage 2)	ast 1	7.39	0.06		0.02	
ENIWETOK At		9.11*	0.08		0.02	
GBR, Bunker Gro	, 3	16.44	0.12	0.07- 0.16	0.06	0.03- 0.08
GBR, Boult Re Bunker Gro	eef, ?	36.10*	0.26		0.09	
GBR, Slasher Reef 7)		16.66	0.28	0.08- 0.81	0.11	0.02- 0.27

<sup>1)</sup> Collected in March, 1970 by D.P. Cheney

<sup>2)</sup> Collected in June, 1970 by D.P.Cheney

<sup>3)</sup> Collected on April 3, 1970 by D.M.Devaney

<sup>4)</sup> Collected on May 21-23, 1970 by K. Marshall

<sup>5)</sup> Collected on December 26, 1969 and February 15, 1970 by R.Pearson

<sup>6)</sup> Collected on December 10, 1969 by R. Pearson

<sup>7)</sup> Collected on July 18, 1970 by R. Pearson

<sup>8)</sup> Collected on June 23, 1971 by L.R.McCloskey

<sup>\*</sup> Aliquot of larger sample composed of gonads from a number of starfish

### DISCUSSION

No marked correlation is evident between amount of organochlorine residues and local abundance of starfish. Material from Guam and the Great Barrier Reef--both with very high starfish populations--clearly also has the highest insecticide residues. But Woleai Atoll has almost no starfish, but surprisingly high mean values of p,p'-DDT. Truk and Rota are also reported to be sites of starfish infestations (CHESHER, 1970), but insecticide residues from starfish collected there are considerably less than Guam--or Woleai. Eniwetok also has a large though localized starfish population, but low pesticide residues. There seems also to be exceptions to the general expectation of higher residues closer to human population or agricultural centers, i.e. Molokai, Hawaii with residue values as low or lower than remote Namoluk and Truk Atolls.

Values given for Acanthaster gonads correspond approximately to p,p'-DDT residues found in phytoplankton from Monterrey Bay, California--0.2 to 0.6 ppm (COX, 1970). D. J. Tranter's data (in WALSH et al, 1971) for Acanthaster from the Great Barrier Reef also agrees with our results.

Of themselves, the residue values are of very limited value in ascertaining their biological significance to Acanthaster. A point we wish not to be overlooked is that there is as yet no way to determine the age of the starfish, and age is very likely to affect residue accumulation. Perhaps much of the variation within samples and even between localities may be due to this or other unknowns.

As well, the effect of the residues on the ecology of Acanthaster cannot be determined. We have been interested to know if biological magnification may occur in Acanthaster predators, particularly Charonia tritonis. We have analyzed the liver of but one specimen of the triton, collected in Guam, and found 0.71 ppm p,p'-DDT. This is in the lower part of the range of values for Acanthaster from Guam, and does not suggest that higher residues are accumulating in the triton. Whether pesticide residues affect the food or predators of the planktonic larvae of the starfish (J.E. RANDALL, personal communication), however, remains a possibility.

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