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**MARINE TOXINS FROM THE PACIFIC II.  
THE CONTAMINATION OF WAKE ISLAND LAGOON**

by Albert H. Banner, Judd C. Nevenzel and Webster R. Hudgins

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## MARINE TOXINS FROM THE PACIFIC II. THE CONTAMINATION OF WAKE ISLAND LAGOON<sup>1</sup>

by Albert H. Banner, Judd C. Nevenzel and Webster R. Hudgins<sup>2/</sup>

Wake Island lies at 19° 18' N and 166° 38' E; it is a small atoll about 6.5 km long and 3 km wide. Its shallow lagoon, with a maximum depth of 25 m, has no connection with the surrounding sea at low water, but at high tide water may enter and leave the lagoon either across the two-mile broad western reef or through a passage on the northern side between Wake Island proper and Peale Island (the original passage between Wake and Wilkes Islands on the southwestern side has been blocked by a causeway). The island is inhabited by about 1500 people who are primarily employed in the servicing of trans-Pacific aircraft.

### Observations

The evening of Sunday, 20 June 1965 was unusually still with a complete cessation of the normal trade winds. Police Officer Earl Harris, on night duty, noticed an unusual, pungent and repulsive odor about midnight, when he was passing over the bridge connecting Wake and Peale Islands. The odor was so strong that he paused and flashed his spotlight into the water below. He was surprised to find that water rushing into the lagoon with a rising tide, instead of being clear as normal, was milky in appearance. At 3 A. M. and again at 6 A. M. he again checked the water, which remained pungent and milky.

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<sup>1/</sup> Contribution No. 305, Hawaii Institute of Marine Biology, University of Hawaii, Honolulu, Hawaii 96822. The second in a continuing series of papers on marine toxins published by a group at the Hawaii Institute of Marine Biology, University of Hawaii; the first 'Advances in the Investigation of Fish Toxins' by Albert H. Banner in *Animal Toxins*, 1967, eds. Russell, F. E., and P. R. Saunders, xiii + 428, Pergamon Press, Oxford.

<sup>2/</sup> A. H. Banner, Department of Zoology, University of Hawaii; J. C. Nevenzel, Department of Biophysics and Nuclear Medicine, University of California, Los Angeles; W. R. Hudgins, Research Laboratory, Allied Chemical Corporation. A. H. Banner responsible for gathering field data and biological observations; W. R. Hudgins for general chemistry; J. C. Nevenzel for detailed chromatographic analyses. Work at Hawaii Institute of Marine Biology supported in part by U. S. Public Health Service Grant EF-00216 and Office of Naval Research Contract Nonr 2289(00), NR 107-405; work at University of California, Los Angeles, by the Atomic Energy Commission Contract AT(04-1) GEN-12 between the Commission and the University.

At 8 o'clock that morning he visited the beach at Peale Island with the Assistant Island Manager, Mr. George La Caille, and the resident physician, Dr. Frederick Goff. They found a band of floating material, perhaps a meter wide and of the consistency of a thin soup, reaching along the lagoon beach of Peale Island as far as the eye could see. Patches of the material were floating far out into the lagoon. The material was light in color with tinges of pink. Most characteristic of the material was its sweet but fetid odor, variously described as "sickeningly sweet," "a combination of a molasses refinery and an exposed oyster flat," or "like sewage." During that day and for several days to come the odor permeated the whole island.

By early afternoon of that day fish were observed to exhibit aberrant behavior. As the material, now being moved from the lagoon towards Peale Island by a slight westerly wind, drifted towards the shore, the small lagoon fish behaved frantically -- moray eels left their holes and swam to deeper water, while others such as wrasses leaped from the water to the beach and lay there flopping in the sand. The smell, the action of the fish, and especially the unknown nature of the flotsam, caused the officials to ban all swimming, boating, and fishing in the atoll waters.

During the next several days the contamination of the lagoon was watched carefully by Dr. Frederick Goff. The original milky material turned pink, and in concentrations along the shoreline, brownish red. So many fish were killed that a sanitary patrol was sent out; it gathered over three barrels of dead fish from the shore of Peale Island. The receding tide left the exposed rocks and bars of the lagoon stained red. Even the birds were afflicted and a number of sooty terns in the colony on Peale Island which were feeding in the western section of the lagoon sickened and died. A frigate bird was seen to dive into a floating mass, presumably after a sick fish; but when it surfaced it was unable to fly and flopped its way to a small sand bar. Within minutes it collapsed and died.

Over four liters of the material were dispatched frozen to the Hawaii Institute of Marine Biology for study; it was received on the afternoon of 24 June (Wake time). Arrangements were completed for the senior author to visit the island as a consultant.

Late in the afternoon of 26 June and the following day, the senior author was able to inspect contaminated beaches and the surrounding waters. No longer was there any floating material in the water -- it was reported to have disappeared by 23 or 24 June. However, near the high tide zone on the beaches at Peale Island were patches of reddish-brown creamy to waxy material. No patches were excessively large, although some were up to a meter wide and seven or more meters long, and with a thickness of a millimeter or so. Other patches were more concentrated, over a centimeter thick for a diameter of more than 30 centimeters. On the lagoon side of Peale Island the patches were most common, but they were also found on the ocean side near the passage as well and correspondingly near both sides of the passage on Wake Island. On Wilkes Island, across the lagoon, a few patches were found. On sandy beaches the material had penetrated into the sand so that when

the top layer was disturbed, the underlying sand had a pink cast. The exposed sand in these areas was of light grey color rather than its characteristic white. Similarly, tufts of attached algae on a shallow tide flat at Peale Island were coated with the pink material. The waxy material on the beach, the sand, and the algae all had the pungent, sickening but sweet, odor.

Along the shores of Wilkes Island were numerous dead fish that obviously had been exposed for some time. One sick tern, unable to fly, was collected on Peale Island on Saturday evening; during the night it died.

On the morning of 27 June the lagoon was inspected by boat and the lagoon and the surrounding waters of the island by air from a U. S. Navy amphibian plane flying at 150 meters. No floating matter was seen, and the coral heads of the western side of the lagoon, exposed by the extreme low water at the time of inspection, seemed unaffected.

At the recommendation of the senior author the lagoon was opened to swimming and boating on Sunday. Bathers reported the water "had a bad taste" but no ill effects were seen from swimming.

#### Laboratory Examination

The frozen material shipped to Honolulu was pink with grey traces. It was a consistency of thick ladled or skimmed cream, with some free water on the bottom of the container. The waxy material collected from the beaches on the 26th and 27th had a consistency of a thick medicinal ointment. Both would adhere to the skin when touched.

Microscopically, the bulk of both materials was without formed structures and was immiscible with water when squashed on a microscope slide. In all material examined there were numerous oil droplets and an amorphous ground substance. The pink masses were equally formless and were scattered as small units through the other material. In the water surrounding the sample shipped to the laboratory were numerous green, spherical cells of a unicellular alga; in the tufts of contaminated algae collected from Peale Island, the cells of the filaments appeared alive; in this material and in the contaminated sand were numerous ciliate protozoans. However, in the consolidated masses of the waxy cream from the beaches there was no life.

The creamy material delivered to the Hawaii Institute of Marine Biology was found, by dehydration at 45° C under reduced pressure, to contain 68.8 per cent water and volatile components, 31.2 per cent solids which obviously included the salts left by the evaporated sea water (estimated at perhaps 2 per cent of the weight of the original sample, a flame test for sodium was positive).

Five hundred grams of the same emulsion, undehydrated, was extracted by occasional shaking for one day in equal volume of 95 per cent ethanol and filtered; the evaporated ethanolic extract yielded 15.3 g of a bright yellow waxy solid. A dry ice trap, followed an ice trap, in the distillation line yielded a small amount of a colorless

volatile liquid with an odor characteristic, in part, of that of the original sample. The residue from the ethanolic extraction was in turn extracted with 500 ml of diethyl ether, and yielded 35 g of a brilliant orange waxy solid that melted slightly below 45° C and that contained scattered microcrystals. It was possible to dissolve and recrystallize the crystals in ethanol; they were white and plate-like, and had a sharp melting point at 51° C. The residue undissolved by the diethyl ether extraction was yellow and fibrous; it gave a positive biuret test for protein.

The toxicity of the mixture and some of its principal components were checked to see if the observed deaths of fish and birds could be attributed to the mixture. The crude cream was emulsified and stirred into an aquarium at about 10 ml per liter and three butterflyfish (*Chaetodon* sp.) were added; the aquarium was continuously aerated. In 40 to 60 minutes the fish, after showing marked respiratory distress, died. Five white mice, weighing from 21 to 24 g, were force-fed some 0.3 to 0.6 ml of emulsified mixture; all exhibited heavy breathing, gasping and listlessness, and three died overnight. Similarly, when the supernatant wax from the original sample was force-fed to mice in similar amounts, three of the six mice died overnight. However, when the ethanolic or ethereal extracts were injected intraperitoneally into mice at the rather massive dosage of 4 mg/g, no deaths resulted. The final fibrous residue caused no deaths when force-fed, but the same residue when injected at 4 mg/g caused one of the two mice to die overnight.

A stoppered vial of the soft beach material with a pink cast was kept at room temperature; after four days it smelled strongly of hydrogen sulfide.

#### Analysis of the Lipid

The total lipid in the original creamy material delivered to the Hawaii Institute of Marine Biology was obtained by extraction into diethyl ether, using centrifugation and methanol to break emulsions. The crude lipid was separated by adsorption column chromatography on silicic acid (Fillerup and Mead, 1953) into the fractions listed in Table I. The identification of the main constituent (60%) of the Wake Island lipid as wax esters (that is, fatty acids esterified with long-chain alcohols) was confirmed by (a) direct comparison in thin-layer chromatography (TLC) with synthetic heptadecyl stearate; (b) gas-liquid chromatography (GLC) of the unhydrolyzed esters (cf. Nevenzel, et al., 1966) -- the results are given in Table II; and (c) hydrolysis to acid and alcohol moieties, which were analyzed by GLC -- the acids as their methyl and phenacyl esters and the alcohols as their trifluoroacetate derivatives (Nevenzel, et al., 1965) -- see Table III.

A comparison of the analysis of the Wake Island lipid with the data of Lederer, et al., 1946, for ambergris (also given in Table I) makes it clear that our material was not ambergris. A direct comparison by TLC of the crude Wake Island lipid with authentic low-grade ambergris from Hawaii further emphasized this point: on Bio-Sil A (Bio-Rad Laboratories, Richmond, California) developed with 10 per cent diisopropyl ether in petroleum ether (b.p. 60-70° C) the Wake Island lipid

showed one main spot of  $R_f$  0.68 (identical to authentic wax esters) and a faint spot of  $R_f$  0.07 while the Hawaiian ambergris had only a faint spot at  $R_f$  0.69 for wax ester or sterol ester, another minor component of  $R_f$  0.31, and the main spot of  $R_f$  0.25 with extensive tailing; authentic octadecanol had an  $R_f$  of 0.13 and cholesterol about 0.05 in this system.

The sterol-containing fraction of Table I was further characterized by TLC, which confirmed the presence of free long-chain alcohols or cholesterol. By GLC analysis of the trifluoroacetate derivatives of these hydroxyl-containing compounds straight chain alcohols  $C_{16}$ - $C_{25}$  were identified together with a trace of a higher molecular weight compound, thought to be a triterpene alcohol or a sterol other than cholesterol. TLC examination of the most polar lipid fraction established the presence of traces of free fatty acids and phospholipid (probably lecithin), but the bulk of the material appeared as a streak with no defined spots and was not characterized further. The yellow color of this sample was due to strong end absorption from a peak below 230  $\mu$ ; no identifiable pigment was present.

### Discussion

To our knowledge, no bloom of an alga, either fixed or planktonic, nor any spawning or other activity of a marine invertebrate or fish has been reported to form an amorphous mass of floating lipids of such volume and nature. Aside from the results of man's activity, the only massive depositions of waxy material in the sea is the voided secretion of the gut of the sperm whale, known commercially as ambergris. As Wake Island lies in the traditional whaling ground for the sperm whale, it was originally thought that the contaminant of the lagoon was a huge mass of ambergris, emulsified and altered in consistency in its passage through the surf. The hypothesis was rendered even more attractive by the musty smell noted on the contaminated beaches.

However, the laboratory studies of one of us (Nevenzel) have destroyed the hypothesis of the other two: the material definitely is not ambergris. On the other hand, only lipids of the sperm whales (family *Physeteridae*, Hilditch and Williams, 1965), the beaked whales (family *Ziphiidae*, Mori, et al., 1964), and some pelagic copepods (Nevenzel, unpublished) and fishes (families *Myctophidae*, Nevenzel and Rodegker, 1965; *Gadidae*, Komori and Agawa, 1955; *Gempylidae*, 1963; and *Trachichthyidae*, Kaufmann and Gottschalk, 1956), and the living fossil (family *Latimeridae*, Nevenzel, et al., 1966) are largely wax esters. Of seven species whose wax esters have been examined in detail, only those of the sperm whale have as low an average chain length as those observed here: Wake Island lipid has 85 per cent esters shorter than  $C_{34}$ , spermaceti wax 90 per cent, but the wax esters from fish have only 4-20 per cent of the total shorter than  $C_{34}$ . The available data on the composition of several sperm whale lipids are given in the Tables for comparison with the corresponding values for the Wake Island lipid. Note that spermaceti is the material which crystallizes from the total sperm whale oil on chilling and consists of the more saturated, longer chain wax esters; it contains only traces of triglycerides or unsaturated components.

The simplest explanation for the origin of the Wake Island material is that a mass of whale oil with some fibrous content floated into the lagoon in an emulsified condition, where it settled out into the floating cream-like layer. In the process it was acted upon by micro-organisms and was subjected to chemical changes (hydrolysis, recombination, oxidation, etc.). In general, the differences in composition between the Wake Island lipid and sperm whale oil (Tables I and III) are consistent with (a) proportionally greater losses of the shorter chain wax esters, due to their greater solubility and greater rate of hydrolysis; (b) the extensive losses of unsaturated components by autoxidation; and (c) the preferential loss of triglycerides because of their higher content of unsaturated fatty acids and greater rate of hydrolysis. The net result of these processes would be the production of a lipid consisting largely of saturated wax esters with a reduced content of C<sub>26</sub> and C<sub>28</sub> components: i.e., something approaching spermaceti. Tables II and III confirm the similarities of Wake Island lipid with spermaceti, although in the former the content of decanoic acid (10:0) is ten times as much as expected for whale oil, and the percentage of palmitic acid (16:0) is also too high. Nor does this explanation account for the toxicity of the samples.

The toxicity probably is the result of bacterial action upon the mixture. When the material was first seen, it was emulsified into a thin milky suspension. In such a state the surface area for bacterial attack would have been tremendous and as the fatty material reconsolidated in the "cream" found floating on the surface, the bacteria were also incorporated. Some probably worked on the lipids, but others could have worked upon the fibrous protein mixed in the mass. By-products from the bacterial decomposition of the mixture could be toxic and account for the death of fish and possibly the birds. An example of such a product is the hydrogen sulfide noted both in the field and the laboratory. The exhaustion of oxygen in the water by bacterial action under the floating "cream" could also account for some deaths of fish, but it cannot account for the death of the butterflyfish in the well-aerated aquarium. The inability of the birds to fly probably was not from the toxicity of the mass but from the oils coating their feathers.

It is more difficult to account for the observed large amount (17.5%) of decanoic acid (C<sub>10</sub>), since in sperm whale oil C<sub>10</sub> is a minor constituent; cf. Table III. One possible suggestion is that the mass reforming from the thin emulsion trapped and incorporated C<sub>10</sub> acids from a bloom of blue-green alga, for Oscillatoria (Trichodesmium) erythraeum is known to contain these (Parker, and Van Baalen, personal communication). However, from interviews of the residents, there was no indication of a previous bloom of alga in the lagoon, and the coincidence of a major bloom of blue-green phytoplankton, a rather rare occurrence, at the moment of the contamination of the lagoon by waxes strains one's credulity.



## Conclusions

As sperm whales do not give up their wax esters voluntarily (unlike their ambrein and sterols) we find the best way<sup>3/</sup> to account for the contamination of Wake Island lagoon is to postulate that some unknown whale factory ship, cruising the whaling grounds discharged its rendering retorts for unknown reasons. This crude oil-wax concentrate, still containing the fibers of the adipose tissue, floated in a coherent mass and probably for a relatively short time, until it was carried by currents to the reef off the northern coast of Wake Island. It arrived in time for the currents of the incoming tide at the shallow entrance to the lagoon. Here the currents carried it through the surf (in spite of the calmness of the night, there would be continuing and possibly heavy surf on the windward reef face). Once the crude mixture was emulsified by the surf, the great surface area permitted rapid bacterial action on all components, speeding a massive alteration of the lipids and decomposing the proteins. A chemical by-product by this action could be the loss of some lipid fractions, thereby concentrating other fractions (such as cetyl alcohol), and the synthesis of C<sub>10</sub> acid. The red cast to the mass observed in the water and on the beaches probably was the result of growth of pigmented sulfur bacteria, which were utilizing the H<sub>2</sub>S given off in other bacterial decomposition.

We further postulate that toxic by-products of the bacterial action killed the fish and rendered the mixture toxic to laboratory mice when fed or injected with it. At the same time, the presence of bacteria caused the ciliate protozoans, which feed on bacteria, to increase and the bacterial release of nutrient salts encouraged the growth of unicellular algae. Possibly some C<sub>10</sub> acids were incorporated into the mass from the phytoplankton. Otherwise we cannot account for their presence.

Finally, the emulsified droplets, floating to the surface, reformed into the waxy mass and was carried to the beaches by the light airs.

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<sup>3/</sup> The senior author alone, having his ambergris hypothesis ruthlessly destroyed by fact, wishes to emphasize that the chromatography proved that this particular combination of wax esters could have come from no known animal or plant. He suggests that we must therefore look for some yet unknown animal, and that perhaps the Great Sea-Serpent, so convincingly described by Oudemans, might be a wax ester producer. Presumably even the sea-serpent does not shed its adipose tissues, but perhaps the magnificent creature might discharge wax ester as a medium for its aura seminalis. He begs to recall the described aura of the Wake Island lagoon.

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TABLE I

## Composition of Wake Island Lipid (Weight %)

Component lipids	Wake Island lipid	Ambergris (1)	Sperm Whale Head Oil (2)
Hydrocarbons	-	3 ± 1	-
Ketones	-	7 ± 1	-
Wax esters	59.9	} 6 ± 2	72.7
Triglycerides	9.5		24.6
Free sterols and alcohols	6.3	78 ± 17	-
Free fatty acids and polar lipids	24.3	5	2.0

(1) Normalized data of Lederer, et al., 1946.

(2) Tateishi, et al., 1958.

TABLE II

Composition of Wax Esters of Wake Island Lipid (Weight %)

Source	Wake Island Lipid	Spermaceti (1)
Homologue (2)		
26:0	10.7	0.6
27:0	-	0.2
28:0	0.8	14.8
29:0	-	1.8
30:0	11.0	36.1
30:1	0.7	-
31:0	-	3.0
32:0	58.2	31.8
32:1	3.7	-
33:0	-	1.8
34:0	4.3	8.2
34:1	8.4	-
36:0	-	1.8
36:1	2.2	-

(1) "Spermaceti, U.S.P.", Fisher Scientific Company, Fair Lawn, N.J.

(2) The number before the colon indicates the total number of carbon atoms, the number following the colon denotes the total number of double bonds in the molecules.

TABLE III

Component Fatty Acids and Alcohols of  
Wake Island Wax Esters (Weight %)

Source	Wake Island Lipid		Spermaceti (1)		Sperm Whale Head Oil	
	Acids	Alcohols	Acids	Alcohols	Acids (2)	Alcohols (3)
Homologues (4)						
10:0	17.5	-	trace	-	1.0	-
12:0	0.2	trace	14	-	11.4	-
12:1	-	-	-	-	4.2	-
14:0	9.3	0.8	39	2.7	17.0	7.7
14:1	-	-	-	-	11.8	-
15:0	0.2	0.1	1.2	1.2	-	0.9
16:0	60.8	88.0	38	80.0	9.8	44.9
16:1	4.1	0.8	trace	-	16.0	5.3
16:2	-	-	-	-	-	1.2
17:0	-	-	1.0	1.8	-	-
18:0	1.0	7.1	5.6	12.9	1.7	3.0
18:1	5.0	1.0	trace	-	17.8	33.4
18:2	0.1	-	-	-	2.0	0.6
20:0	0.1	1.0	trace	0.4	0.6	-
20:1	0.8	0.6	-	-	5.8	1.8

(1) "Spermaceti, U.S.P.", Fisher Scientific Company, Fair Lawn, N.J.

(2) Data of Tateishi, *et al.*, 1958, recalculated to estimate value for 18:2.

(3) Data of Mori, *et al.*, 1964, for Arctic Sperm Whale, head oil.

(4) The number before the colon indicates the number of carbon atoms, that following the number of double bonds in the acid or alcohol.



Figure 1. Intertidal area at Peale Island, showing contamination of beach. The normal beach color is dazzling white, as shown in the fore- and back-ground; the crescentic darkened area, reddish-brown in hue, is of the waxy material well over a meter wide but only a few millimeters thick.



Figure 2. Intertidal area at Peale Island, showing a "glob" of contaminant. The thick material, dark red-brown in color, is of the consistency of a thick ointment and covers the shells and coral fragments on the beach variously from 1 to 4 cm thick. The scale may be interpreted from the wrist watch on the upper left.