# MARINE NATURAL PRODUCTS:

# INTERFERENCE IN PESTICIDE RESIDUE ANALYSIS

ABSTRACT:

Naturally-occurring halogenated compounds produced by marine organisms are shown to interfere with pesticide and PCB residue analysis. Using the accepted gas chromatographic methods for chlorinated hydrocarbon analyses, extracts from a variety of marine organisms show natural halogenated compounds with retention times easily confused with DDT, its metabolites and the PCB's.

Synthetic chlorinated hydrocarbons such as DDT and the PCB's are recognized as accumulating pollutants in sea water (1), as well as in marine-related organisms, plankton (1), invertebrates (2), fish (3), birds (4) and mammals (5). The quantitative estimations of these chlorinated pollutants rely almost exclusively on the use of gas-liquid chromatography, coupled with electron-capture detection (ECGC). The electron-capture detector provides very high sensitivity toward organo-halogen compounds, as well as selectivity in complex mixtures. It has therefore permitted measurements of chlorinated pollutants to be made at very low levels in relatively unpurified samples. While sensitive and selective detection is an obvious benefit in environmental pollution studies, ECGC suffers from an obvious problem in that it provides little qualitative information concerning the halogen-containing substances being measured (6). With an understanding of these drawbacks, numerous analytical modifications have been described to improve the specificity of chlorinated hydrocarbon analysis (7). Even though sample contamination during chlorinated hydrocarbon analysis with natural substances has been discussed by many investigators, the origins of these naturally-halogenated compounds and their chromatographic behaviors are not well understood.

In recent years, many halogen-containing natural products have been isolated and described from marine organisms (8). These chlorine-, bromine-, and iodine-containing compounds are biosynthetically produced from seawater components by many red seaweeds, sponges, molluscs and marine bacteria. Hundreds of

natural halogen-containing compounds of diverse types are recognized, with some being structurally similar to synthetic pesticide molecules. The unusually high concentrations of these natural compounds often reaches 1-5% of the dry weight of the organisms, which indicates the prominence of halogenation processes in marine organisms.

In an attempt to assess the thermal stability and chromatographic behavior of the natural halogens, and, hence, their potential to interfere in PCB and DDT analysis, we made hexane extracts of six locally available organisms already known to contain natural halogens. The extracts (9), when subjected to ECGC analysis under standardized conditions (10), showed peaks with retention times easily confused with those of DDD, DDE, DDT and two PCB mixtures (Figure 1). Four red seaweeds (Laurencia pacifica (11), L. subopposita (12), Chondria californica (13) and Plocamium cartilagineum (14)), an abundant local sponge (Verongia sp. (15)) and one bacterium (Chromobacterium sp. (16)) were selected, since each, with the exception of Chondria, is known to synthesize bromine- and chlorine-containing compounds. In each case (see respective references) the major halogen-containing compounds have been isolated and structurally described. All six extracts showed compounds with the same detectability as pesticides which fall within the retention time limits associated with common PCB's (Arochlor 1254 and Arochlor 1242). Laurencia and Chromobacterium contain substances that could easily be confused with DDT or its metabolites.

Since the marine algae are potentially the largest source for naturally-occurring halogenated compounds (17), we studied the chemical composition of an isolated tide pool containing large amounts of the red seaweeds Plocamium and Corallina, inter alia, as a function of time. Twenty-liter samples were collected every 1.5 hours and immediately extracted with one liter of purified hexane, and four microliter aliquots of these extracts were systematically compared via ECGC (Figure 2). Extreme care was taken to exclude contaminating material from all glassware by baking, followed by rinsing with nanograde hexane. After three hours, significant quantities of electron-capture detectable compounds were found to have accumulated in these samples. Co-injection with known pesticide standards showed the major peaks to be naturally-occurring. The concentration of the major compound after three hours (Figure 2, trace C) is estimated at .0005 ppm, which is at least 500 times the typical content of DDT and its metabolites in seawater (1). In a recent study, the bromine-containing phenol, lanosol, originally reported as a natural component of the red seaweed Polysiphonia lanosa, was shown to be a dissolved constituent of seawater (18). These combined observations provide convincing evidence that natural halogens are being released into the marine environment.

The strong correlation of the retention times of the natural compounds reported here with those of DDT, its metabolites, and the PCB's shows conclusively that natural substances can be interpreted as pollutants, using solely ECGC methods. The real potential for these compounds to interfere in pollution analysis

lies in samples taken from the coastal environment and will probably be minimized in open ocean studies.

While there is little doubt that industrial halogens are broad-scale environmental pollutants, accurate analyses of these compounds in marine samples must be obtained by more specific methods. Chromatographic pretreatments are successful in removing many contaminants; however, the constituents noted here are of broad structural types, from non-polar hydrocarbons to very polar hydroxyl-containing metabolites, and, hence, no single treatment can be deemed entirely successful. We second the earlier suggestion that ECGC be abandoned in favor of combined gas chromatography-mass spectrometry methods, which provide both qualitative and quantitative information (19).

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References and Notes

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9. The algae Plocamium cartilagineum, Laurencia pacifica, L. subopposita and Chondria californica were extracted by placing 25 gm of drained plant in 50 ml of nanograde hexane for 12 hours. The sponge Verongia sp. was extracted in 95% ethanol. The ethanol was evaporated and the extract taken up in hexane. The Chromobacterium sp. was extracted in ether. The ether was evaporated and the extract taken up in hexane.

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20. This work is a result of research sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grant #04-3-158-22. The U. S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

Figure 1. Electron capture gas chromatographic traces of pesticide standards and hexane extracts from marine organisms: A) DDT, B) DDE (first peak) and DDD, C) Laurencia pacifica, D) Laurencia subopposita, E) Chromobacterium sp, F) Aroclor 1242, G) Aroclor 1254, H) Plocamium cartilagineum, I) Verongia sp., J) Chondria californica. See notes 8 and 9 for extraction procedure and chromatographic conditions.

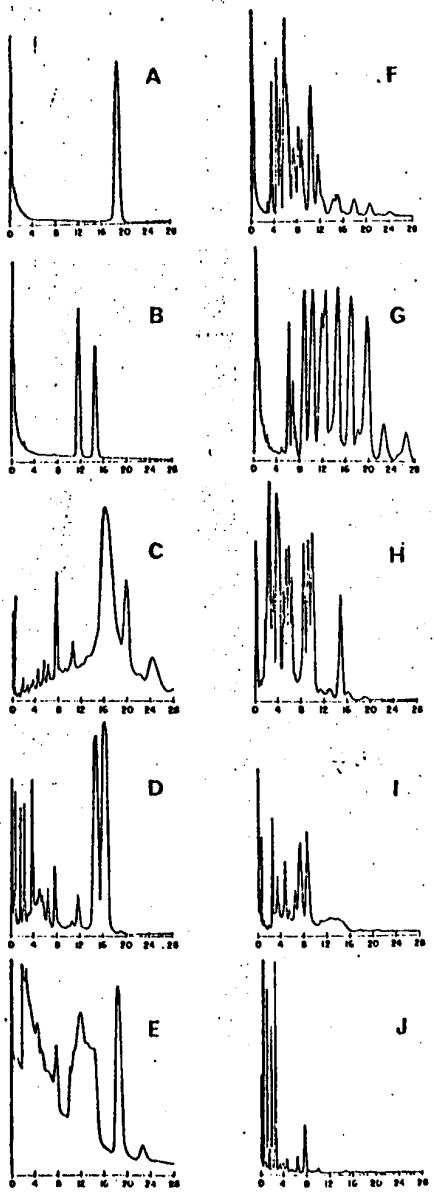
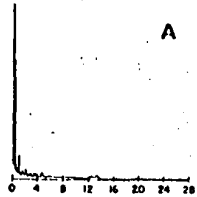
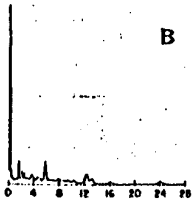
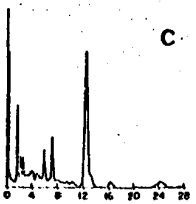


Figure 2. Electron capture gas chromatographic traces of hexane extracts of tidepool water. The water samples were obtained from an isolated tidepool at low tide and extracted immediately. At the time of sampling, the air temperature was 27°, the water temperature was 18.5° and the day was partly cloudy. Neither nanograde hexane nor 1 liter of nanograde hexane condensed to 5 ml contained any contaminating material. A) Time zero, 4µl; B) 1.5 hours, 4 µl; C) 3 hours, 4 µl.




MONS 045239