# Presence of lipid-soluble chlorinated hydrocarbons in marine oils

**ARTICLE** *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · FEBRUARY 1975

Impact Factor: 5.33 · DOI: 10.1021/es60100a009

# Presence of Lipid-Soluble Chlorinated Hydrocarbons in Marine Oils

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■ The total amount of chlorine present as lipid-soluble chlorinated hydrocarbons is determined in marine oils from fish, shrimp, and whale. The oils are isolated by heat treatment and also by extraction with suitable organic solvents. The level of chlorine varies from about 20–650 ppm in the samples investigated. It is concluded that the larger part of this lipid-soluble organic-bound chlorine can hardly be accounted for by known substances. They may in part be synthesized by natural means in the marine environment. The absorption of unidentified chlorinated pollutants is another possible source. Bromine and iodine have been determined as well to allow comparison with previously obtained data.

It has been shown previously that marine oils contain a variety of trace elements present as lipid-soluble organic compounds. Among these should be mentioned arseno lipids (*1, 2*), seleno lipids (*3*), and also compounds containing bromine and iodine (*1*). Comparable compounds have so far not been detected in oils of vegetable origin, or in oils from animals that have been fed a terrestrial diet (*4*). It has been shown, however, that seabirds may contain a certain amount of lipid-soluble bromo organic compounds that presumably originate from the fish in their diet (*4*).

Results from investigations over the bromo organic compounds in marine oils show that bromine is not present as one, or a particular group of compounds, but rather that this element is distributed between a wide variety of chemical classes. The fairly large amounts detected may point to a synthesis in the marine food chain as their origin (*4*). In the above-mentioned investigation (*1*), the presence of chlorine was detected as well, but further studies were not at that time pursued.

In recent years, interest in chlorinated hydrocarbons in the environment has increased dramatically. Most of the work carried out so far relates to chlorinated pesticides, chlorinated industrial, compounds such as the polychlorinated biphenyls (PCBs), and various chlorinated substances released in industrial waste and from various other sources. Some of these compounds, such as PCB and DDT and their metabolites, have been the subject of a wide variety of investigations, directed in particular to their occurrence in foods and biologic material. This type of halogenated compounds is concentrated extensively in aquatic organisms (*5*). One may assume that other man-made lipid-soluble chlorinated organic compounds in a similar way may be absorbed and enriched in organisms living in water.

At present, very little is known about the complex mixture of halogenated (especially chlorinated) hydrocarbons introduced into the environment. If these compounds are to be studied, it is important to have available general methods for the determination of the total level of organic-bound chlorine that, combined with systematic fractionation of raw samples into defined chemical groups, provide a tool for further characterization of these halogenated compounds by chemical means. As in the case of bromine (*1*), chlorinated hydrocarbons may also, to some extent, be synthesized in the marine food chain. Work on specific halogenated compounds of marine origin has recently been reviewed (*6, 7*). Such compounds are, however, still considered to be rare.

Determination of chlorine and bromine has previously been carried out in commercial marine oils processed to edible fats (*8, 9*). Representative results from these investigations are given in Table I. The purpose of the present investigation is to gain further insight regarding the level of organic-bound chlorine in the lipid phase of marine organisms and also to extend the sample material.

Determination of total concentration of chlorine is based on neutron activation and subsequent gamma-spectrometry for the isotope $^{38}Cl$ with a half-life of 37.2 min. Bromine and iodine may be determined simultaneously by means of the isotopes $^{80}Br$ and $^{128}I$ with 17.6 min and 25.0 min half-life, respectively.

*Experimental*

**Sample Material.** In selection of the sample material, emphasis was placed on obtaining material from a number of different localities. In addition to fish, some samples of shrimp have been included. Two samples of factory-produced whale oil were also added. The oils were produced either by extraction with organic solvents or by means of heat treatment. Extractions were performed with a hexane/isopropanol mixture (50/50), as follows: The fish material was homogenized in a Waring Blendor, and solvent was added in equal volumes. The mixture was sonicated for 15 min and then further extracted in a shaker for 2 hr at 20 °C. The solvent mixture was subsequently filtered off. This extraction was repeated twice. The filtrates were combined, and the hexane was isolated by addition of water. Oil was isolated from the hexane phase by evaporation of the hexane in a Büchii Rotavapor apparatus.

Oil was prepared by heat treatment by addition of one part of distilled water to one part of homogenized fish raw

**Table I. Total Chlorine, Bromine, and Iodine (PPM) in Marine Oils Produced from Mackerel and Herring (10)**

| Sample | Locality[a] | Preparation[b] | Cl | Br | I |
|---|---|---|---|---|---|
| Mackerel | Bergen, W.N., 1972 | Hexane | 125 | 2,8 | 4,3 |
| Mackerel | Bergen, W.N., 1972 | Hex/iso | 126 | 3,3 | 6,5 |
| Herring | Oslo Fiord, S.N., 1972 | Hexane | 85 | 3,2 | 4,3 |
| Herring | Oslo Fiord, S.N., 1972 | Hex/iso | 51 | 2,8 | 4,4 |
| Herring | Bergen, W.N., 1971 | Hexane | 93 | 2,2 | 3,5 |
| Mackerel | Southern Norway, 1972 | Cyclohex/eth | 65 | 2,5 | 1,0 |

[a] W.N. = Western Norway, S.N. = Southern Norway. [b] Hex/iso = hexane–isopropanol, cyclohex/eth = cyclohexane–ethanol.

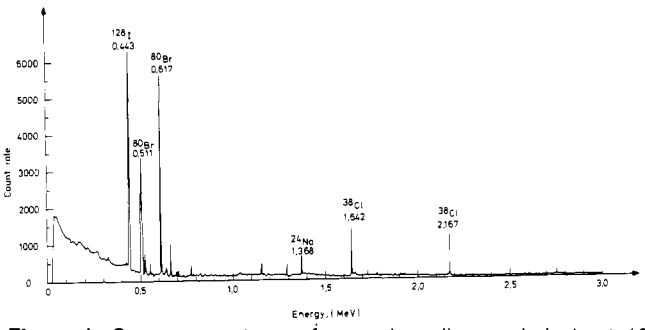


**Figure 1.** Gamma-spectrum of a marine oil recorded about 10 min after irradiation

material. The mixture was heated to 100°C and kept at this temperature for 60 min. When the oil was fully liberated, the samples were washed twice with distilled water to remove inorganic halides and were centrifuged. This washing process has been previously described (*10, 11*). Neutron activation of the oil was carried out at a flux of about $1.5 \times 10^{13}$ n/cm$^2$ sec. The oil samples were sealed in polyethylene vials and transferred to the reactor core by means of a "rabbit" system. Most samples were irradiated for 5 min together with standards for chlorine, bromine, and iodine. The induced radioactivity was registered immediately after irradiation by means of a 22-cm$^3$ Ge-Li detector and a multichannel analyzer. Figure 1 shows a $\gamma$-spectrum as registered about 10 min after the irradiation.

*Results and Discussion*

The results from the present analysis of halogens are shown in Table II. The values for chlorine from this and from the previous work (Table I) show that marine oils contain far more chlorine as lipid-soluble organic compounds than can possibly be accounted for by known compounds such as the PCBs and DDTs. Recent studies indicate a concentration of ~1.0 ppm for PCB and ~1.30 ppm for the DDTs in herring caught along the Norway coast (*12*). One may assume that some of this organic-bound chlorine is due to chlorinated pollutants other than the above-mentioned ones. However, such pollution hardly makes up the whole amount found in the oils, as several of the samples that come from areas considered to be relatively uncontaminated, still show fairly high results for chlorine. One must conclude, therefore, as with lipid-soluble bromine compounds (*4*), that a fair amount of these chlorinated hydrocarbons are produced in the marine environment, either outside or in the marine food chain.

The results indicate that the method of preparation of the oil does not influence the level of chlorinated hydrocarbons in it, and therefore that these substances are probably not bound to, or associated with, any particular fraction in the organism in the same way as are, for instance, the phospholipids. Rather, they appear to be uniformly dissolved in the body lipids.

The values found for bromine and iodine are generally in agreement with those found in earlier investigations (*1*). Comparing the ratio of chlorine to bromine and iodine in the oils, for instance in cod liver (Cl/Br/I, 40/6/12) to that in seawater (Cl/Br/I, $10^3$/65/0.06) (*13*), it is evident that there is a strong enrichment of the heavier halogens relative to the lighter ones. This indicates that especially the iodine compounds may be essential to the organisms.

At present, it is not possible to state what kind of chlorinated compounds are present in the marine oils beyond the known chlorinated hydrocarbons, as for example, PCB and DDT, that latter making up only for a minor part of

**Table II. Total Chlorine, Bromine, and Iodine (PPM) in Marine Oils**

| Sample | | Locality[a] | Preparation[b] | Cl | Br | I |
|---|---|---|---|---|---|---|
| Cod liver | *Gadus morhua* | Lofoten N.N. | Heat treatment | 28 | 5.9 | 9.2 |
| Cod liver | *Gadus morhua* | Lofoten N.N. | Heat treatment | 25 | 5.2 | 9.4 |
| Cod liver | *Gadus morhua* | More W.N. | Heat treatment | 38 | ... | ... |
| Cod liver | *Gadus morhua* | More W.N. | Heat treatment | 40 | ... | ... |
| Cod liver | *Gadus morhua* | Bergen W.N. | Heat treatment | 75 | 40 | 12 |
| Cod liver | *Gadus morhua* | Bergen W.N. | Heat treatment | 74 | 50 | 14 |
| Herring | *Clupea harengus* | Stavanger W.N. | Hex/iso | 46 | ... | ... |
| Herring | *Clupea harengus* | Bergen W.N. | Heat treatment | 60 | 8.2 | 3.3 |
| Herring | *Clupea harengus* | Skagerak S.N. | Heat treatment | 36 | 9.2 | 5.5 |
| Herring | *Clupea harengus* | Shetland | Heat treatment | 38 | 4.2 | 1.6 |
| Herring | *Clupea harengus* | Iceland | Heat treatment | 24 | ... | ... |
| Capelin | *Mallotus villosus* | Northern Norway | Hex/iso | 32 | 5.6 | 0.9 |
| Capelin | *Mallotus villosus* | Northern Norway | Hex/iso | 28 | 4.7 | 1.1 |
| Menhaden | *Brevooratia tyrannus* | N. America | Heat treatment | 21 | 3.0 | 3.4 |
| Dab | *Limanda limanda* | Skagerak S.N. | Heat treatment | 84 | 2.8 | 4.1 |
| Dab | *Limanda limanda* | Skagerak S.N. | Heat treatment | 79 | 8.5 | 5.0 |
| Plaice | *Pleuronectes platessa* | Skagerak S.N. | Heat treatment | 44 | 2.8 | 4.1 |
| Plaice liver | *Pleuronectes platessa* | Skagerak S.N. | Heat treatment | 657 | 25 | 15 |
| Haddock liver | *Melanogrammus aeglefinus* | Skagerak S.N. | Heat treatment | 74 | 13 | 12.2 |
| Rosefish | *Sebastes marinus* | Skagerak S.N. | Heat treatment | 39 | 5.1 | 4.2 |
| Halibut | *Hippoglossus hippoglossus* | East Greenland | Hex/iso | 48 | 6.1 | 6.6 |
| Halibut | *Hippoglossus hippoglossus* | East Greenland | Hex/iso | 35 | 7.2 | 2.8 |
| Tunny | *Thunnus thynnus* | Bergen W.N. | Hex/iso | 68 | 4.0 | 2.9 |
| Tunny | *Thunnus thynnus* | Bergen W.N. | Hex/iso | 114 | 2.8 | 2.3 |
| Finback | *Balaenoptera physalus* | Antarctica, 1962 | Pf | 32 | 2.1 | ... |
| Sperm whale | *Physeter macrocephalus* | Antarctica, 1962 | Pf | 32 | 0.7 | ... |
| Shrimp | *Pandalus borealis* | Oslo Fiord S.N. | Hex/iso | 36 | ... | ... |
| Shrimp | *Crangon vulgaris* | Oslo Fiord S.N. | Hex/iso | 33 | ... | ... |

[a] W.N. = Western Norway, S.N. = Southern Norway, N.N. = Northern Norway. [b] Hex/iso = hexane-isopropanol, pf = produced in factory.

the amount demonstrated. Work is now in progress to get more information as to the origin of these unknown chlorinated hydrocarbons.

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*Received for review April 19, 1974. Accepted September 30, 1974. Work supported by the Royal Norwegian Council for Scientific and Industrial Research.*


# Mechanism of Autoxidation of Manganese in Aqueous Solution


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■ Analytical data indicate MnOOH to be the primary product resulting from manganese autoxidation in aqueous solution. Mechanisms for the formation of oxides of varying composition consistent with the initial formation of MnOOH are discussed.


The removal of manganous manganese from aqueous solution by oxidation with dissolved molecular oxygen at pH of about 9.0 has previously been shown to be autocatalytic in nature (*1*). Since the reaction production is solid, the autocatalysis is heterogenous and exact kinetic analysis of the reaction is difficult. Nevertheless at constant pH and under constant partial pressure of oxygen, an autocatalytic rate expression of the type

$$-\frac{d[\mathrm{Mn(II)}]}{dt} = k_0[\mathrm{Mn(II)}] + k_1[\mathrm{Mn(II)}][\mathrm{MnO}_x]$$

was shown to be obeyed during the initial stages of the oxidation.

In this rate expression $MnO_x$ is used to denote a general empirical formula for the oxidation product, so that the values of $x$ of 1, 1.5, and 2.0 would correspond to Mn(II), Mn(III), and Mn(IV) oxides, respectively, without concern for the degree of hydration. Experimentally the products have varied in composition from $MnO_{1.3}$ to $MnO_{1.9}$, depending on the conditions of reaction (*2*). Such conditions involved, for instance, supersaturation of the reaction solution with respect to manganous carbonate during the use of buffer systems similar to those in natural waters, or supersaturation with respect to manganous hydroxide at high pH.

The formation of these variable reaction products indicates that a mixed removal mechanism may have been occurring. To characterize the products of oxidation alone, a study was undertaken where conditions were such that no supersaturation with respect to any species in the initial reaction solution could be predicted from existing thermodynamic data (*1*). On the basis of such data, no precipitation was expected to occur in a carbonate-free system containing $5 \times 10^{-4}$ mol of Mn(II) per liter at pH of approximately 9.3 or below at 25°C. Reactions were therefore carried out in 25°C ammonia buffer solutions of maximum pH 9.02.

Colorimetric procedures were used to determine the number of oxidizing equivalents per mole of suspended manganese when removal of soluble manganese through reaction with oxygen was complete. This enabled the calculation of empirical formulas $MnO_x$. A combined gravimetric/volumetric technique was also used to determine equivalent weights of filtered product, and finally the possibility of continued oxidation of the precipitated product was also investigated.

*Experimental*

ACS-certified reagents were used in makeup of reaction solutions and as reagents for subsequent analysis, except for manganous perchlorate, obtained from the G. F. Smith Chemical Co. USP-grade oxygen was used to presaturate reaction solutions.

Two colorimetric procedures (*3*) were used in the characterization of suspended oxidation products. Oxidizing equivalents of manganese were estimated by the *o*-tolidine method. Spectrophotometer readings were taken within 2 min of the mixing of sample with reagents to reduce error arising from color fade. Total manganese was determined by the formaldoxime method. Here the order of addition of reagents to samples was important for reproducibility. The procedure depends upon the formation of a colored complex between manganese and formaldoxime at high pH. Analysis of a typical 10-ml sample of approximately $5 \times 10^{-4}M$ $Mn^{2+}$ solution involves addition of 2 ml of reagent and 5 ml of $5N$ NaOH solution, followed by dilution to 100 ml and colorimetric measurement at 450 mμ. Consistent color formation could be obtained only if reagent was added after base addition to the sample.