



## NRC Publications Archive Archives des publications du CNRC

### **Extractable organically bound chlorine (EOCL) in fish and sediments, the unidentified 85%: a literature review**

Boyd, Robert K.

For the publisher's version, please access the DOI link below./ Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

#### **Publisher's version / Version de l'éditeur:**

<https://doi.org/10.4224/23001226>

*IMB Technical Report, 1993*

#### **NRC Publications Record / Notice d'Archives des publications de CNRC:**

<https://nrc-publications.canada.ca/eng/view/object/?id=d260c5ea-df67-4145-9c41-8ae29735b1a2>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=d260c5ea-df67-4145-9c41-8ae29735b1a2>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

#### **Questions?** Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

**Vous avez des questions?** Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.



main  
gft  
B

**EXTRACTABLE ORGANICALLY BOUND  
CHLORINE (EOCL) IN FISH AND  
SEDIMENTS; THE UNIDENTIFIED 85%.**

**A LITERATURE REVIEW.**

**Issued as IMB Technical Report No. 69  
NRCC No. 34876.**

**July, 1993.**

**ANALYZED**

**R.K. Boyd,**

**Institute for Marine Biosciences,  
National Research Council,  
1411 Oxford Street, Halifax, Nova Scotia,  
CANADA B3H 3Z1.**

**© 1993**

618225321 EP

## INTRODUCTION

The subject of this literature review is one of the ongoing mysteries of environmental analytical chemistry. Essentially the problem amounts to a large discrepancy between the total chlorine concentration in an appropriate organic extract (usually cyclohexane) of fish tissue (lake and marine sediments have also been investigated), as determined by neutron activation analysis (NAA), and the concentrations of targeted organochlorines such as PCBs, chlorinated pesticides (DDT, Toxaphenes, Mirex, etc.), chlorophenols, etc. The chlorine bound in identified pollutants typically contributes less than 10-15% of the total extractable organochlorine (EOCL) in fish, and about 5% in sediments [1-3].

Some recent Swedish and Norwegian work has concentrated upon trying to identify major components in this unknown 85% of the EOCL. There are two major groups involved, apparently working independently of one another. One group is at the Swedish Environmental Research Institute in Stockholm, and the other at the University of Lund. There is also some related work from a group at the Center for Industrial Research in Oslo, Norway, which has connections with the Lund group.

The following account is essentially a somewhat expanded version of summaries published by the Lund group [4-7] on work on chlorinated lipids. Some additional work by other groups on other potential chlorinated residues, which might *possibly* account for some of the thus far unidentified EOCL, has been added.

## CHLORINATED FATTY ACIDS

This section deals largely with the recent Scandinavian work [1-7]. However, at this point it is appropriate to mention some early Canadian connections. In 1978, Leach and Thakore [8] of British Columbia Research in Vancouver identified chlorinated stearic acids and chlorinated resin acids in pulp mill waste streams. In 1982, Richard Addison of the Bedford Institute of Oceanography proposed [9] that chlorinated fatty acids might be incorporated into fish lipids. In 1985 Voss and Rapsomatiotis [10], of the Pulp and Paper Research Institute in Pointe Claire, confirmed the findings of Leach and Thakore, mentioned above.

Early work (1988) on the problem of identification of EOCL in fish used gel-permeation chromatography (GPC) to show [11,12] that about 60% of the total EOCL was associated with material having molecular weight greater than about 300 Da. Also in 1988 it was shown [13] that EOCL from rainbow-trout liver eluted from the GPC column together with triacylglycerols (molecular weights around 850 Da) and with substances having molecular weights around 2000 Da. The related problem of organically bound bromine (EOBR) in marine fish was studied very early (1972) by Lunde [14], who found this EOBR to behave similarly to triacylglycerols and sterol esters. In 1980 it was shown [15] that 60-80% of the EOBr in marine fish was likely associated with brominated fatty acids. Up to this point, all identifications were based upon comparisons of chromatographic retention behaviour with that of synthesised standards.

By about 1990, serious applications of modern analytical methods to this problem began to appear in the literature. In 1990 and 1991 Remberger *et al.* [16,17] published some excellent work on the analysis of EOCL fractions from marine sediments (not fish tissues). Full details are given of extraction procedures, of synthetic methods for standards, of quantitation by GC/FID and qualitative identification by GC/MS. Free organochlorine compounds were dominated by chlorinated long-chain alkanolic acids and chlorinated resin acids. (Resin acids are triterpenes produced as byproducts of pulp mill operations, see Leach and Thakore [8]). Transesterification with anhydrous sodium methoxide in methanol yielded additional chlorinated alkanolic acids (as their methyl esters) ranging from 40-100% of the free acids. For example, one sediment contained 9,10-dichloro-octadecanoic acid at 148 mg/kg in the free form, and 154 mg/kg as the bound form liberated by transesterification. The chlorinated resin acids identified and quantified were chlorinated forms of dehydroabietic acid, present entirely in the free forms (no increase upon transesterification) at levels often higher than those of the chlorinated alkanolic acids. This indicates that some of the chlorinated fatty acids were bound as glycerides. Chlorophenolic compounds were also investigated. Chloroguaiacols were present, mostly (not entirely) bound in a manner from which they were released by treatment with KOH. Chlorocatechols and chlorophenols were also observed and quantitated, but these were all apparently in the free form (no increases on treatment with KOH). The authors [16] give the following summary for identified components in the most polluted of the sediment samples:

Analysis of an EOCL extract from a polluted sediment [16] (all concentrations expressed as mg of chlorine per kg of organic carbon):

total chlorinated resin acids, 105;

total chlorinated fatty acids, 68;

total chlorinated phenolic compounds, 9.5;

chloroform, 1.3;

PCBs, 0.4;

EOCL, 2180.

These papers [16,17] by the Stockholm group, although dealing with sediments and not fish tissues, are extremely informative and helpful. (It has been shown [18,19] that sediments containing chlorinated pollutants can be a source of chlorinated substances in fish). The authors emphasise that, despite all their efforts, not more than 8% of the EOCL fractions were identified in terms of known compounds. With the exception of the PCBs, all of the identified compounds were established constituents of pulp bleach effluents.

Also in 1990 the Lund and Oslo groups published an excellent short paper [4] on characterization of EOCL in fish tissue (cod liver) and marine sediment. Again an excellent level of experimental detail was included. The EOCL extracts were split into portions, only one of which was hydrolyzed by lipase. In each case neutral and acidic

fractions were obtained by partitioning between cyclohexane and an alkaline buffer, *i.e.* a total of 4 fractions from each original extract. Each of the cyclohexane fractions was washed with aqueous  $\text{H}_2\text{SO}_4$  (pH 2), and the aqueous layers discarded. The aqueous alkaline fractions were acidified with  $\text{H}_2\text{SO}_4$  to pH 2, and extracted with cyclohexane. All of these final cyclohexane solutions were washed with distilled water, dried over  $\text{Na}_2\text{SO}_4$ , and the total chlorine content determined by NAA. Recoveries of chlorophenols were determined by spiking standards into the first fraction in alkaline buffer. In the case of the cod livers, no free chlorophenols or chlorinated fatty acids were detected in the control portion not subjected to enzymatic hydrolysis. All of the EOCL (by NAA) was recovered in the neutral fraction. After hydrolysis about 40% of the original EOCL was recovered in the neutral fraction, 30% in the acidic fraction, and 30% could not be accounted for. In the case of the sediment, the portion not hydrolysed again showed all the EOCL in the neutral fraction. After enzymatic hydrolysis about 20% of the EOCL was recovered (in the neutral fraction only), and the remainder could not be accounted for. Thus, the vast majority of the EOCL in both sediment and cod liver was affected by lipase hydrolysis. Acidic chloroorganics were released, in addition to substances which could not be re-extracted by cyclohexane following acidification of the aqueous buffer. Possibly some EOCL was dechlorinated in the hydrolysis, but this was pure speculation. The lipase used is known to not be specific for acylglycerols, e.g. p-nitrophenol fatty acid esters can be hydrolysed, so it is possible that chlorophenol conjugates of fatty acids were present and hydrolysed. It has been reported that such conjugates are found in rat liver, involving pentachlorophenol [20] and hydroxylated DDT [21]. For this reason the chlorophenol

spiking studies were done, and good recoveries obtained in the alkaline extract (containing acidic substances) except for those compounds (catechols) known to be susceptible to oxidation.

The main conclusion from this work [4] was thus that the acidic chloroorganics (about 30% of total EOCL in the cod liver) are chlorinated fatty acids and/or chlorinated phenols. The loss of EOCL upon enzymatic hydrolysis could be due to uncontrolled degradation (e.g. for chlorinated catechols), or could be a measure of chlorinated compounds liberated by hydrolysis but which are too hydrophilic to be extracted into the cyclohexane layer (e.g. short-chain chlorinated organic acids).

The Lund group published two major contributions in 1992. The first [5] was a study of analytical methods using model compounds, *viz.* chlorinated alkanoic acids and their triacylglycerol (TAG) esters. Methodologies investigated included reverse-phase HPLC (refractive index detector) and LC/MS (plasmaspray interface) for the TAGs. The chlorinated acids themselves were analysed by GC, with FID, ECD, and mass spectrometric detection (EI and positive-ion ammonia CI for methyl esters, and isobutane negative-ion CI for pentafluorobenzyl esters). Again, the level of experimental detail provided is excellent. The HPLC with refractive index detection was very insensitive. Plasmaspray LC/MS of the TAGs gave low sensitivity, with no molecular ions. For methyl esters of chlorinated octadecanoic acids, GC/FID and GC/ECD detection limits ( $S/N = 3$ ) were about 1ng and 0.5ng, respectively. Positive-ion GC/MS (electron impact) mass



spectra of these same monochloro esters were dominated by loss of HCl, so that the spectra were very similar to those of the corresponding product unsaturated (non-chlorinated) esters. The extremely low molecular ion intensities made this approach useless for quantitation by selected ion monitoring (SIM). However, positive ion ammonia CI gave intense  $(M + NH_4)^+$  signals, with SIM detection limits of about 0.1ng. Negative ion CI of the same methyl esters gave clean spectra but much poorer detection limits. However, the pentafluorobenzyl esters of the same chlorinated acids gave negative ion CI detection limits about the same as those for the corresponding methyl esters in positive ion CI. In the same paper, experiments were also described which investigated recoveries of the chloroalkanoic acids upon chemical hydrolysis (both acid and base catalysed), upon lipase hydrolysis, and upon transesterification (both acid and base catalysed). All 4 chemical methods liberated 100% of the fatty acids or methyl esters from tri-9,10-dichlorooctadecanoylglycerol (synthetic method described), although the base-catalysed hydrolysis caused a chlorine loss of about 5%. The enzymatic hydrolysis liberated about 80% of the free acids. This paper [5] is extremely helpful.

The second 1992 paper [6] by the Lund group (again in collaboration with the group in Stockholm) described the actual detection of chlorinated fatty acids in fish lipids using enzymatic hydrolysis and analysis by GC with electrolytic conductivity detection (Hall detector). The detection limits obtained for reference compounds were disappointing, about 50 pg of chlorine (corresponding to about 0.4 ng of monochloro and 0.25 ng of dichloro octadecanoic acid methyl esters). The GC/FID detection limits were

only slightly higher. However, useful results were obtained for several samples of both eel and flounder, taken from waters both close to kraft mills and from waters remote from such point sources of chlorinated organics. The more polluted eel samples contained 1200 ppm of EOCL, of which the majority appeared to be chlorinated fatty acids (liberated both by transesterification and by lipase hydrolysis). In particular 9, 10-dichlorostearic acid was confirmed to some degree by co-injection of a synthesised standard.

In 1993 the Lund group published a two-page abstract [7] summarising their work to date and describing work in progress. The new data shown are for the polluted eel sample mentioned above [6], for which GC/MS using positive-ion ammonia CI in SIM mode allowed confirmation of 9, 10-dichlorohexadecanoic acid, 9,10-dichlorooctadecanoic acid, and 9, 10, 12, 13-tetrachlorooctadecanoic acid (all as methyl esters, of course). The dichloro compounds were found in both the TAGs and in the phospholipids, but the tetrachloro compound only in the latter. In addition, these same 3 compounds were seen in the phospholipid fraction from herring purchased at a local market. The authors also refer to, but give absolutely no details of, batch procedures developed to selectively concentrate chlorinated fatty acid methyl esters (manuscript in preparation). They also acknowledge that the origins of these chlorinated fatty acids are unclear. Their concentrations are appreciably higher in fish exposed to chlorine-bleach pulp mill effluents, but are also non-zero in fish from remote areas. The authors believe that chlorinated fatty acids bound in TAGs have little physiological impact, but that these compounds in phospholipids might cause membrane damage. (In fact, it has been shown

[22, 23] that chlorinated lipids have detrimental effects as judged by various bioassays). The authors refer to another manuscript in preparation, in which they will describe the effects of phospholipids containing chlorinated fatty acids on ATP leakage from cultured mammalian cells. On a related point, some information has been published [24,25] on the effects of brominated fatty acids on liver and hearts of rats fed either pure standards or brominated corn oil (brominated vegetable oils have been used as food additives and as flame retardants).

The chlorinated fatty acid hypothesis thus appears likely to account for at least part of the unidentified EOCL load, at least in fish exposed to pulp bleach effluents. It remains to be determined to what extent the hypothesis can account for all such contamination.

It is worthwhile to summarise here some information on naturally occurring marine lipids containing chlorine. White and Hager [26] found fatty acid chlorohydrins (*i.e.* containing the  $-\text{CHCl}-\text{CH}(\text{OH})-$  group) in the lipids of a species of jellyfish. Elovson and co-workers [27,28] characterised some unusual  $\text{C}_{22}$  and  $\text{C}_{24}$  1,14-diol disulphates containing up to 6 chlorine atoms, found in the chrysomonad *Ochromonas danica*. Both groups speculated on the biogenesis of these unusual compounds. Though not concerned with fish lipids a review [29] of naturally occurring halogenated organics in the *Rhodophyta* marine red algae, and a monograph on biohalogenation [30], are of interest if only because they emphasise that organochlorines are not unknown in nature.

## CHLORINATED RESIN ACIDS

The chlorinated resin acids were mentioned above in the context of the Stockholm group's work [16,17] on marine sediments. The diterpene chlorinated dehydroabietic acids were shown to be toxic byproducts of kraft pulpmill bleach plants by Leach and Thakore [31] in 1975. A synthetic procedure for standards was described by Kutney and Dimitriadis [32].

## CHLORINATED PARAFFINS

Chlorinated paraffins with at least 10 carbon atoms are widely used as plasticisers, lubricating oil additives, paint additives, fire retardants, *etc.* In 1980, the annual world consumption was estimated at 230,000 tonnes. In North America these compounds are produced mainly by the Diamond Shamrock Co. under the tradename Chlorowax, and in the U.K. by ICI under the tradename "Cereclor xy", where "xy" is a number denoting the approximate weight percent of chlorine.

These compounds are extremely difficult to analyse due to the large numbers of isomers and to their involatility and thermal instability which precludes GC analysis. Early methods due to Zitko used column chromatography followed by microcoulometric

detection [33], and a confirmatory method [34] in which the chloroparaffins are dechlorinated by reduction with sodium *bis*(2-methoxyethoxy) aluminum hydride, and the resulting hydrocarbons analysed by GC. Both methods are insensitive and non-selective. Friedman and Lombardo [35] described a GC method applicable to chloroparaffins which are slightly volatile, based on microcoulometric detection and photochemical elimination of chlorinated aromatics which would otherwise interfere. Hollies *et al.* [36] were unable to reproduce this latter technique, and devised a method based upon thin-layer chromatography with detection limits stated to be in the range 50 µg/kg to 16 ng/kg for sediments and biota, for chloroparaffins with chlorine contents in the range 42-45%. This method [36], which distinguishes only between long carbon chains (C<sub>20</sub>-C<sub>30</sub>) and shorter chains (C<sub>13</sub>-C<sub>17</sub>) was subsequently used in a study [37] of these compounds in the environment around the U.K. (very low or not detected) and of their toxicities (very low) to fish, shellfish and birds [38].

In 1982 the first mass spectrometric method for chloroparaffins was described [39], in which samples were introduced to a negative-ion (methane) chemical ionisation source by direct probe. The sample investigated contained only C<sub>10</sub>-C<sub>13</sub> molecules with a chlorine content of 70%, and thus corresponded to the more volatile molecules of this type and should have contained molecules with molecular weights over 900. However, the mass spectra thus obtained peter out at about *m/z* 550, in agreement with general findings about the volatilities of these compounds. Nonetheless, the method was capable of demonstrating the same mass spectrometric fingerprint in a cyclohexane extract of fish

exposed to the same chloroparaffin preparation in a controlled feeding study. Later [40,41] a GC/MS method was described using negative-ion CI, and again all mass spectra terminated above  $m/z$  550. Nonetheless this was the best attempt yet, and demonstrated chloroparaffin levels of approximately 30 ppm, 200 ppb and 5 ppb in samples of sewage sludge, human fat, and lake sediment, respectively. Very recently a preliminary report of development of an LC/MS method, by DFO (Winnipeg) and the University of Manitoba, was presented [42]. The LC/MS interface used was a VG Plasmaspray (a variant of Thermospray, in which the sample is indeed subjected to considerable heating), and the only mass spectrum shown [42] again terminated at  $m/z$  520 or so. A summary of work done on toxicology of chloroparaffins, up to 1984, is given in [41].

## CHLORINATED POLYCYCLIC AROMATIC HYDROCARBONS

The case of polychlorinated naphthalenes, manufactured as industrial formulations, is dealt with separately below. The possibility of chlorination of PAHs during disinfection of contaminated water was investigated by Carlson and his collaborators [43,44]. The earlier work [43] was devoted to devising an HPLC/GC method for analysis, and demonstrating the production of chlorinated PAHs at low pH. The more recent work [44] derived a rate expression for reaction of phenanthrene over the pH range 3-10, and characterised the major reaction products, viz. the arene oxide phenanthrene 9,10-oxide at  $\text{pH} > 4$ , and phenanthrene-9,10-dione plus 9-chlorophenanthrene at  $\text{pH} \leq 4$ , with other oxygenated and/or chlorinated products making minor contributions. A kinetic mechanism was proposed, consistent with the experimental findings for phenanthrene. A few experiments were also conducted using the non-alternant PAHs fluorene and fluoranthene, at low pH. Monochloro-substituted PAHs were the major products, with some evidence of higher degrees of chlorination.

## CHLORINATED HETEROCYCLIC AROMATICS

The susceptibility of organic materials present in wastewater to chemical transformations during disinfection by chlorination has been studied for several structural types, including PAHs by Carlson *et al.* [43,44] as summarised above. Carlson later extended this study to include several aromatic heterocyclic compounds [45], including indoles and carbazoles, pyridine and quinoline, benzothiophenes, *etc.* Chlorinated water (hypochlorous acid and hypochlorite) gave products arising from both chlorination and oxygenation, while  $\text{ClO}_2$  in general yielded only oxygenation products. Chloramine was in general quite unreactive under the conditions used, except at low pH where the reactivity may have been due to a significant conversion to hypochlorous acid.

The case of chlorinated dibenzofurans and dibenzodioxins is so well appreciated that there is no reason for its inclusion here. Chlorinated thiophenes have been identified [46,47] in effluents from kraft bleach plants, and shown to have a weak mutagenic activity. At the ultra-trace level, chlorinated dibenzothiophenes (sulfur analogues of chlorinated dibenzofurans) have been observed in environmental samples, including crab pancreas [48-53].

No reference was found to chlorinated indoles or carbazoles in environmental samples. It was found by Lin and Carlson [45] that chlorination of indole and carbazole with hypochlorite did produce chlorinated as well as oxygenated products. The literature



on preparative chlorination of indole [54-57] emphasises the use of non-aqueous solvents. Some work has been published [58,59] on chlorination of tryptophan (an amino acid with an indole sidechain), with reference to both food processing and more general environmental concerns. In the work of Trehy *et al.* [58] dichloroacetonitrile and chloral were shown to be major products; chlorinated aromatic intermediates were detected and characterized in the case of tyrosine, but were not reported for tryptophan. The work of Sen *et al.* [59] was concerned with kinetic and mutagenicity studies, without characterizing the products of the chlorination.

#### CHLORINATED NAPHTHALENES.

Polychlorinated naphthalenes (PCNs) have been manufactured as technical formulations for at least as long as have PCBs, although production never rose to the levels of the latter. PCNs are also present as contaminants in PCB formulations, and are produced in municipal waste incineration, in chlorine production, *etc.* PCNs are now as widespread in the environment as PCBs, and have been shown [60] to represent a similar health hazard. A detailed study of PCBs and PCNs in Swedish environmental samples has been published recently [61].

## CONCLUSIONS

This brief review has been concerned only with what is known (in summer 1993) or speculated about the 80-85% of organochlorines in contaminated fish (and sediments) which have *not* thus far been identified and/or quantified. Some of the speculations involve organochlorines known to be present in the environment, but which are not amongst the compounds normally analyzed due to lack of reliable analytical techniques (*e.g.* the chloroparaffins). Others (*e.g.* chlorinated fatty acids) correspond to compound classes whose presence has been known or suspected for some time, but which have not, for one reason or another, been extensively investigated up till now. Thus, nothing has been said here about PCBs, chlorinated pesticides, *etc.*, and little about chlorophenols, which together constitute the 10-15% of the EOCL which have been identified and are regularly analyzed. Also, the main emphasis has been placed upon chemical identification of the unknown 85%, with almost nothing noted about possible sources and sinks for these compounds, nor about their possible deleterious effects. An excellent review of literature concerned with distribution, fate and persistence in the environment of organochlorine compounds from pulp mills, has been published [17].

Any review on this subject will almost certainly be outdated as soon as written. However, it is believed that the present effort covers the relevant literature available as of July, 1993.

## REFERENCES.

1. Gether, J.; Lunde, G.; Steiness, E.  
Determination of the total amount of organically bound chlorine, bromine and iodine in environmental samples by instrumental neutron activation analysis.  
*Anal. Chim. Acta.* **108**, 137-147 (1979).
2. Lunde, G.; Gether, J.; Steiness, E.  
Determination of volatility and chemical persistence of lipid-soluble halogenated organic substances in marine organisms.  
*Ambio* **5**, 180-182 (1976).
3. Södergren, A.; Bengtsson, B.E.; Jonsson, P.; Lagergren, S.; Larsson, Å.; Olsson, M.; Renberg, L.  
Summary of results from the Swedish project Environment/Cellulose.  
*Wat. Sci. Technol.* **20**, 49-60 (1988).
4. Wesén, C.; Carlberg, G.E.; Martinsen, K.  
On the identity of chlorinated organic substances in aquatic organisms and sediments.  
*Ambio* **19**, 36-38 (1990).
5. Sundin, P.; Larsson, P.; Wesén, C.; Odham, G.  
Chlorinated triacylglycerols in fish lipids? Chromatographic and mass spectrometric studies of model compounds.  
*Biol. Mass Spectrom.* **21**, 633-641 (1992).
6. Wesén, C.; Mu, H.; Kvernheim, A.L.; Larsson, P.  
Identification of chlorinated fatty acids in fish lipids by partitioning studies and by gas chromatography with Hall electrolytic conductivity detection.  
*J. Chromatogr.* **625**, 257-269 (1992).
7. Sundin, P.; Wesén, C.; Mu, H.; Odham, G.; Björn, H.  
GC-MS detection of chlorinated fatty acids bound in triacylglycerols and phospholipids extracted from fish.  
*Proceedings of the Kyoto '92 International Conference on Biological Mass Spectrometry*, Sept. 20-24, 1992, Kyoto, Japan, (Matsuo, T., Ed.); Jan-ei Publishing Co., Kyoto; pp. 120-121 (1993).
8. Leach, J.M.; Thakore, A.N.  
Compounds toxic to fish in pulp mill waste streams.  
*Progr. Water Technol.* **9**, 787-798 (1978).

9. Addison, R.F.  
Organochlorine compounds and marine lipids.  
*Progr. Lipid Res.* **21**, 47-71 (1982).
10. Voss, R.H.; Rapsomatiotis, A.  
An improved solvent-extraction based procedure for the gas chromatographic analysis of resin and fatty acids in pulp mill effluents.  
*J. Chromatogr.* **346**, 205-214 (1985).
11. Wesén, C.  
Chemical characterization of chlorinated lignin derivatives in organisms, sediment and air.  
*Water Sci. Technol.* **20**, 185-188 (1988).
12. Martinsen, K.; Kringstand, A.; Carlberg, G.E.  
Methods for determination of sum parameters and characterization of organochlorine compounds in spent bleach liquors from pulp mills and water, sediment and biological samples from receiving waters.  
*Water Sci. Technol.* **20**, 13-24 (1988).
13. Hemming, J.; Lehtinen, K.-J.  
Extractable organic chlorine (EOCL) in fish exposed to combined mill effluents from bleached kraft pulp production.  
*Nord. Pulp Paper Res. J.* **4**, 185-190 (1988).
14. Lunde, G.  
Analysis of arsenic and bromine in marine and terrestrial oils.  
*J.Am. Oil Chem. Soc.* **49**, 44-47 (1972).
15. Tinsley, I.J.; Lowry, R.R.  
Bromine content of lipids of marine organisms.  
*J.Am. Oil Chem. Soc.* **57**, 31-33 (1980).
16. Remberger, M.; Hynning, P.; Neilson, A.  
Gas chromatographic analysis and gas chromatographic-mass spectrometric identification of components in the cyclohexane-extractable fraction from contaminated sediment samples.  
*J. Chromatogr.* **508**, 159-178 (1990).
17. Neilson, A.H.; Allard, A.-S.; Hynning, P.; Remberger, M.  
Distribution, fate and persistence of organochlorine compounds formed during production of bleached pulp.  
*Toxicol. Environ. Chem.* **30**, 3-41 (1991).

18. Larsson, P.  
Zooplankton and fish accumulate chlorinated hydrocarbons from contaminated sediments.  
*Can. J. Fish. Aquat. Sci.* **43**, 1463-1466 (1986).
19. Carlberg, G.E.; Kringstad, A.; Martinsen, K.; Nashaug, O.  
Environmental impact of organochlorine compounds discharged from the pulp and paper industry.  
*Paperi ja puu.* **69**, 337-341 (1987).
20. Leightly, E.G.; Fentiman, A.F.  
Conjugation of pentachlorophenol to palmitic acid by liver microsomes.  
*Bull. Environ. Contam. Toxicol.* **28**, 329-333 (1982).
21. Leightly, E.G.; Fentiman, A.F.; Thompson, R.M.  
Conjugation of fatty acids to DDT in the rat; possible mechanisms for retention.  
*Toxicol.* **15**, 77-82 (1980).
22. Cunningham, H.M.  
Toxicology of compounds resulting from the use of chlorine in food processing.  
"Water Chlorination: Environmental Impact and Health Effects; Vol. 3 "; Ann Arbor Science Publishers, Ann Arbor, Michigan (1980); pp. 995-1005.
23. Hakansson, H.; Sundin, P.; Andersson, T.; Brunström, B.; Dencker, L.; Engwall, M.; Ewald, G.; Gilek, M.; Holm, G.; Honkasalo, S.; Idestam-Almquist, J.; Jonsson, P.; Kautsky, N.; Lundberg, G.; Lund-Kuernheim, A.; Martinsen, K.; Norrgren, L.; Personen, M.; Rundgren, M.; Stalberg, M.; Tarkepea, M.; Wésen, C.  
*In vivo* and *in vitro* toxicity of fractionated fish lipids, with particular regard to their content of chlorinated organic compounds.  
*Pharmacol. Toxicol.* **69**, 459-471 (1991).
24. Jones, B.A.; Tinsley, I.J.; Lowry, R.R.  
Bromine levels in tissue lipids of rats fed brominated fatty acids.  
*Lipids* **18**, 319-326 (1983).
25. Jones, B.A.; Tinsley, I.J.; Wilson, G.; Lowry, R.R.  
Toxicology of brominated fatty acids: metabolite concentration and heart and liver changes.  
*Lipids* **18**, 327-334 (1983).
26. White, R.H.; Hager, L.P.  
Occurrence of fatty acid chlorohydrins in jellyfish lipids.  
*Biochemistry* **16**, 4944-4948 (1977).

27. Elovson, J.; Vagelos, P.R.  
Structure of the major species of chlorosulfolipid from *Ochromonas danica*.  
2,2,11,13,16,16-hexachloro-n-docosane 1,14-disulfate.  
*Biochemistry* **9**, 3110-3126 (1970).
28. Elovson, J.  
Biosynthesis of chlorosulfolipids in *Ochromonas danica*. Origin of primary and  
secondary hydroxyl groups determined by  $^{18}\text{O}$  incorporation *in vivo*.  
*Biochemistry* **13**, 2105-2109 (1974).
29. Fenical, W.  
Halogenation in the *Rhodophyta*: a review.  
*J. Phycol.* **11**, 245-259 (1975).
30. Neidleman, S.L.; Geigert, J.  
"Biohalogenation: Principles, Basic Roles and Applications".  
Ellis Horwood, Chichester, U.K., (1986).
31. Leach, J.M.; Thakore, A.N.  
Isolation and identification of constituents toxic to juvenile rainbow trout (*Salmo gairdneri*) in caustic extraction effluents from kraft pulpmill bleach plants.  
*J. Fish. Res. Board Can.* **32**, 1249-1257 (1975).
32. Kutney, J.P.; Dimitriadis, E.  
Studies related to biological detoxification of kraft pulp mill effluent, V. The  
synthesis of 12- and 14-chlorodehydroabietic acids and 12,14-  
dichlorodehydroabietic acid, fish-toxic diterpenes from kraft pulp mill effluent.  
*Helv. Chim. Acta* **65**, 1351-1358 (1982).
33. Zitko, V.  
Chromatography of chlorinated paraffins on alumina and silica columns.  
*J. Chromatogr.* **81**, 152-155 (1973).
34. Zitko, V.  
Confirmation of chlorinated paraffins by dechlorination.  
*J. Assoc. Off. Anal. Chem.* **57**, 1253-1259 (1974).
35. Friedman, D.; Lombardo, P.  
Photochemical technique for the elimination of chlorinated aromatic interferences  
in the gas-liquid chromatographic analysis for chlorinated paraffins.  
*J. Assoc. Off. Anal. Chem.* **58**, 703-706 (1975).

36. Hollies, J.I.; Pinnington, D.F.; Handley, A.J.; Baldwin, M.K.; Bennett, D.  
The determination of chlorinated long-chain paraffins in water, sediment and biological samples.  
*Anal. Chim. Acta* **111**, 201-213 (1979).
37. Campbell, I.; McConnell, G.  
Chlorinated paraffins and the environment, 1. Environmental Occurrence.  
*Env. Sci. Technol.* **14**, 1209-1214 (1980).
38. Madeley, J.R.; Birtley, R.D.N.  
Chlorinated paraffins and the environment, 2. Aquatic and avian toxicology.  
*Env. Sci. Technol.* **14**, 1215-1221 (1980).
39. GjØs, N.; Gustavsen, K.O.  
Determination of chlorinated paraffins by negative ion chemical ionization mass spectrometry.  
*Anal. Chem.* **54**, 1316-1318 (1982).
40. Müller, M.D.; Schmid, P.P.  
GC/MS analysis of chlorinated paraffins with negative ion chemical ionization.  
*J. High Resolut. Chromatogr. Chromatogr. Commun.* **7**, 33-37 (1984).
41. Müller, M.D.; Schmid, P.P.  
Trace level detection of chlorinated paraffins in biological and environmental samples using gas chromatography/mass spectrometry with negative-ion chemical ionization.  
*J. Assoc. Off. Anal. Chem.* **68**, 427-430 (1985).
42. Tomy, G.T.; Muir, D.C.G.; Westmore, J.B.; Stern, G.A.  
Liquid chromatography/plasma spray mass spectrometry for the characterization of a commercial chlorinated n-paraffin mixture.  
*Proc. 41st ASMS Conf. Mass Spectrom. Allied Topics*, San Francisco, June 1993 (in press).
43. Oyler, A.R.; Bodenner, D.L.; Welch, K.J.; Liukkonen, R.J.; Carlson, R.M.; Kopperman, H.L.; Caple, R.  
Determination of aqueous chlorination reaction products of polynuclear aromatic hydrocarbons by reversed phase high performance liquid chromatography-gas chromatography.  
*Anal. Chem.* **50**, 837-842 (1978).

44. Oyler, A.R.; Liukkonen, R.J.; Lukasewycz, M.T.; Heikkila, K.E.; Cox, D.A.; Carlson, R.M.  
Chlorine disinfection chemistry of aromatic compounds. Polynuclear aromatic hydrocarbons: rates, products and mechanisms.  
*Environ. Sci. Technol.* **17**, 334-342 (1983).
45. Lin, S.; Carlson, R.M.  
Susceptibility of environmentally important heterocycles to chemical disinfection: reactions with aqueous chlorine, chlorine dioxide, and chloramine.  
*Environ. Sci. Technol.* **18**, 743-748 (1984).
46. Carlberg, G.E.; Johnsen, S.; Landmark, L.H.; Bengtsson, B.E.; Bergstroem, B.; Skramstad, J.; Storflor, H.  
Investigations of chlorinated thiophenes: a group of bioaccumulable compounds identified in the effluents from kraft bleaching.  
*Water Sci. Technol.* **20**, 37-48 (1988).
47. Lunde, A.; Skramstad, J.; Carlberg, G.E.  
Identification, mutagenicity and origin of chlorinated thiophenes in kraft bleaching effluent.  
*Paperi ja puu.* **73**, 522-526 (1991).
48. Sinkkonen, S.; Koistinen, J.  
Chlorinated and methylated dibenzothiophenes; preparation of the model compounds and their analysis from some environmental samples.  
*Chemosphere* **21**, 1161-1171 (1990).
49. Sinkkonen, S.; Paasivirta, J.; Koistinen, J.; Tarhanen, J.  
Tetra- and pentachlorodibenzothiophenes are formed in waste combustion.  
*Chemosphere* **23**, 583-587 (1991).
50. Sinkkonen, S.  
Isolation of chlorinated dibenzothiophenes by high-performance thin-layer chromatography.  
*J. Chromatogr.* **553**, 453-457 (1991).
51. Buser, H.-R.; Rappe, C.  
Determination of polychlorodibenzothiophenes, the sulfur analogues of polychlorodibenzofurans, using various gas chromatographic/mass spectrometric techniques.  
*Anal. Chem.* **63**, 1210-1217 (1991).



52. Tong, H.Y.; Giblin, D.L.; Lapp, R.L.; Monson, S.J.; Gross, M.L.  
Mass profile monitoring in trace analysis by gas chromatography/mass spectrometry.  
*Anal. Chem.* **63**, 1772-1780 (1991).
53. Cai, Z.; Giblin, D.E.; Ramanujam, V.M.S.; Gross, M.L.  
Identification of polychlorodibenzothiophenes in crab tissues from Raritan/Newark Bay.  
*Proc. 41st ASMS Conf. Mass Spectrom. Allied Topics*, San Francisco, June 1993 (in press).
54. Palmer, M.H.  
*The Structure and Reactions of Heterocyclic Compounds*, St. Martin's Press, New York (1967), p.317.
55. Remers, W.A.  
Properties and reactions of indoles.  
*The Chemistry of Heterocyclic Compounds: Indoles, Part One*, Houlihan, W.J., Ed.; Wiley-Interscience, New York (1972), pp.77-78.
56. Powers, J.C.  
Haloindoles and organometallic derivatives of indoles.  
*The Chemistry of Heterocyclic Compounds: Indoles, Part Two*, Houlihan, W.J., Ed.; Wiley-Interscience, New York (1972), pp.155-159.
57. De Rosa, M.; Alonso, J.L.T.  
Studies of the mechanism of chlorination of indoles. Detection of N-chloroindole and 3-chloro-3H-indole as intermediates.  
*J. Org. Chem.* **43**, 2639-2643 (1978).
58. Trehy, M.L.; Yost, R.A.; Miles, C.J.  
Chlorination byproducts of amino acids in natural waters.  
*Environ. Sci. Technol.* **20**, 1117-1122 (1986).
59. Sen, A.C.; Owusu-Yaw, J.; Wheeler, W.B.; Wei, C.I.  
Reactions of aqueous chlorine and chlorine dioxide with tryptophan, N-methyltryptophan, and 3-indolacetic acid: kinetic and mutagenicity studies.  
*J. Food Sci.* **54**, 1057-1060 (1989).
60. Kover, F.  
Environmental hazard assessment report: chlorinated naphthalenes.  
*U.S. Environmental Protection Agency Report 560/8-75-001*; U.S. EPA, Washington, D.C. (1975).

61. Järnberg, U.; Asplund, L.; de Wit, C.; Grafström, A.-K.; Haglund, P.; Jansson, B.; Lexén, K.; Strandell, M.; Oisson, M.; Jonsson, B.  
Polychlorinated biphenyls and polychlorinated naphthalenes in Swedish sediment and biota: levels, patterns and time trends.  
*Environ. Sci. Technol.* **27**, 1364-1374 (1993).