

# Natural Chlorine Updates – No. 1

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## I. Introduction

This literature review is the first in a series of periodic updates to the natural halogen literature, with a particular focus on organochlorine compounds.

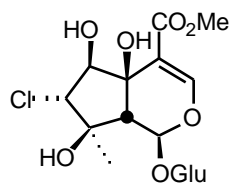
The coverage is approximately from the end of 1994 to mid-June 1995. Of special importance was the TAPPI (Technical Association of the Pulp and Paper Industry) Environmental Conference in Atlanta, Georgia, May 7-10, 1995.

Coverage of novel naturally-occurring organohalogen compounds begins where a recent comprehensive survey of the field ends (1). Several other reviews of various aspects of natural organohalogen compounds have been published (2).

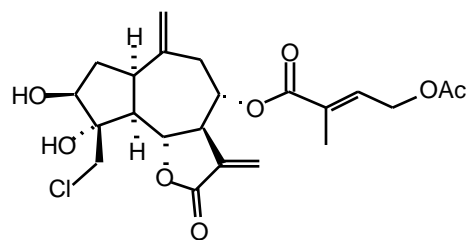
## II. New Natural Organohalogens

Both terrestrial and marine organisms continue to be a source of novel organohalogen compounds. The potential of marine algae as a source of biologically active natural products, many of which contain halogen, has been reviewed (3).

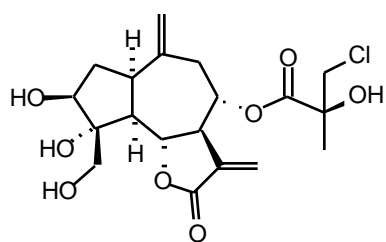
A new iridoid, phoyoside II (1), has been isolated from the plant *Phlomis younghusbandii*, bringing the total number of chlorinated iridoids to about 15 (4). The sesquiterpene lactone chloroscoparin (2) is a newly discovered metabolite of the plant *Centaurea scoparia* (5). Similarly, *C. glatifolia* has yielded the novel guaianolide epicebellin J (3) (6), as well as the previously known chlorine-containing cebellin J, episolstiolid, 19-desoxy-15-chlorojanerin, centaurepensin, and epicentaurepensin (4) (7). The latter compound was not described in the previous review (1). No chlorinated solvents or hydrochloric acid was used in the extraction-isolated process.



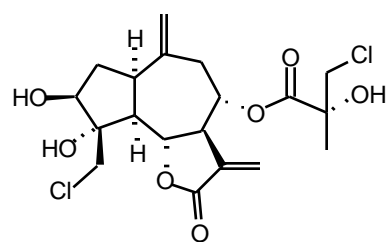
1 (phoyoside II)



2 (chloroscoparin)

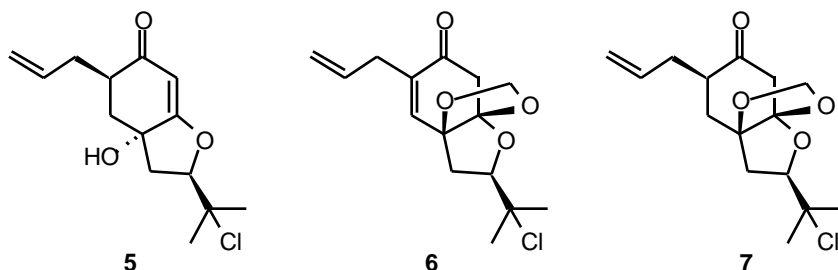


3 (epicebellin)

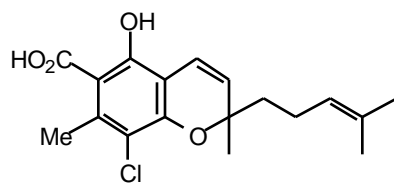
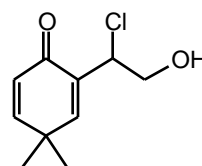


4 (epicentaurepsin)

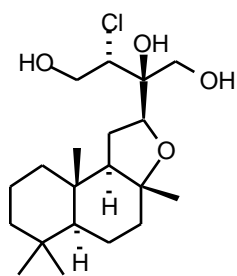
The novel sesquiterpenoid 12-chloroillifunone C (**5**) has been found in the plant *Illicium tashiroi* (**8**), and a later study of this same plant has revealed the two similar compounds 12-chloroillicinone E (**6**) and **7** (**9**). The latter metabolite increases choline acetyltransferase activity and thus may find use in the treatment of Alzheimer's disease. The parasitic fungus *Cylindrocarpon olidum* produces 8-chlorocannabiorcichromenic acid (**8**), which has antifungal activity (**10**). The known monoterpene **9** has been found for the first time in a blue-green alga, *Lyngbya majuscula* (**11**). The North American "Cup Plant" (*Silphium perfoliatum*), which was used in folk medicine by Native Americans, contains the labdane diterpene chlorosilphanol A (**10**) (**12**). The plant *Triphasia trifolia* contains the novel coumarin derivative **11** (**13**).

5  
(12-chloroillifunone C)6  
(12-chloroillicinone E)

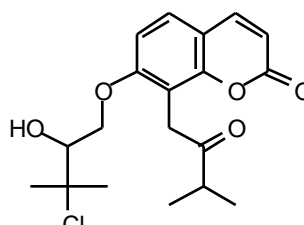
7

8  
(8-chlorocannabiorcichromenic acid)

9



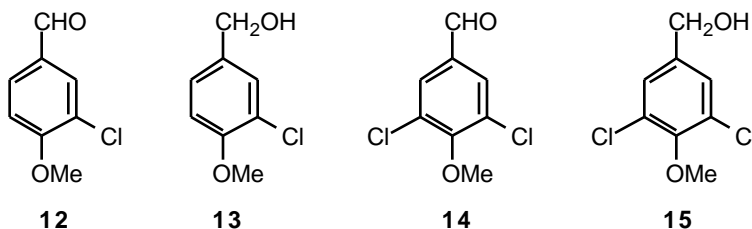
10 (chlorosilphanol A)



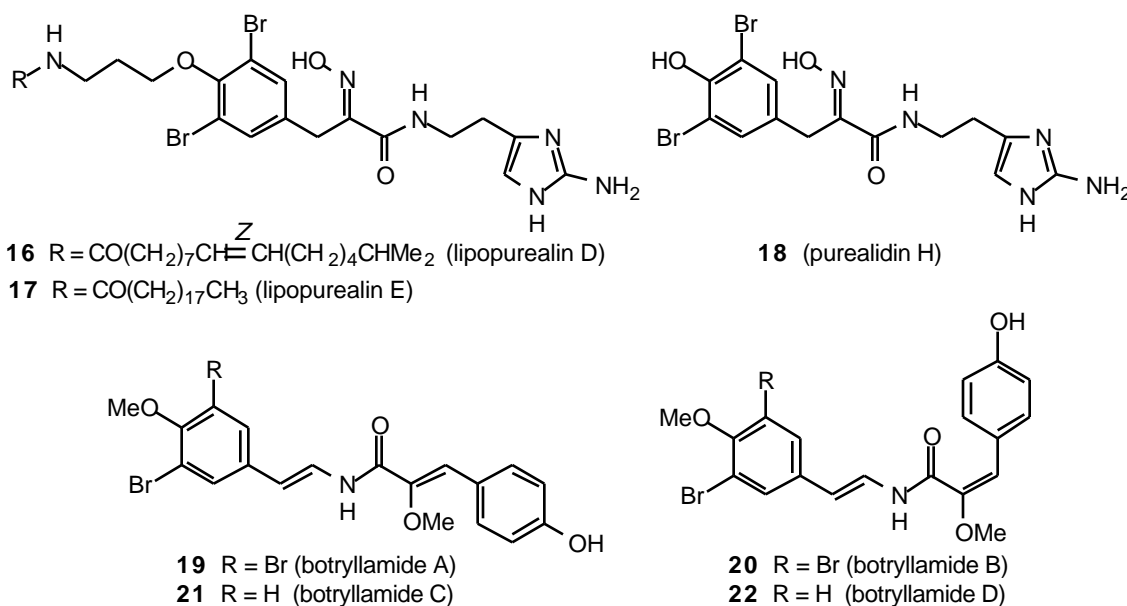
11

A detailed examination of the white-rot fungus *Bjerkandera adusta* has revealed the presence

of 3-chloro-4-methoxybenzaldehyde (**12**), 3-chloro-4-methoxybenzyl alcohol (**13**), 3,5-dichloro-4-methoxybenzaldehyde (**14**), and 3,5-dichloro-4-benzyl alcohol (**15**) (14). This fungus also produces diiodomethane and chloriodomethane in the presence of added iodide. The production of these halogenated alkanes is related to the formation of halogenated aromatic compounds. More volatile halogenated alkanes, if present, could not be detected under the experimental conditions.

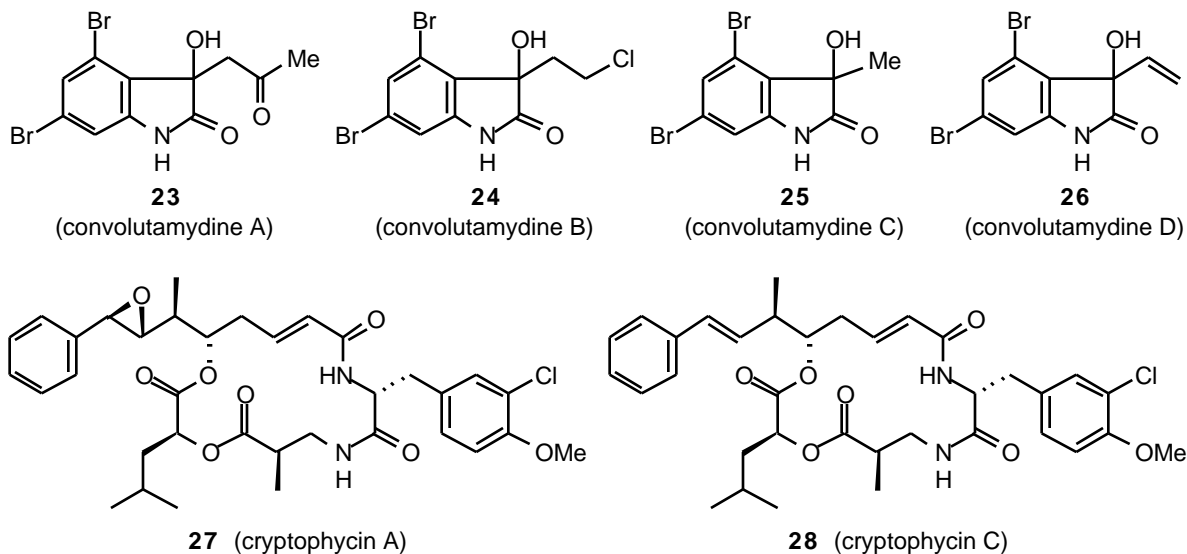


The Okinawan sponge *Psammaphysilla purea* produces the two new lipopurealins D (**16**) and E (**17**) as well as purealidin H (**18**) (15). The bromotyrosine derivatives botryllamides A-D (**19-22**) are found in the ascidian *Botryllus* sp. and *B. schlosseri* from the Philippines and the Great Barrier Reef, respectively (16). Botryllamide D shows marginal activity against the human colon cancer cell line HCT 116.

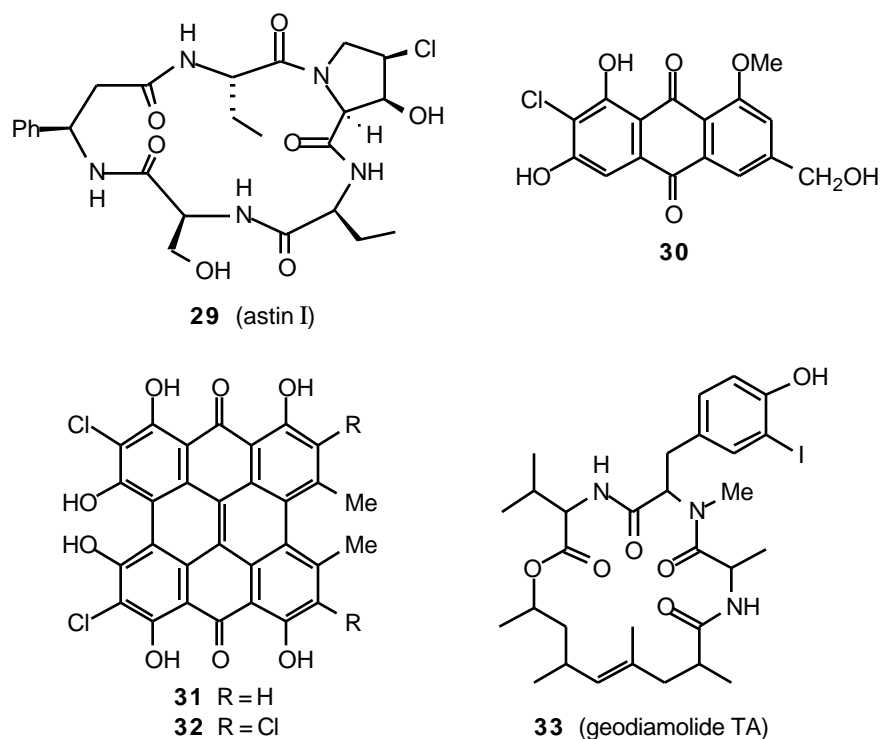


The marine bryozoan *Amathia convoluta* produces the novel convolutamydines A-D (**23-26**), which display differentiation of HL-60 cells (17). The structures of cryptophycins A (**27**) and C (**28**), which are produced by the blue-green alga *Nostoc* sp., have been revised to those shown, reflecting the presence of the D-configuration of the chloro-*O*-methyltyrosine unit (18). It is of interest to note that cryptophycin A has extraordinary activity against solid tumors and clinical

evaluation and utility seem likely.



Another antitumor cyclic peptide from the plant *Aster tataricus* has been isolated and shown to be the structure **29** (Astin I) (19). This metabolite possesses the unusual amino acid  $\alpha$ -hydroxy- $\beta$ -chloroproline. The lichen *Nephroma laevigatum* produces the new anthraquinone **30** as well as the novel **31** and **32**, in addition to some known chlorinated anthraquinones (20). The South African sponge *Hemiasterella minor* produces the cytotoxic cyclic peptide geodiamolide TA (**33**) in addition to the known jaspamide (21). The relative configuration of **33** appears to be the same as that found in geodiamolides A-F, which also contain halogen.



### III. Biohalogenation

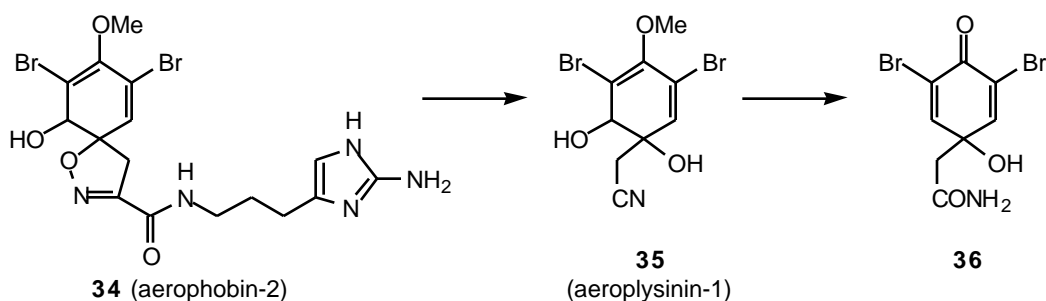
The mechanisms whereby organisms synthesize organohalogen metabolites continue to be of interest and a review of haloperoxidase enzymes has appeared (22). These enzymes, which are ubiquitous in our biosphere, catalyze the incorporation of halogen into organic molecules.

An extensive investigation of the production of chlorinated aromatic compounds by fungi has revealed that this biogenesis is common to many types of common wood- and forest litter-degrading fungi (23). These compounds (**12-15**) are found at concentrations of 75 mg/kg of wood in the environment. Not only are these compounds found in the fungi, they are present in the surrounding environment (leaves, twigs, branches, nut husks, rotting logs). However, these chlorinated aromatics were not found in fresh forest litter or intact wood where fungi are not present. Indeed, rotting wood or leaf litter adjacent to the fruiting bodies of these fungi contain chlorinated aromatics in "concentrations high enough to be considered hazardous according to Dutch environmental regulations concerning chlorophenols in soil ( > 10 mg/kg)" (23). Likewise, these chlorinated anisyl metabolites were found in the edible mushroom *Lepista nuda* at a level of 0.8 mg/kg. The authors conclude that these chlorinated aromatic producing fungi are widespread in the environment and the resulting organochlorines are likely transformed into chlorine-containing humic substances, which are known to be present in pristine surface waters and groundwaters (24).

### IV. Function

The fungal production of chlorinated aromatics seems to be involved in the generation of hydrogen peroxide for the lignin-degrading peroxidases, since the chlorinated anisyl alcohols **13** and **15** are excellent substrates for extracellular aryl alcohol oxidases (25).

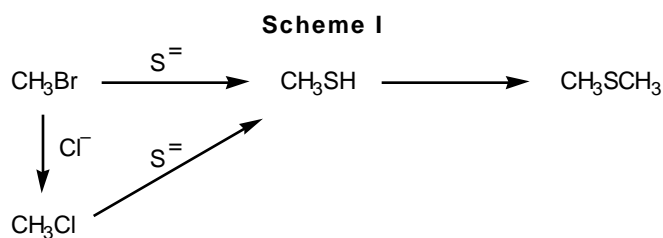
A recent study of the biotransformation of the brominated compounds in the marine sponge *Verongia aerophoba* has led the authors to propose an induced chemical defense for this organism to prevent overgrowth by fouling organisms (26). Thus, inactive compounds (*e.g.*, **34**) seem to be enzymatically converted into the biologically active (repellent) compounds (*e.g.*, **35, 36**) following the disruption of cells.



## V. Biodegradation

When an organism dies, its organic material must be reconverted to simpler compounds for reuse. Obviously, this natural process must also include organohalogen compounds and studies of such degradation continue to be reported. For example, a review of the mechanism whereby white rot fungi degrade organic compounds has recently appeared (27).

The white-rot fungus *Trametes versicolor* is capable of degrading chlorinated lignin derivatives in a combined bleach plant effluent from sulfite pulping to the extent of 45% reduction of adsorbable organohalogen (AOX) (28). Several other fungi were also effective. Chlorophenols are degraded under anaerobic conditions by sulfate-reducing enrichment cultures (29). The enzymatic oxidative coupling of chlorophenols has been found to result in the loss of chloride, both in the polymerization process and in the binding to humic substances (30). The degradation of pentachlorophenol by polyurethane-immobilized *Flavobacterium* sp. cells has been studied and found to be an excellent method for the removal of pentachlorophenol from highly contaminated water (31). The degradation of mono- and polyhalomethanes by *Methylosinus trichosporium* follows the reactivity order  $\text{CH}_3\text{X} > \text{CH}_2\text{X}_2 > \text{CHX}_3$  (where X = Cl, Br) (32). In these biodegradation reactions, loss of halide follows the insertion of oxygen into the C-H bond, leading to the ultimate formation of carbon dioxide. The responsible enzyme in these rapid and relatively non-selective reactions is methane monooxygenase. Methyl bromide is degraded anaerobically in saltmarsh sediments after reaction with sulfide to yield methanethiol (33). Further reaction affords dimethyl sulfide. Moreover, under these conditions methyl bromide reacts with chloride to form methyl chloride, which can also react with sulfide. This chemistry is summarized below in Scheme I. Thus, sulfide-rich sediments, such as those in coastal saltmarshes, are likely to be a natural sink for atmospheric methyl bromide.



The mechanism of dehalogenation of dichloromethane and  $\text{CH}_2\text{FCl}$  with the enzymes dichloromethane dehalogenase from *Methylophilus* sp. and rat liver glutathione *S*-transferases involves nucleophilic attack of glutathione on the substrate to produce a halomethylthioether intermediate, which subsequently hydrolyzes to formaldehyde and HCl or HF (34).

## VI. Pulp Chlorination

The formation and fate of organochlorines in the bleaching of wood pulp with either chlorine or chlorine dioxide continue to be of intense interest.

The source of polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) that are formed in the chlorination of pulp is most likely a combination both of the chlorination of dibenzo-*p*-dioxin (DBD) and dibenzofuran (DBF), which are present in the wood pulp, and of the chlorination of precursors. In the case of PCDF, the predominant pathway is the chlorination of DBF (35). A study of the organohalogen content of mussels from an unpolluted lake *vis-à-vis* a pulp mill recipient lake has revealed that significant quantities of organohalogens are found in the former group of mussels (36). Chlorinated fatty acids represent the highest concentration of known identified compound classes in these mussels. Although the organohalogen content is higher in the mussels exposed to discharges from the pulp mill than those mussels found in pristine lakes, obviously, another source of organohalogens must account for their presence in those mussels not exposed to pulp mill discharge. Whether this source is the biogenic synthesis of organohalogens or the atmospheric deposition of natural or anthropogenic organohalogen compounds remains to be seen.

## VII. TAPPI International Environmental Conference

Several important papers dealing with natural organochlorine and other natural organohalogen compounds were presented at the 1995 TAPPI International Environmental Conference in Atlanta,

Georgia, May 7-10.

Dr. Frederick Archibald of the Pulp and Paper Research Institute of Canada put into perspective natural vs. man-made organochlorines with regard to the kraft pulp mill industry in Quebec (37). This paper summarizes the recent evidence as to the formation of chlorinated humic substances in pristine lakes and the fact that these same compounds are very similar to those produced by pulp mills. Indeed, probably all natural lakes, rivers, and streams contain organochlorines. Measurements of AOX in the Montreal region (lakes, rivers, springs, wells) show these compounds to be ubiquitous. Many of the waters containing high levels of pollution had AOX levels of only about twice that of pristine, remote lakes. There is strong evidence that chloroperoxidase enzymes, which are naturally present in soil, are responsible for at least some of these natural organochlorine compounds (e.g., chlorinated humic and fulvic acids).

Archibald also summarized the little-known fact that the mammalian immune system utilizes chlorination (and other halogenation reactions) to kill foreign invaders. Thus, the chloride that is normally present in blood and lymph is oxidized by myeloperoxidase or eosinophil peroxidase in the presence of hydrogen peroxide to hypochlorite. This active form of chlorine then chlorinates invading bacteria, fungi, and perhaps tumor cells. The chlorine-containing products of these reactions are presumably the AOX that is found in mammalian tissues and blood. These organochlorine compounds have yet to be identified. When mice are stimulated with a bacterial endotoxin, Archibald found that their blood cell and plasma AOX was elevated significantly.

Professor Douglas Reeve, Department of Chemical Engineering and Applied Chemistry, and the Director of the Pulp and Paper Center at the University of Toronto, delivered two papers. The first, "Chlorine Mythology," presented an excellent overview of the misconceptions and myths about chlorine and organochlorine compounds that are perpetuated by some environmental groups (38). Reeve's second paper dealt with the Ontario Ministry of the Environment and its newly announced regulations regarding AOX discharge by the pulp and paper industry (39). In particular, the comparison of chlorine to chlorine dioxide in the bleaching process was reviewed in some depth.

In his presentation, Dr. Bruce Fleming of Boise Cascade Corporation summarized the natural occurrence of organochlorine compounds and also presented striking data to indicate the ubiquity

of organochlorines in foodstuffs, water, soil, clothing, and vegetation (40). The several Tables that follow are from Fleming. Several studies have demonstrated that large quantities of adsorbable organochlorine compounds (AOX), which are not yet well-characterized, are discharged by pristine rivers and streams into lakes. For example, many rivers that feed into Lake Superior contain AOX (Table 1). In a study of Swedish rivers and lakes, the quantities of AOX ranged from 20-60  $\mu\text{g/L}$  with a high of 200  $\mu\text{g/L}$  in some cases (41). Most of this AOX is believed to be due to the action of microorganisms and chloroperoxidase in soil and peat bogs, and a correlation exists between AOX concentrations and the quantity of humic substances present in the water (42). Furthermore, the production of organochlorines by the incubation of soil samples has been demonstrated (43). A compilation of AOX quantities in various soil samples is shown in Table 2.

**Table 1.** Naturally Occurring AOX in Rivers Feeding Lake Superior (40, 41)  
(Samples taken May 1991)

<u>Location</u>	<u>AOX <math>\mu\text{g/L}</math></u>	<u>Location</u>	<u>AOX <math>\mu\text{g/L}</math></u>
Arrow and Pigeon Rivers	15	Prairie River	8
Kaministiquia River	13	Little Pic River	9
Current River	17	Pic and Black Rivers	10
McKenzie River	16	White River	8
Wolf River	21	Magpie River	7
Black Sturgeon River	22	Michipicoten River	4
Nipigon River	3	Old Woman River	7
Jackfish River	16	Baldhead River	2
Jackpine River	13	Sand River	4
Gravel River	11	Agawa River	3
Pays Plat River	11	Montreal River	5
Aguasabon River	5	Batchawana River	3
Steel River	6	Chippewa River	2
		Goulais River	6

**Table 2.** Organochlorine in Soil Samples (40)

<u>Sampling Site</u>	<u>Vegetation</u>	<u>OX, <math>\mu\text{g Cl/g dry matter}</math></u>	<u>OX/TOC <math>\text{mg Cl/gC}</math></u>
Alberta, Canada	Peat Bog	254 - 72	0.7-0.22
Japan	Bamboo Forest	114	1.3
Britain	Meadow	224 - 62	1.9 - 0.9
Sweden	Garden	29	1.0

Sweden	Pine Forest	360	0.84
Sweden	Beech Forest	348 - 196	2.4 - 2.8
Iceland	Arctic Heath	130 - 290	1.1 - 1.3

Fleming also described studies that show that the organochlorine quantities in oysters near a pulp mill are similar to those in oysters living in unpolluted waters (Table 3).

Table 3. Organochlorine in Animal Tissue (40)

<u>Site</u>	<u>µg/g</u>
Oysters near pulp mill, 1978-79	85 - 504
Oysters from a non-industrial site	53 - 174
Antarctic krill, Wedell Sea	10 - 25
"Unpolluted" mussels, Lake Gatow, Germany	16 - 23

It is now well established that wood, vegetation, and unbleached kraft pulp contain organochlorines, and Table 4 summarizes a study of several samples of vegetation and their organochlorine content.

Table 4. Organochlorine Content of Vegetation Samples (40)

<u>Sample</u>	<u>µg/g dry material</u>
Beech Leaves	10 - 20
Norway Spruce Needles	20 - 60
Sphagnum Moss	60 - 80
Grass	60 - 100

Fleming also presented data to indicate that certain foodstuffs contain organochlorine compounds, particularly chlorinated fatty acids in bakery products (Table 5) (44-46).

Table 5. Chloro Fatty Acids in Foodstuffs (40, 44-46)

<u>Food</u>	<u>Chloro Fatty Acid, ppm</u>
Sugar Cookies	10 - 48
Yellow Cake	8 - 43
Biscuits	8 - 43
Chocolate chip cookies	8 - 41
Blueberry muffins	4 - 21
Pancakes	0.6 - 3
English muffins	0.3 - 3

Fleming has confirmed the results in Table 5 and has also found that unbleached flour can

contain organochlorines to the extent of 29  $\mu\text{g Cl/g}$  (40). Only C-18 fatty acids were analyzed in the foodstuffs listed in Table 5. Fleming found that yellow cake mix contains 210  $\mu\text{g Cl/g}$  of organochlorine (40). The origin of these chlorinated fatty acids remains unknown.

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