Lorenz Adrian · Frank E. Löffler   *Editors*

Organohalide- Respiring Bacteria

Springer

Organohalide-Respiring Bacteria

Lorenz Adrian · Frank E. Löffler
Editors

Organohalide-Respiring Bacteria

 Springer

Editors
Lorenz Adrian
Department of Isotope Biogeochemistry
Helmholtz Centre for Environmental
 Research—UFZ
Leipzig
Germany

Frank E. Löffler
Department of Microbiology,
 Department of Civil and Environmental
 Engineering, Center for Environmental
 Biotechnology
University of Tennessee
Knoxville, TN
USA

and

Biosciences Division
Oak Ridge National Laboratory
Oak Ridge
TN, USA

Contents

Part I
Introduction

Chapter 1
Organohalide-Respiring Bacteria—An Introduction

Lorenz Adrian and Frank E. Löffler

Abstract Organohalide-respiring bacteria (OHRB) "breath" halogenated compounds for energy conservation. This fascinating process has received increasing attention over the last two decades revealing the physiological, biochemical, genomic, and ecological features of this taxonomically diverse bacterial group. The discovery of OHRB enabled successful bioremediation at sites impacted with toxic chlorinated compounds, and has drawn researchers with diverse science and engineering backgrounds to study this process. Chapters discussing fundamental and applied aspects of OHRB demonstrate a vibrant research field that will continue to spur scientific discovery and innovate practice.

The realization in the 1970s of potentially harmful impacts of chlorinated organics on human and environmental health triggered extensive research on degradation mechanisms and pathways. At the time, chloroorganic compounds were considered to be predominantly of anthropogenic origin, and it was somewhat surprising that microbes capable of degrading many chlorinated chemicals were found in diverse environments. Carbon–chlorine bond breakage is mediated by dehalogenating enzyme systems (i.e., dehalogenases), and three main mechanisms were discovered: hydrolytic

---

L. Adrian (✉)
Department Isotope Biogeochemistry, Helmholtz Centre for Environmental
Research—UFZ, Leipzig, Germany
e-mail: lorenz.adrian@ufz.de

F.E. Löffler (✉)
Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
e-mail: frank.loeffler@utk.edu

F.E. Löffler
University of Tennessee and Oak Ridge National Laboratory (UT-ORNL),
Joint Institute for Biological Sciences (JIBS), Oak Ridge, TN 37831, USA

F.E. Löffler
Center for Environmental Biotechnology, Department of Microbiology,
Department of Civil and Environmental Engineering,
University of Tennessee, Knoxville, TN 37996, USA

dehalogenases replace the halogen substituent with a hydroxyl group derived from water, oxygenolytic dehalogenases replace the halogen substituent with a hydroxyl group derived from oxygen, and reductive dehalogenases replace the halogen substituent with a hydrogen atom. The degree of chlorine substitution has a strong effect on hydrolytic and oxygenolytic dehalogenation, and highly chlorinated compounds such as polychlorinated dibenzodioxins, polychlorinated biphenyls, hexachlorobenzene, lindane, or tetrachloroethene appeared recalcitrant. Reductive dechlorination was an interesting alternate mechanism as it acted on polychlorinated pollutants but the process was slow, incomplete, and presumably co-metabolic. A scientific breakthrough was the discovery of the bacterium *Desulfomonile tiedjei* (DeWeerd et al. 1990), an organism that derived all its energy required for growth from the reductive dechlorination of 3-chlorobenzoate to benzoate (Suflita et al. 1982). Then, this type of metabolism was described in a mixed culture capable of reductive dechlorination of the priority groundwater contaminant tetrachloroethene (Holliger et al. 1993), which was the prelude to the discovery of a diversity of bacteria capable of using chloroorganic compounds as electron acceptors. Due to pressing environmental problems, practical interests, at least in the United States, mostly drove research on reductive dechlorination, and support from the U.S. Department of Defense was crucial for developing this field. The observation of complete reductive dechlorination of tetrachloroethene to environmentally benign ethene in a mixed culture was a seminal contribution (Freedman and Gossett 1989) that lead to the discovery of *Dehalococcoides* (Maymó-Gatell et al. 1997). A growing research community has contributed substantially to our understanding of microbial taxa capable of using halogenated compounds as terminal electron acceptors. A key feature of these organisms is their ability to couple reductive dehalogenation to energy conservation and growth. In the case of *Dehalococcoides*, no other electron acceptors support growth, which gives these organisms a selective advantage at sites impacted with chloroorganic contaminants and makes them ideal agents for bioremediation.

A few years ago, experts in the field coined the term 'Organohalide Respiration' to describe the respiratory reductive dehalogenation process. This term is analogous to terms describing other respiratory processes such as nitrate respiration, fumarate respiration, or sulfate respiration and has replaced ambiguous expressions such as "halorespiration", "dehalorespiration" and "chlororespiration". Organohalide respiration accurately describes the process under study, has been widely adopted in the peer-reviewed literature, and is used in this book.

Organohalide respiration is a mode of energy conservation under anoxic conditions. Organohalide-respiring bacteria (OHRB) "breathe" halogenated organic molecules (called organohalides or organohalogens) just like humans breathe oxygen. In biochemical terms, OHRB use organohalides as terminal electron acceptors in a respiratory chain, which is coupled to vectorial proton movement across the cell membrane and energy conservation. The required electrons stem from external electron donors such as molecular hydrogen or other oxidizable compounds. In respiratory processes, none of the participating compounds themselves

provide energy but rather is energy available as an electric potential difference (voltage) between the participating redox couples. The term "substrate" is therefore imprecise when describing respiratory processes and the electron donor and the electron acceptor (i.e., the redox couple) should be indicated.

Although reductive dehalogenation is thermodynamically favorable, the actual growth yields of OHRB are low, a likely reason why obligate OHRB are minor components of natural microbial assemblies. It is now established that many organohalides occur naturally in low concentrations in diverse environments, which might explain the widespread distribution of OHRB. At contaminated sites, where chlorinated pollutants are present in elevated concentrations, a modest OHRB population size can turn over substantial amounts of the contaminants. Since many contaminated aquifers and sediments are anoxic and the priority pollutants recalcitrant to aerobic degradation, the reductive dechlorination process mediated by OHRB has had a transformative impact on remediation practice. Of note, bioaugmentation strategies with OHRB have been successfully implemented at many sites demonstrating that science-driven engineering can substantially accelerate contaminated site clean-up. The practical successes may have overshadowed the diverse contributions of the research field to basic science. Knowledge of microbial strategies for energy capture is crucial for understanding, modeling and predicting ecosystems function and responses to perturbation. For example, evidence that OHRB are members of deep subsurface environments is accumulating suggesting that natural organohalides serve as an energy source in such isolated ecosystems. OHRB are phylogenetically diverse and some taxa have deep branching points suggesting that organohalide respiration is an evolutionary old process. More than 2000 putative RDase genes are deposited in open-access databases and a dynamic classification system currently separating 46 different ortholog clusters has been proposed (Hug et al. 2013). Several of the confirmed reductive dehalogenase genes show propensity for horizontal gene transfer, an exciting field of study with great potential for discovery of novel principles of adaptation. Recently, heterologous expression of functional reductive dehalogenases (Mac Nelly et al. 2014; Parthasarathy et al. 2015) and their structural characterization (Bommer et al. 2014; Payne et al. 2015) combined with electron density modelling of dehalogenation reactions (Cooper et al. 2015) and detailed studies of the effects of the essential cobamide prosthetic group on reductive dechlorination activity (Yan et al. 2015) revealed new roles for coenzyme B_{12} in catalysis. These examples demonstrate how research driven by practical needs (e.g., contaminated site clean-up) has developed into a field of scientific endeavor with great potential for transformative discoveries.

The chapters in this book discuss environmental, physiological, biochemical, genomic, ecological, evolutionary, practical, and last but not least, historical aspects. The content of these chapters should help students to learn about OHRB and the breadth of the field, provide useful information to engineers and practitioners, and also serve as a valuable resource for experts.

References

Bommer M, Kunze C, Fesseler J, Schubert T, Diekert G, Dobbek H (2014) Structural basis for organohalide respiration. Science 346(6208):455–458. doi:10.1126/science.1258118

Cooper M, Wagner A, Wondrousch D, Sonntag F, Sonnabend A, Brehm M, Schüürmann G, Adrian L (2015) Anaerobic microbial transformation of halogenated aromatics and fate prediction using electron density modelling. Environ Sci Technol 49(10):6018–6028. doi:10.1021/acs.est.5b00303

DeWeerd KA, Mandelco L, Tanner RS, Woese CR, Suflita JM (1990) *Desulfomonile tiedjei* gen. nov. and sp. nov., a novel anaerobic dehalogenating, sulfate-reducing bacterium. Arch Microbiol 154:23–30. doi:10.1007/BF00249173

Freedman DL, Gossett JM (1989) Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl Environ Microbiol 55(9):2144–2151

Holliger C, Schraa G, Stams AJM, Zehnder AJB (1993) A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Appl Environ Microbiol 59:2991–2997

Hug LA, Maphosa F, Leys D, Löffler FE, Smidt H, Edwards EA, Adrian L (2013) Overview of organohalide-respiring bacteria and a proposal for a classification system for reductive dehalogenases. Philos Trans R Soc Lond B Biol Sci 368(1616):20120322. doi:10.1098/rstb.2012.0322

Mac Nelly A, Kai M, Svatoš A, Diekert G, Schubert T (2014) Functional heterologous production of reductive dehalogenases from *Desulfitobacterium hafniense* strains. Appl Environ Microbiol 80(14):4313–4322. doi:10.1128/aem.00881-14

Maymó-Gatell X, Chien YT, Gossett JM, Zinder SH (1997) Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 276:1568–1571. doi:10.1126/science.276.5318.1568

Parthasarathy A, Stich TA, Lohner ST, Lesnefsky A, Britt RD, Spormann AM (2015) Biochemical and EPR-spectroscopic investigation into heterologously expressed vinyl chloride reductive dehalogenase (VcrA) from *Dehalococcoides mccartyi* strain VS. J Am Chem Soc 137(10):3525–3532. doi:10.1021/ja511653d

Payne KAP, Quezada CP, Fisher K, Dunstan MS, Collins FA, Sjuts H, Levy C, Hay S, Rigby SEJ, Leys D (2015) Reductive dehalogenase structure suggests a mechanism for B12-dependent dehalogenation. Nature 517(7535):513–516. doi:10.1038/nature13901

Suflita JM, Horowitz A, Shelton DR, Tiedje JM (1982) Dehalogenation: a novel pathway for the anaerobic biodegradation of haloaromatic compounds. Science 218:1115–1119. doi:10.1126/science.218.4577.1115

Yan J, Simsir B, Farmer AT, Bi M, Yang Y, Campagna SR, Löffler FE (2015) The corrinoid cofactor of reductive dehalogenases affects dechlorination rates and extents in organohalide-respiring *Dehalococcoides mccartyi*. ISME J. doi:10.1038/ismej.2015.197

Chapter 2
Natural Production of Organohalide Compounds in the Environment

James A. Field

Abstract More than 5000 natural organohalogen compounds have been identified. In terrestrial environments, the bulk of the organochlorine is locked up in humic polymers, collectively accounting for a global organochlorine storage of several million Gg. Natural sources are primarily responsible for the global budget of chloromethane and chloroform. Basidiomycete fungi involved in the decomposition of forest litter produce large quantities of chlorinated phenolic methyl ethers. In marine environments naturally occurring chlorinated and brominated bipyrroles as well as methoxypolybrominated phenyl ethers biomagnify in sea mammals. There are at least five distinct halogenating enzyme systems: (1) methyl transferases; (2) heme haloperoxidases; (3) vandadium haloperoxidases; (4) flavin-dependent halogenases and (5) α-ketoglutarate/Fe(II) dependent halogenases. Natural halogenated phenolic metabolites are subject to biotransformation including *O*-demethylation and organohalide respiration. Naturally occurring phenolics are also polymerized by oxidative enzymes to dioxins and chlorohumus.

2.1 Scope

The natural production of organohalogens is an important component of the halogen biogeochemical cycles. Over 5000 natural halogenated compounds have been identified so far as of 2012 (Gribble 1996, 2010, 2012). These naturally halogenated compounds are formed by living organisms such as microalgae, sponges, fungi, bacteria, higher plants, insects, and animals. Likewise such compounds are formed by abiotic process such as volcanoes, forest fires, and abiotic oxidation of soil organic matter. Over half of the natural organohalogens

J.A. Field (✉)
University of Arizona, Tucson, USA
e-mail: jimfield@email.arizona.edu

described contain chlorine, about half contain bromine, and several hundred contain both chlorine and bromine. As of 2004, there were also approximately 110 natural organoiodine and 30 organofluorine compounds described (Gribble 2004a).

The formation of organochlorine in terrestrial environments is known to be tightly linked to the decomposition of organic matter and processes of soil humus formation (Leri et al. 2007; Myneni 2002). The use of in situ X-ray spectroscopic techniques has revealed that inorganic chloride in plant leaves is initially converted to aliphatic and aromatic organochlorine structures. At advanced stages of humification, the chlorinated aromatic fraction continues to increase whereas the chlorinated aliphatic fraction is stable (Leri et al. 2007; Leri and Myneni 2010; Myneni 2002; Reina et al. 2004). The measured organohalogen or organochlorine content of soils measured around the world ranges from 12 to 340 mg organochlorine kg^{-1} soil dry weight (dwt) (Redon et al. 2013; Öberg 2003). The median organochlorine-to-organic carbon ratio in soils has been reported to be 2.3 mg organochlorine g^{-1} soil C (Öberg 2003). This value, when multiplied by the global organic carbon content of soils, indicates a global storage of organochlorine in the pedosphere of 3.35×10^6 Gg (Öberg 2003). Field measurements of organochlorine formation rates in forest soils indicate a production rate of 0.35–0.5 kg organohalogen $ha^{-1} y^{-1}$ (Öberg and Bastviken 2012).

2.2 Evidence

There are multiple lines of evidence to support the large scope of natural organohalogen storage and production in terrestrial environments. Many of the studies evaluating soil organic matter utilize micro-coulometric measurement to assess organohalogens based on the corrosion of silver needed to replace silver ions precipitated by halides. Adsorbable organohalogens (AOX) measures the water soluble organic halogens that are adsorbable onto activated carbon and liberated by combustion (Asplund et al. 1989). Total organohalogen (TOX) measures the halogens in samples directly combusted (Hjelm et al. 1995). In both cases, the combustion is performed after rinsing away inorganic halides with a nitrate/nitric acid solution. A variation of AOX and TOX is to directly measure halides captured from the combustion with ion chromatography (Biester et al. 2004; Putschew et al. 2003). In some studies, halides are measured by neutron activation analysis in sequentially extracted samples at a research nuclear reactor facility (Redon et al. 2013). Lastly, isotopes such as radioactive $^{36}Cl^-$ are spiked into soil and the radioactivity is monitored over time in sequentially extracted soils (Gustavsson et al. 2012) or peat samples (Silk et al. 1997).

A critique of total organohalogen methods based on detecting halides in soil residue is that they do not account for intracellular cytoplasmic chloride of micro-organisms (Bastviken et al. 2007; Putschew et al. 2003; Rohlenova et al. 2009). Thus measures of total organohalogens in soil samples can potentially overestimate the actual value. Freezing and thawing of samples prior to extraction has been proposed as a means of lowering this type of interference by enabling the

extraction of intracellular inorganic halides (Rohlenova et al. 2009). Nonetheless, there is no doubt that extensive chlorination of organic matter occurs during plant litter decay and soil humus formation. The measurement of organohalogens in water soluble humic fractions extracted from soil is not burdened by interferences caused by intracellular halides. Extensive incorporation of ^{36}Cl into humic and fulvic acids of soils has been demonstrated (Rohlenova et al. 2009). Likewise AOX measurements in natural organic matter (NOM) of pristine surface water and ancient groundwater provides additional evidence that natural occurring organohalogens are associated with humic materials (Asplund et al. 1989).

To add to the evidence, several research groups have utilized techniques to selectively fragment high molecular NOM or lignin into low molecular structures amendable to gas chromatography—mass spectrometry (GC-MS) or microwave induced plasma atomic emission detection (GC-AED). The techniques require derivatization of free OH groups. These techniques demonstrate aromatic substructures that are halogenated in decomposing wood, leaf litter, sphagnum peat, soil, or NOM recovered from surface water and groundwater. The corresponding nonderivatized substructures are shown in Fig. 2.1. The fragments reveal that 3-chloro- and 3,5-dichloro-*p*-hydroxybenzyl structures, 3,5-dichloro-*p*-anisyl, 5-chlorovanillyl as well as 2-chloro- and 2,6-dichlorosyringyl structures in addition to dichloro- and
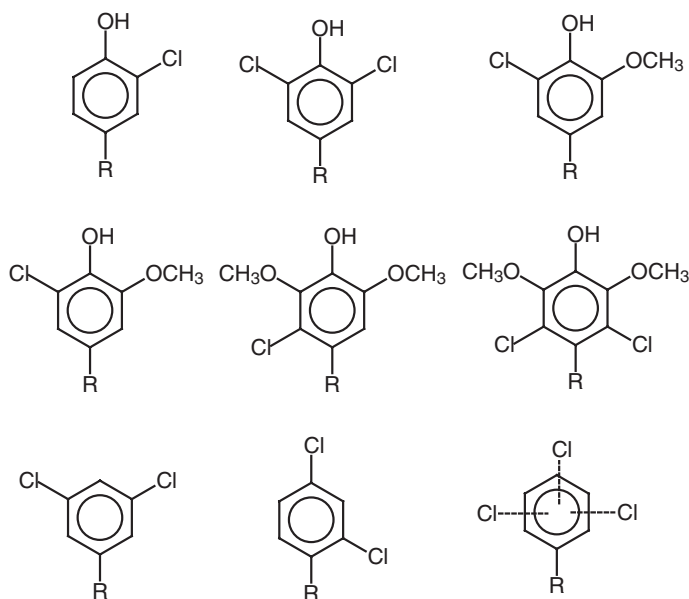
Fig. 2.1 Chlorinated aromatic moieties determined after fragmenting humus in natural organic matter or lignin subjected to microbial decomposition (Dahlman et al. 1993; Flodin et al. 1997; Ortiz-Bermudez et al. 2007). From *left* to *right*, *top row* 3-chloro- and 3,5-dichloro-*p*-hydroxybenzyl structures, 3,5-dichloro-*p*-anisyl; *middle row* 5-chlorovanillyl as well as 2-chloro- and 2,6-dichlorosyringyl. *Bottom row* to 3-5-dichloro- and 2,4-dichlorobenzyl and trichlorobenzyl

trichlorobenzyl structures are embedded in high MW NOM and decomposing lignin (Dahlman et al. 1993; Flodin et al. 1997; Ortiz-Bermudez et al. 2007).

Most recently, synchrotron enabled technique such as near edge X-ray absorption fine structure (NEXAFS) spectroscopy and extended X-ray absorption fine structure spectroscopy (EXAFS) provide spectroscopic evidence of organochlorine formation during forest litter decomposition and humus formation (Leri et al. 2007; Myneni 2002; Reina et al. 2004). These techniques can distinguish between unique spectroscopic signatures for inorganic chloride from those for aliphatic and aromatic organochlorines.

The de novo synthesis of organohalogens by living organisms provides compelling evidence for the natural formation of organohalogens. Basidiomycete fungi are the main group of organisms responsible for the decay of lignocellulosic forest litter (Frankland et al. 2009). A total of 191 Basidiomycete fungal strains were tested on defined medium for the production of AOX and half of them were shown to significantly produce AOX beyond background levels. High levels (5–67 mg AOX L^{-1}) were produced in 9 % of the strains. Fungi belonging to the genus *Hypholoma* had the highest specific production with AOX equivalent to 1.8–3.1 % of their mycelium dwt. The fungi also produced AOX on natural substrates such as forest litter and wood with levels of AOX reaching 61, 115, and 193 mg kg^{-1} dwt substrate on wood, straw, and forest litter, respectively (Verhagen et al. 1996; Öberg et al. 1997). Thus, the synthesis of organohalogen metabolites is associated with the decomposition of forest litter.

2.3 Halogenated Metabolites

Living organisms produce a multitude of halogenated metabolites (Gribble 1996, 2010). Reviews of organohalogen metabolites produced by marine algae (Ballschmiter 2003; Cabrita et al. 2010; Vetter 2006) and fungi (de Jong and Field 1997; Field and Wijnberg 2003; Rezanka and Spizek 2005) are available. The diversity of metabolites found in bacteria, sponges, lichens, higher plants, insects, and mammals can be found in reviews by Gribble (2003a, b, 2004b, 2012). Due to the large diversity of halometabolite structures, a focus will be placed on metabolites which are either identical or structurally similar to halogenated pollutants susceptible to organohalide respiration or other mechanisms of anaerobic bacterial dehalogenation. The categories of compounds to be considered will include halomethanes, chloroethenes, chloroacetic acids, chlorophenols, polychlorinated dibenzodioxins/furans, and polybrominated diphenylethers.

2.3.1 C1 and C2 Metabolites

Chloromethane Fungi, plants and marine algae are known sources of chloromethane (compound 1) (Harper 1985, 2000; Harper and Hamilton 2003).

Polypore white rot fungi are an important biological source of chloromethane, especially those of the *Hymenochaetaceae* family (Harper and Hamilton 2003). The highest producers are within the genus *Phellinus*, which convert up to 80–90 % of inorganic chloride in the growth medium to chloromethane. The rate of chloromethane formation is as high as 20 mg chloromethane kg^{-1} fresh weight (fwt) mycelium d^{-1} (Harper and Hamilton 1988). *Phellinus* spp. are also capable of methylating bromide and iodide (Harper and Hamilton 2003).

Tropical plants have also been implicated in an extensive production of chloromethane (Yokouchi et al. 2002). The flux of chloromethane from the leaves of selected tropical plants is reported to range from 1680 to 44,160 mg kg^{-1} dwt d^{-1}. Likewise microalgae and microalgae from marine environments produce low levels of chloromethane as well as bromo- and iodomethanes (Harper and Hamilton 2003; Harper 2000; Gribble 2010).

The median estimate of the global production of chloromethane and released to the atmosphere is 3000 Gg y^{-1} (Keppler et al. 2005). The anthropogenic contribution is about 5 % (industrial, coal combustion, and incineration). The contribution due to tropical plants is estimated at 910 Gg y^{-1} (or 30 % of global production). The contribution from fungi is estimated to be about 160 Gg y^{-1} (or 5 % of global production, which is equivalent to the anthropogenic contribution).

Chloroform There is strong evidence that chloroform is produced naturally. Several organisms have been shown to produce chloroform. Pure cultures of the fungus *Caldariomyces fumago*, well known for producing chloroperoxidase, were shown to produce chloroform at rates ranging from 0.07–70 µg L^{-1} culture fluid d^{-1}, depending on the culture conditions. Additionally a few basidiomycetes, *Mycena metata* and *Peniophora pseudopini*, were also shown to produce chloroform in pure cultures at rates of 0.7–40 ng L^{-1} culture fluid d^{-1} (Hoekstra et al. 1998b). Termites have been shown to produce chloroform (Khalil et al. 1990). Marine macroalgae and microalgae were also implicated in the production of chloroform (Nightingale et al. 1995; Scarratt and Moore 1999). The highest rate of chloroform production in macroalgae reaching 200 µg kg^{-1} dwt biomass d^{-1} was observed with brown seaweed, *Laminaria saccharina* (Nightingale et al. 1995). Macroalgae are however much better at producing bromoform. *L. saccharina* was also the highest producer with a bromoform production rate of 30 mg kg^{-1} dwt biomass d^{-1} (Nightingale et al. 1995). Bromoform is the most dominant volatile organohalocarbon produced by marine macroalgae accounting for 79–92 % of all volatile organohalogens this organism group produces, depending on region (Laturnus 2001).

Formation of chloroform has been noted in soil and peat in several studies (Gron et al. 2012; Haselmann et al. 2000a, b, 2002; Simmonds et al. 2010). The evidence is strengthened by de novo production when soil samples are incubated in closed bottles in the laboratory (Gron et al. 2012). In one study, the top layer of soil was spiked with $^{37}Cl^-$ NaCl and subsequently chloroform enriched with ^{37}Cl was detected, providing conclusive proof of de novo chloroform formation in top soil (Hoekstra et al. 1998a).

The enzyme chloroperoxidase (CPO) is responsible for the formation of chloroform when it is incubated with humic substances. Reactions of CPO with aquatic NOM and humic acids from peat produced up to 240 µg L^{-1} of chloroform (Breider and Hunkeler 2014a). The maximum rates of chloroform production with CPO incubated with humic acids were 1.4 mg L^{-1} d^{-1} (Breider and Hunkeler 2014b). Mechanistically, CPO is responsible for the formation of hypochlorous acid (HOCl) and phenolic moieties in humus become chlorinated in the chemical reaction with HOCl. Reaction schemes have been proposed for phenol (Breider and Hunkeler 2014b) and resorcinol (Hoekstra et al. 1999a) as shown in Fig. 2.2. In support of this hypothesis is the detection of CPO in forest soil and decomposing wood (Ortiz-Bermudez et al. 2007; Laturnus et al. 1995; Asplund et al. 1993).

The global production of chloroform and released to the atmosphere is estimated at 700–820 Gg y^{-1} (Laturnus et al. 2002; Gribble 2010). The known anthropogenic sources only account for 60–73 Gg y^{-1}, thus 90 % or more of the estimated annual chloroform production is natural. The most important natural sources are oceans, soil, termites, and microalgae.

Trichloroacetic acid The natural occurrence of trichloroacetic acid (TCAA) has been inferred by its presence in bog water (Niedan and Schöler 1997; Haiber et al. 1996) and in pristine forest soils (Hoekstra et al. 1999a; Frank 1988). Biological formation of TCAA occurs when CPO is incubated with humic substances or simple organic acids (Haiber et al. 1996; Niedan et al. 2000). A mechanism of TCAA formation during the chlorination of the phenolic moiety, resorcinol, is shown in Fig. 2.2.

Chloroethanes and chloroethenes Marine algae have been reported to produce trichloroethene (TCE) and tetrachloroethene (PCE) (Abrahamsson et al. 1995; Collen et al. 1994). Rates up to 81.6 and 0.2 mg kg^{-1} fresh wt d^{-1} TCE and PCE, respectively, were recorded in the highest producing red algae species. However, these results have only been reported from one research group. Another research group made an extensive attempt to confirm biogenic production from the highest producing algae but was unsuccessful (Marshall et al. 2000). TCE and PCE were also
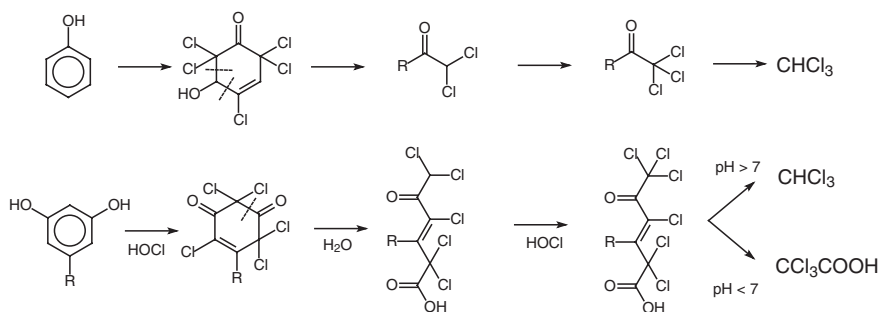

Fig. 2.2 Proposed reaction schemes for the conversion of phenolic and resorcinolic moieties in humus to chloroform and trichloroacetic acid due to their oxidation by hypochlorous acid generated by chloroperoxidase (Breider and Hunkeler 2014a; Hoekstra et al. 1999a)

reported in salt lake sediments at concentrations up to 1.65 and 8.53 µg kg^{-1} fresh wt (Weissflog et al. 2005). Incubations of salt lake sediment samples in closed bottles in the laboratory indicated de novo production was taking place, reaching levels as high as 25 and 50 µg kg^{-1} fresh wt, respectively, after 6 weeks (Weissflog et al. 2005).

2.3.2 Phenolic Compounds, Benzoates and Their Methyl Ethers

Simple phenols A selection of simple phenols that is known to be produced by living organisms are shown in Fig. 2.3. 2,6-dichlorophenol has been identified as a natural product (sex hormone) in many species of ticks (Gribble 1996). The metabolite 2,4-dichlorophenol was shown to be produced by the soil fungus *Penicillium* sp. (Ando et al. 1970). A litter-degrading fungus, *Lepista nuda*, was shown to produce low levels of 2,6-dichloroanisole (Hjelm et al. 1999). Another litter-degrading fungus from the tropics, *Mycena* sp., produced tetrachlorocatechol and tetrachloroguaiacol (Daferner et al. 1998) are as shown in Fig. 2.3. Lastly, three trichlorinated phenols were observed as metabolites in the sphagnum moss inhabiting fungus *Hypholoma elongatum* (Swarts et al. 1998). These metabolites were 2,4,6-trichloro-3-methoxy-phenol, 3,5,6-trichloro-2,4-dimethoxyphenol and 3,4,6-trichloro-2,5-dimethoxy-phenol (Fig. 2.3). In marine environments, red, green and brown macroalgae were shown to produce various congeners of bromophenols, with 2,4,6-tribromophenol being one of the most predominant congeners found (Paul and Pohnert 2011).

Strong evidence for the natural formation of chlorophenols was obtained by spiking forest soil with ^{37}Cl$^-$ and subsequently detecting chlorophenols enriched with ^{37}Cl. Using this method, the natural formation of 4-chlorophenol, 2,4-(or 2,5-)dichlorophenol, 2,6-dichlorophenol, and 2,4,5-trichlorophenol was confirmed
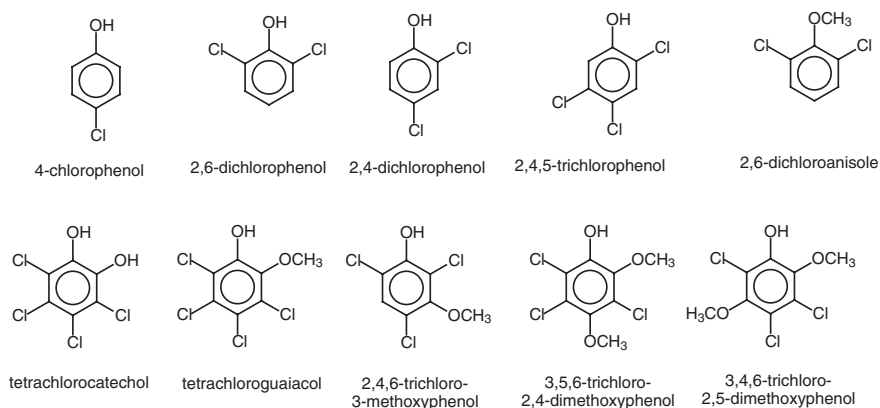

Fig. 2.3 Examples of naturally occurring chlorophenol compounds and a chloroanisole compound. References of each illustrated compound are provided in the text

(Hoekstra et al. 1999b) (Fig. 2.3). The strongest enrichment in ^{37}Cl was observed for 4-chlorophenol. The sum of all simple chlorophenols detected in forest soils ranged up to 71 µg kg^{-1} dwt.

Chlorinated anisyl metabolites A family of metabolites known as the chlorinated anisyl metabolites (CAM) are produced in large quantities by numerous basidiomycete fungi (de Jong and Field 1997; de Jong et al. 1994; Field et al. 1995; Field and Wijnberg 2003; Swarts et al. 1997). The family is composed of 3-chloro- or 3,5-dichloro- *p*-anisyl alcohols and aldehydes (Fig. 2.4). Also the benzoic acid (anisate) form of these metabolites has also been found in the litter-degrading fungus *L. nuda* (Hjelm et al. 1996) and in culture fluids of white rot fungi of to the *Bjerkandera* genus (Swarts et al. 1996) and *Hypholoma fasciculare* (Verhagen et al. 1998b). The production of CAM metabolites is significant, with levels of CAM commonly ranging between 2 and 37 mg CAM L^{-1} in the broths of pure cultures of CAM-producing basidiomycetes (de Jong et al. 1994; Verhagen et al. 1998b), with one exceptional fungus, *H. elongatum*, producing up to 108 mg CAM L^{-1} (Swarts et al. 1997). Likewise, CAM metabolites are detected at relatively high concentrations in the field. CAM concentrations of 7–180 mg kg^{-1} dwt of litter or wood colonized by CAM-producing fungi have been observed (de Jong et al. 1994; Hjelm et al. 1996). Composite forest litter has measurable concentrations of CAM up to 4.5 mg kg^{-1} dwt (de Jong et al. 1994). Estimates of CAM production indicate approximately 300 g ha^{-1} y^{-1} in Dutch forests
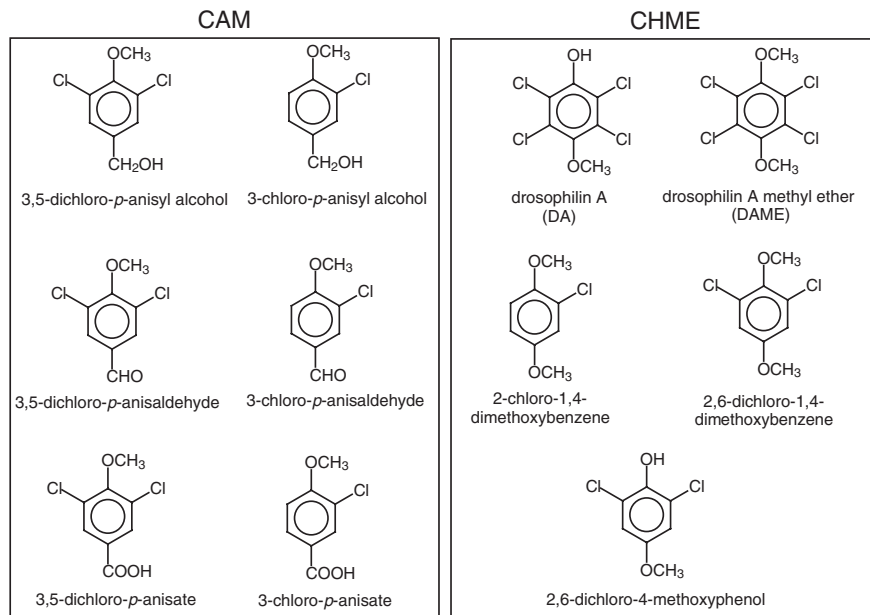

Fig. 2.4 Chlorinated anisyl metabolites (*CAM*) and chlorinated hydroquinone methyl ethers (*CHME*). References are provided in the text

(Field and Wijnberg 2003). This value corresponds to 100 g AOX ha^{-1} y^{-1} for just one family of metabolites, and provides a quantity equivalent to 20–29 % of the field measured organochlorine formation rates in forests (Öberg and Bastviken 2012).

Chlorinated hydroquinone methyl ethers Another important group of fungal chlorophenolic metabolites are the chlorinated hydroquinone methyl ethers (CHME) shown in Fig. 2.4. Tetrachlorinated drosophilin A (DA) and drosophilin A methyl ether (DAME) are found in numerous species of basidomycetes (Teunissen et al. 1997; de Jong and Field 1997), including *Agaricus bisporus*, the common store-bought white button mushroom. Concentrations of DA and DAME are typically found in the culture fluid in the range of 0.14–0.7 mg L^{-1}, with one exceptional white rot fungal species, *Phellinus fastuosus*, producing 11 mg L^{-1} (Swarts et al. 1998; Teunissen et al. 1997). Pure crystals of DAME have been found in the heartwood of mesquite trees being degraded by the basidiomycete, *Phellinus badius* (Garvie et al. 2015). In addition to DA and DAME, 2-chloro-1,4-dimethoxybenzene, 2,6-dichloro-1,4-dimethoxybenzene and 2,6-dichloro-4-methoxyphenol have been identified as fungal metabolites (Hjelm et al. 1996; Swarts et al. 1996; Spinnler et al. 1994; de Jong and Field 1997). The environmental importance of DA has recently come to light when it was reported that it potentially biomagnifies along the food chain. DA was found at concentrations of 1 mg kg^{-1} lipids in the meat of wild boars in Germany (Hiebl et al. 2011). Wild mushroom are considered an important component of the wild boar diet.

Chlorinated benzoic acids Evidence for the natural formation of chlorinated benzoic acids is limited to a few environmental measurements and several examples of chlorinated benzoic acids as metabolites. Structures of some of the naturally occurring chlorinated benzoic acids are shown in Fig. 2.5. The environmental evidence is based on the occurrence of 2,4-dichlorobenzoic acid in bog water and sediments from pristine environments (up to 0.48 µg L^{-1} and 3.4 mg kg^{-1} dwt, respectively) (Niedan and Schöler 1997). A cyanobacterium, *Fischerella ambigua*, was shown to produce 2,4-dichlorobenzoic acid with a yield of 135 mg kg^{-1} dwt cell biomass (Wright et al. 2005). Two hydroxylated chlorinated benzoic acid metabolites, 3-chloro-*p*-benzoic acid and 3,5-dichloro-*p*-benzoic acid, were observed in the culture fluids of the white rot fungus *Bjerkandera* (Swarts et al. 1996).
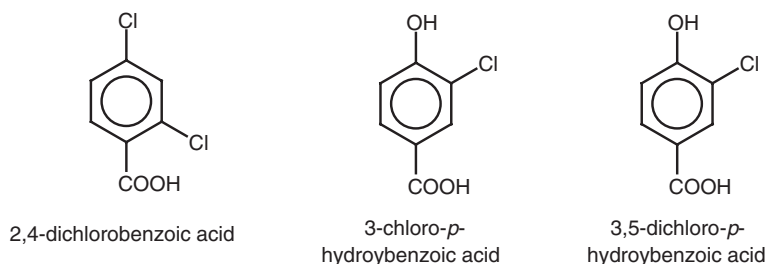

2,4-dichlorobenzoic acid   3-chloro-*p*-hydroybenzoic acid   3,5-dichloro-*p*-hydroybenzoic acid

Fig. 2.5 Naturally occurring chlorinated benzoic acids. References are provided in the text

2.3.3 Multi-ring Phenolic Metabolites

Diphenyl ethers A large number of marine sponges produce polybrominated diphenyl ethers (PBDEs) (Gribble 1992, 2010). Examples of natural PBDEs from sponges such as 4,5,6,2′,4′-pentabromo-2-hydroxydiphenyl ether and 3,5,4′,5′,6′-2,2′-dimethoxydiphenyl ether are shown in Fig. 2.6. PBDEs have also been detected in macroalgae samples (Haraguchi et al. 2010). Archived samples of whale oil from 1921 contain PBDEs, providing supporting evidence of their natural occurrence since 1921 predates the industrial production of PBDEs (Gribble 2010). Methoxy-PBDEs have also been found in fish and sea mammals and strong evidence is presented that they bioaccumulate (Vetter 2006; Teuten et al. 2005). Polychlorinated diphenyl ethers are known from the freshwater fungus *Krischsteiniothelia* (Poch et al. 1992) and the cyanobacterium *F. ambigua* (Wright et al. 2005) (e.g. ambigol B in Fig. 2.6). Mushrooms of the basidomycete fungus *Russula subnigricans* contain several chlorinated polyphenyl ethers such as russuphelin-A (shown in Fig. 2.6), which was recovered at a concentration of 247 mg kg^{-1} fresh wt of mushrooms (de Jong and Field 1997; Takahashi et al. 1992).

Chlorinated xanthones and anthraquinones A large variety of chlorinated xanthones are produced by lichens (Gribble 1996, 2010). The metabolite, 4,5-dichloronorlichexanthone (Fig. 2.6) is produced by the largest diversity of lichens. Both fungi and lichens are well known for their production of chlorinated anthraquinones (Gribble 1996, 2010). The metabolite 7-chloroemodin (Fig. 2.6) is produced by the greatest diversity of fungi.

Chlorinated dibenzodioxins/furans Chlorinated dibenzodioxins/furans have been detected in archived soil samples from 1880 and in deep peat bog sediment layers, including those deposited up to 5000 years ago, indicating that dibenzodioxins/furans have a natural origin (Green et al. 2001, 2004; Silk et al. 1997). Important congeners in deep bog layers include 2468-TCDF and 1379-TCDD (Fig. 2.6) (Green et al. 2004; Silk et al. 1997). Natural chlorinated dibenzodioxin/furan formation has been attributed to the oxidation of chlorinated phenols catalyzed by peroxidases and clay. The formation of dioxins from the oxidation of simple chlorophenols has been demonstrated with chloroperoxidase (Silk et al. 1997), horseradish peroxidase (Wittsiepe et al. 1999; Öberg and Rappe 1992), lactoperoxidase (Öberg and Rappe 1992), myeloperoxidase (Wittsiepe et al. 1999) and manganese peroxidase (Munoz et al. 2014). Likewise certain clays with Fe(III) were shown to oxidize chlorophenols to chlorinated dibenzodioxins (Gu et al. 2011; Holmstrand et al. 2006; Horii et al. 2008). The best evidence for natural formation of chlorinated dibenzodioxins comes from spiking forest soil with ^{37}Cl^{-}, and demonstrating the natural formation of ^{37}Cl enriched precursors, chlorophenols, as well as, ^{37}Cl enriched chlorinated dibenzodioxins (Hoekstra et al. 1999b). Recently, the peroxidase oxidation of bromophenols was also shown to oxidize the naturally occurring 2,4,6-tribromophenol (in marine environments) to 1,3,6,8-tertrabromodibenzodioxin by bromoperoxidase (Arnoldsson et al. 2012).
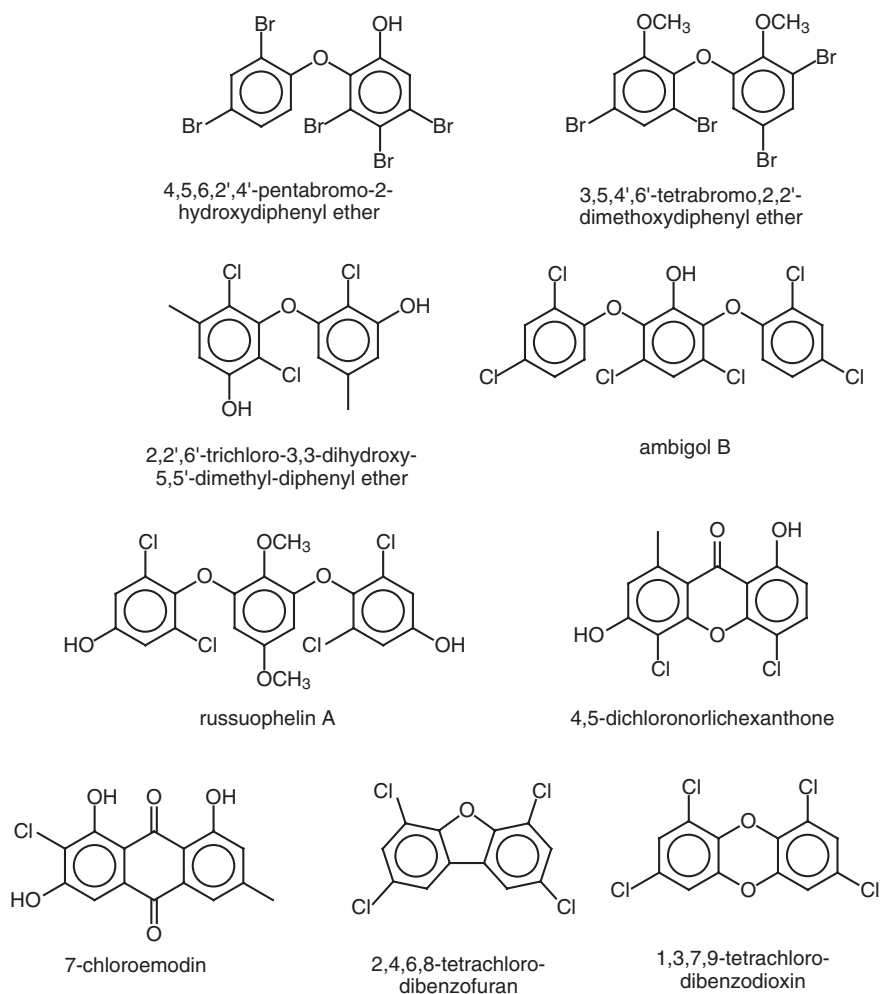
Fig. 2.6 Naturally occurring multi-ring phenolic compounds. References are provided in the text

2.3.4 Chlorinated and Brominated Bipyrroles

A heptachlorometabolite of putative natural origin has been found to accumulate in the lipids of sea mammals (Vetter et al. 2001; Vetter 2006). This compound has been designated Q1 and has been found in the blubber of dolphins at concentrations ranging from 450 to 9100 μg kg^{-1} lipids (Vetter et al. 2001). The chemical structure of Q1 has been elucidated as 2,3,3′,4,4′,5,5′-heptachloro-1′-methyl-1,2′-bipyrrole (Fig. 2.7) (Wu et al. 2002). Passive samplers placed around the Great Barrier Reef indicate that Q1 is produced naturally in that ecologically rich system (Vetter et al. 2009).

2,3,3',4,4',5,5'-heptachloro-
1'-methyl-1,2'-bipyrrole (Q1)

5,5'-dichloro-1,1'-dimethyl-3,3',4,4'-
tetrabromo-2,2'-bipyrrole

Fig. 2.7 Halogenated bipyrrole compounds. References are provided in the text

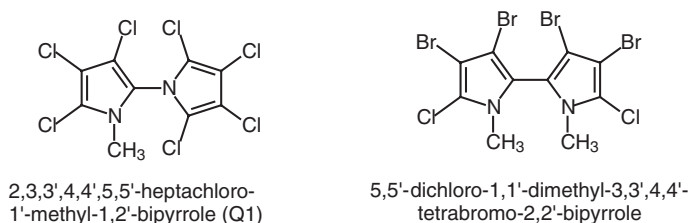A tetrabrominated and dichlorinated metabolite similar in structure to Q1 has also been identified in as a putative natural marine metabolite biomagnified in seabird eggs (Tittlemier et al. 1999). Its structure has been elucidated as 5,5′-dichloro-1,1′-dimethyl-3,3′,4,4′-tetrabromo-2,2′-bipyrrole (Fig. 2.7). This compound together with related halogenated dimethyl-bipyrrole compounds have been detected in a variety of sea mammals and there is evidence of their biomagnification with trophic level in the food chain (Vetter 2006).

2.4 Abiotic Formation Chlorinated Compounds in Soil

During the diagenesis of soil, there are important mechanisms leading to the abiotic formation of chlorinated compounds. A series of studies has demonstrated formation of simple chlorinated C1 and C2 compounds due to the oxidation of humus in soil by Fe(III) in the presence and absence of H_2O_2. The first of these studies demonstrated that chloromethane, bromo- and iodomethane were produced in response to the oxidation of soil organic matter with Fe(III) (Keppler et al. 2000). A similar pattern was also observed using guaiacol (2-methoxyphenol) as a model for soil organic matter together with ferrihydrite as a model for Fe(III) minerals. Chloromethane, bromomethane, or iodomethane were each produced depending on whether 10 mM KCl, KBr or KI was provided as the halide.

The natural formation of vinyl chloride (chloroethene) was indicated in a study where top soil air was observed to be highly enriched in vinyl chloride concentration compared to ambient air (60–850 times greater) (Keppler et al. 2002). In contrast, no enrichment of trichloroethene was observed in soil air. The abiotic oxidation of soil organic matter with Fe(III) was shown to form vinyl chloride (Keppler et al. 2002). Vinyl chloride, chloromethane, chloroethane and chloropropane production was also observed in a model system with Fe(III), KCl and catechol as a model for humus. The vinyl chloride production in soil or in the catechol model system increased if the reaction mixture also contained H_2O_2.

Oxidation of catechol with Fe(III) also resulted in the formation of chloroacetylene (chloroethyne) (Keppler et al. 2006) and the addition of H_2O_2 to the reaction mixture increased the amount of chloroacetylene formed. Like in the case of vinyl
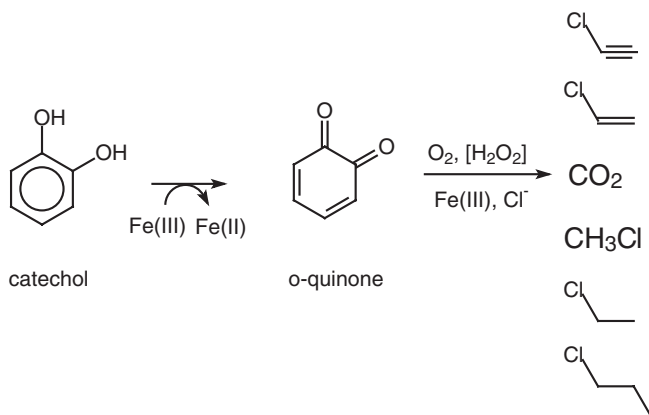
Fig. 2.8 Proposed mechanism for the abiotic formation of chloromethane, vinyl chloride, chloroacetylene, chloroethane and chloropropane by oxidation of catechol with Fe(III) (Keppler et al. 2002, 2006)

chloride, enrichment of chloroacetylene was also observed in topsoil air (Keppler et al. 2006). An overall reaction scheme for the oxidation of catechol to chlorinated alkanes, vinyl chloride, and chloroacetylene is shown in Fig. 2.8.

The addition of Fe(III) and H_2O_2 together with NaCl, humic acid or phenolic model substrates led to the formation of dichloroacetic acid (DCAA) and to a lesser extent TCAA (Fahimi et al. 2003). After the addition of Fe(III), NaCl, and H_2O_2 to soil, the collective formation of chloroacetic acids reached 1.5 mg kg^{-1} soil. In similar experiments, the formation of chloroform was also reported when oxidizing soil or phenolic model compounds with Fe(III), KCl, and H_2O_2 (Huber et al. 2009). Bromoform and trihalomethanes substituted with both chlorine and bromine were observed when KBr was added to the model systems or when soils containing bromide were used.

Plants with pectin and pectin itself serve as a methyl donor to form chloromethane when heated in the presence of chloride (Hamilton et al. 2003). In short-term temperature ramping experiments, significant formation of chloromethane starts after surpassing 170 °C. By the time the temperature was ramped up to 270 °C, 35 g CH_3Cl kg^{-1} pectin was produced while all the chloride was consumed. Long term experiments with pectin containing horse chestnut leaves allowed to slowly dry, rates of 30–50 μg CH_3Cl kg^{-1} d^{-1} dwt leaf mass were observed at 40 °C.

2.5 Biosynthesis of Natural Organohalogens

There are multiple enzyme systems responsible for the formation of organohalogens. Simple halomethanes are formed by methyl transferases, which transfer a methyl group from S-adenosylmethionine (SAM) to a chloride, bromide or, iodide ion

(Murphy 2003). Heme-containing haloperoxidases such as chloroperoxidase (CPO) uses peroxide (H_2O_2) to convert halides to hypohalous acids, such as hypochlorous acid (HOCl) which in turn chemically halogenate organic molecules (Murphy 2003; Butler and Sandy 2009). There are also haloperoxidases without heme groups, such as vanadium peroxidase, which uses H_2O_2 to oxidize bromide to form hypobromous acid (HOBr) that in turn chemically brominates organic molecules (Wever and van der Horst 2013).

In contrast to haloperoxidases, there are halogenating enzymes that catalyze more specific halogenation reactions during metabolite biosynthesis. One such group of halogenating enzymes are the flavin-dependent halogenases (Murphy 2003; Butler and Sandy 2009; Chen and van Pee 2008). Flavin-dependent halogenases are implicated in the synthesis of halogen-containing intermediates during microbial antiobiotic biosynthesis such as tryptophan 7-halogenase in the biosynthesis of pyrrolnitrin by *Pseudomonas fluorescens* (Chen and van Pee 2008). During the enzyme catalytic cycle, a flavin peroxide is formed that oxidizes chloride to HOCl. The chlorine of HOCl is enzymatically inserted into tryptophan (as opposed to the random chemical reaction induced by CPO).

Another type of specific halogenase is the α-ketoglutarate-dependent halogenase. This type of halogenase requires α-ketoglutarate and Fe(II) and is responsible for the halogenation of peptides of nonribosomal synthetic origin (Chen and van Pee 2008; Butler and Sandy 2009). The α-ketoglutarate-dependent halogenases are the only halogenases that attach chloride (or other halides) onto carbons without double bonds.

Some of the most abundant halogenated secondary metabolites in terrestrial environments belong to the CAM family produced by basiomycete fungi. Through the use of isotopically labeled precursors (Mester et al. 1997; Silk et al. 2001) or fluorinated precursors (Silk and Macaulay 2003; Lauritsen and Lunding 1998), the biosynthetic precursors of CAM have been identified. L-phenylalanine is the common precursor, which is metabolized to benzoic acid and subsequent hydroxylation in the *para* position yields 4-hydroxybenzoic acid. This intermediate becomes chlorinated in the *ortho* position(s) of the hydroxyl group and is subsequently methylated to form chlorinated *p*-anisic acids which are reduced by aryl alcohol dehydrogenases to CAM (Field and Wijnberg 2003).

2.6 Biodegradation and Biotransformation of Natural Organohalogens

Natural organohalogens are subject to biodegradation and biotransformation. The anaerobic and aerobic biodegradation of the significant naturally occurring chlorinated methanes, chloromethane, and chloroform have been reviewed elsewhere (Field and Sierra-Alvarez 2004; Harper 2000). A review of aerobic and anaerobic biodegradation of the ubiquitous and naturally occurring TCAA and DCAA

has also been provided earlier (Field and Sierra-Alvarez 2004). Interestingly, TCAA is an electron acceptor for the organohalide-respiring bacterium, *Geobacter* (formerly *Trichlorobacter*) *thiogenes* (De Wever et al. 2000).

The anaerobic biotransformation of CAM to chlorophenols by anaerobic mixed cultures has been documented. The CAM 3,5-dichloro-*p*-anisyl alcohol was transformed progressively by demethylation and oxidation of the benzylic carbon leading to accumulation of 3,5-dichloro-*p*-hydroxybenzoic acid, which in turn became decarboxylated to yield 2,6-dichlorophenol (Verhagen et al. 1998a). During the biotransformation, there is a chemical coupling reaction of 3,5-dichloro-*p*-hydroxybenzyl alcohol to yield a tetrachlorinated dimer, bis(3,5-dichloro-4-hydroxyphenyl)methane (Verhagen et al. 1998a).

The anaerobic biotransformation of CHME has also been documented. An anaerobic enrichment culture was responsible for demethylating DAME and DA yielding tetrachloro-1,4-hydquinone, this intermediate was in turn progressively dechlorinated yielding 1,4-hydroquinone (Milliken et al. 2004b). Organohalide respiring bacterial isolates of *Desulfitobacterium*, initially dechlorinated DA to 3′,5-dichloro-4-methoxyphenol. Subsequently that intermediate was demethylated to 2′,6-dichloro-1′,4-hydroquinone and the desulfitobacterium strains subsequently continued to dechlorinate the molecule, ultimately yielding 1′,4-hydroquinone (Milliken et al. 2004b). *Desulfitobacterium* sp. strain PCE1 and a microbial consortium from Baltimore Harbor sediments were also discovered that demethylate, reductively dehydroxylate, and dechlorinate DA to chlorophenols (Milliken et al. 2004a). The main chlorophenol congener that accumulated was 2,3,5,6-tetrachlorphenol and to a lesser extent 2,3,5-trichlorophenol accumulated as well.

Under aerobic conditions, the CAM, was readily mineralized to inorganic chloride and CO_2 by *Burkholderia* spp. bacterial soil isolates from an oak forest soil. The organic chlorine and carbon in 3,5-dichloro-*p*-anisyl alcohol supplied at 350 mg L^{-1} were converted to Cl^- (100 % recovery) and CO_2 (60 % recovery), respectively, within 20 days (Field and Wijnberg 2003).

Chlorinated xanthones, such as 2,7-dichloroxanthone and 5,7-dichloro-1,3-dihydroxylxanthone, were dechlorinated by soil microbial cultures in anaerobic microcosms (Krzmarzick et al. 2014). The chlorinated xanthones were dechlorinated by two of the soil samples tested with the concomitant formation of the nonchlorinated daughter products, xanthone and 1,3-dihydroxylxanthone, respectively. Bacteria belonging to the *Firmicutes* "Gopher group" were enriched in the dechlorinating cultures compared to control cultures. The "Gopher group" is a group of *Firmicutes* related to the known organohalide-respiring genera *Dehalobacter* and *Desulfitobacterium*.

Another study by the same research group found widespread occurrence of *Dehaloccocoides*-like *Chloroflexi* 16S rRNA genes in pristine soil samples (Krzmarzick et al. 2012). The numbers of *Dehaloccocoides*-like *Chloroflexi* 16S rRNA genes were positively correlated with the organochlorine/total organic carbon ratio of soil samples, whereas no such correlation could be found for universal bacterial 16S rRNA genes. Moreover, CPO-synthesized chlorohumus was shown to be reductively dechlorinated by soil microorganisms as evidenced by chloride

release and enrichment of the number of *Dehaloccocoides*-like *Chloroflexi* 16S rRNA genes when the microcosms were incubated anaerobically.

During the decomposition of soil litter, humus forming reactions occur due to the polymerization of phenolic compounds originating from the decay of plants or as secondary metabolites of microorganisms. Chlorinated secondary aromatic metabolites such as CAM and CHME will become copolymerized into the chlorohumus by extracellular phenol oxidizing enzymes (peroxidases or laccases) or mineral oxidants (e.g. MnO_2) (Ruttimann-Johnson and Lamar 1996; Pizzigallo et al. 1995; Lassen et al. 1994). The microbial demethylation of methoxy-groups yielding, chlorinated phenols from CAM and CHME will activate such molecules for the oxidative polymerization reactions catalyzed by enzymes and/or MnO_2. An alternative synthesis of chlorohumus is via the activity of extracellular CPO. The chlorination of aromatic moieties in fulvic acid with CPO has been demonstrated (Niedan et al. 2000). In nature, probably both mechanisms play an important role in the formation of chlorohumus.

2.7 Conclusions

The evidence presented in this chapter indicates an extensive scope of natural organohalogen production in the earth's biosphere with over 5000 natural organohalogen compounds described and an estimated terrestrial storage of several million Gg of organochlorine. The vast majority of the identified organohalogen compounds were shown to be produced as natural metabolites by organisms such as fungi, algae, bacteria, and plants. Likewise abiotic mechanisms are also known. The types of natural organohalogens produced are halogenated methanes, halogenated acetic acids, methyl ethers of chlorophenolic compounds, polychlorinated dibenzodioxins/furans, and polybrominated diphenylethers. In the terrestrial environments, wood and litter-degrading fungi produce large quantities of chlorinated anisyl and chlorinated hydroquinone methyl ether metabolites. In marine environments, polybrominated diphenylethers and chlorinated/brominated bipyrroles of presumed natural origin are consistently detected in the lipids of sea mammals. Diverse enzymatic and abiotic mechanisms are available in nature to account for the halogenation of the natural organohalogen metabolites. The enzymatic mechanisms include: (1) methyl transferases; (2) heme haloperoxidases (3) vandadium haloperoxidases; (4) flavin-dependent halogenases and (5) α-ketoglutarate/Fe(II) dependent halogenases. The natural organohalogen metabolites are subject biotransformation and biodegradation including organisms responsible for halorespiration. Chlorinated anisyl and chlorinated hydroquinone methyl ether metabolites are subject to *O*-demethylation generating chlorinated phenols as biotransformation products that can be oxidatively polymerized into humus forming so called "chlorohumus". Chlorohumus can also be formed by direct reaction of chloride with humus by chloroperoxidases. Chlorohumus may be representative of the species responsible for the large quantities of terrestrial organochlorine in Earth's soil.

References

Abrahamsson K, Ekdahl A, Collen J, Pedersen M (1995) Marine algae—a source of trichloroethylene and perchloroethylene. Limnol Oceanogr 40(7):1321–1326

Ando K, Kato A, Suzuki S (1970) Isolation 2,4-dichorophenol from a soil fungus and its biological significance. Biochem Biophys Res Commun 39(6):1104. doi:10.1016/0006-291x(70)90672-8

Arnoldsson K, Andersson PL, Haglund P (2012) Formation of environmentally relevant brominated dioxins from 2,4,6,-tribromophenol via bromoperoxidase-catalyzed dimerization. Environ Sci Technol 46(13):7239–7244. doi:10.1021/es301255e

Asplund G, Grimvall A, Pettersson C (1989) Naturally produced adsorbable organic halogens (AOX) in humic substances from soil and water. Sci Total Environ 81–2:239–248. doi:10.1016/0048-9697(89)90130-7

Asplund G, Christiansen JV, Grimvall A (1993) A chloroperoxidase-like catalyst in soil—detection and characterization of some properties. Soil Biol Biochem 25(1):41–46. doi:10.1016/0038-0717(93)90239-8

Ballschmiter K (2003) Pattern and sources of naturally produced organohalogens in the marine environment: biogenic formation of organohalogens. Chemosphere 52(2):313–324. doi:10.1016/s0045-6535(03)00211-x

Bastviken D, Thomsen F, Svensson T, Karlsson S, Sanden P, Shaw G, Matucha M, Oberg G (2007) Chloride retention in forest soil by microbial uptake and by natural chlorination of organic matter. Geochim Cosmochim Acta 71(13):3182–3192. doi:10.1016/j.gca.2007.04.028

Biester H, Keppler F, Putschew A, Martinez-Cortizas A, Petri M (2004) Halogen retention, organohalogens, and the role of organic matter decomposition on halogen enrichment in two Chilean peat bogs. Environ Sci Technol 38(7):1984–1991. doi:10.1021/es0348492

Breider F, Hunkeler D (2014a) Investigating chloroperoxidase-catalyzed formation of chloroform from humic substances using stable chlorine isotope analysis. Environ Sci Technol 48(3):1592–1600. doi:10.1021/es403879e

Breider F, Hunkeler D (2014b) Mechanistic insights into the formation of chloroform from natural organic matter using stable carbon isotope analysis. Geochim Cosmochim Acta 125:85–95. doi:10.1016/j.gca.2013.09.028

Butler A, Sandy M (2009) Mechanistic considerations of halogenating enzymes. Nature 460(7257):848–854. doi:10.1038/nature08303

Cabrita MT, Vale C, Rauter AP (2010) Halogenated compounds from marine algae. Mar Drug 8(8):2301–2317. doi:10.3390/md8082301

Chen XP, van Pee KH (2008) Catalytic mechanisms, basic roles, and biotechnological and environmental significance of halogenating enzymes. Acta Biochim Biophys Sin 40(3):183–193. doi:10.1111/j.1745-7270.2008.00390.x

Collen J, Ekdahl A, Abrahamsson K, Pedersen M (1994) The involvement of hydrogen-peroxide in the production of volatile halogenated compounds by *Meristiella gelidium*. Phytochemistry 36(5):1197–1202. doi:10.1016/s0031-9422(00)89637-5

Daferner M, Anke T, Hellwig V, Steglich W, Sterner O (1998) Strobilurin M, tetrachloropyrocatechol and tetrachloropyrocatechol methyl ether: new antibiotics from a Mycena species. J Antibiot 51(9):816–822

Dahlman O, Morck R, Ljungquist P, Reimann A, Johansson C, Boren H, Grimvall A (1993) Chlorinated structural elements in high-molecular-weight organic-matter from unpolluted waters and bleached-kraft mill effluents. Environ Sci Technol 27(8):1616–1620. doi:10.1021/es00045a018

De Wever H, Cole JR, Fettig MR, Hogan DA, Tiedje JM (2000) Reductive dehalogenation of trichloroacetic acid by Trichlorobacter thiogenes gen. nov., sp nov. Appl Environ Microbiol 66(6):2297–2301. doi:10.1128/aem.66.6.2297-2301.2000

de Jong E, Field JA (1997) Sulfur tuft and turkey tail: biosynthesis and biodegradation of organohalogens by basidiomycetes. Annu Rev Microbiol 51:375–414. doi:10.1146/annurev.micro.51.1.375

de Jong E, Field JA, Spinnler HE, Wijnberg J, Debont JAM (1994) Significant biogenesis of chlorinated aromatics by fungi in natural environments. Appl Environ Microbiol 60(1):264–270

Fahimi IJ, Keppler F, Schöler HF (2003) Formation of chloroacetic acids from soil, humic acid and phenolic moieties. Chemosphere 52(2):513–520. doi:10.1016/s0045-6535(03)00212-1

Field JA, Sierra-Alvarez R (2004) Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds. Rev Environ Sci Bio/Technol 3(3):185–254. doi:10.1007/s11157-004-4733-8

Field JA, Wijnberg JBPA (2003) An update on organohalogen metabolites produced by basidiomycetes. In: Gribble G (ed) Natural production of organohalogen compounds, vol 3. Springer, Berlin, pp 103–119

Field JA, Verhagen FJM, de Jong E (1995) Natural organohalogen production by basidiomycetes. Trends Biotechnol 13(11):451–456. doi:10.1016/s0167-7799(00)89001-0

Flodin C, Johansson E, Boren H, Grimvall A, Dahlman O, Morck R (1997) Chlorinated structures in high molecular weight organic matter isolated from fresh and decaying plant material and soil. Environ Sci Technol 31(9):2464–2468. doi:10.1021/es960374l

Frank H (1988) Trichloressigsaure im Boden: eine Ursache neuartiger Waldschaden. Nachr Chem Tech Lab 36:889

Frankland J, Hedger JN, Swift MJ (2009) Decomposer basidiomycetes: their biology and ecology. Cambridge University Press, Cambridge

Garvie LAJ, Wilkens B, Groy TL, Glaeser JA (2015) Substantial production of drosophilin A methyl ether (tetrachloro-1,4-dimethoxybenzene) by the lignicolous basidiomycete *Phellinus badius* in the heartwood of mesquite (*Prosopis juliflora*) trees. Naturwissenschaften 102(3–4):18

Green NJL, Jones JL, Johnston AE, Jones KC (2001) Further evidence for the existence of PCDD/Fs in the environment prior as 1900. Environ Sci Technol 35(10):1974–1981. doi:10.1021/es0002161

Green NJL, Hassanin A, Johnston AE, Jones KC (2004) Observations on historical, contemporary, and natural PCDD/Fs. Environ Sci Technol 38(3):715–723. doi:10.1021/es034599p

Gribble GW (1992) Naturally occurring organohalogen compounds–a survey. J Nat Prod 55(10):1353–1395. doi:10.1021/np50088a001

Gribble GW (1996) Naturally occurring organohalogen compounds–a comprehensive survey. Prog Chem Org Nat Prod 68:1–423

Gribble G (2003a) The diversity of naturally produced organohalogens. In: Gribble G (ed) Natural production of organohalogen compounds, vol 3. Springer, Berlin, pp 1–15

Gribble GW (2003b) The diversity of naturally produced organohalogens. Chemosphere 52(2):289–297. doi:10.1016/s0045-6535(03)00207-8

Gribble GW (2004a) Amazing organohalogens. Am Sci 92:342–349

Gribble GW (2004b) Natural organohalogens. Science Dossier 6. Euro Chlor, Brussels

Gribble GW (2010) Naturally occurring organohalogen compounds—a comprehensive update. Progress in the chemistry of organic natural products, vol 91, pp 1–613. doi:10.1007/978-3-211-99323-1_1

Gribble GW (2012) Recently discovered naturally occurring heterocyclic organohalogen compounds. Heterocycles 84(1):157–207. doi:10.3987/rev-11-sr(p)5

Gron C, Laturnus F, Jacobsen OS (2012) Reliable test methods for the determination of a natural production of chloroform in soils. Environ Monit Assess 184(3):1231–1241. doi:10.1007/s10661-011-2035-5

Gu C, Liu C, Ding YJ, Li H, Teppen BJ, Johnston CT, Boyd SA (2011) Clay mediated route to natural formation of polychlorodibenzo-p-dioxins. Environ Sci Technol 45(8):3445–3451. doi:10.1021/es104225d

Gustavsson M, Karlsson S, Oberg G, Sanden P, Svensson T, Valinia S, Thiry Y, Bastviken D (2012) Organic matter chlorination rates in different boreal soils: the role of soil organic matter content. Environ Sci Technol 46(3):1504–1510. doi:10.1021/es203191r