REVIEW

Chloromethane production by wood-rotting fungi and an estimate of the global flux to the atmosphere

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The production of chloromethane (CH_3Cl) by wood rotting fungi of the Hymenochaetaceae is discussed with particular emphasis on emissions by species of *Phellinus* and *Inonotus*. Recent work on the metabolic role of CH_3Cl as a methyl donor in the biosynthesis of secondary metabolites both in the Hymenochaetaceae and other families of white-rot fungi is reviewed. The parameters affecting the fungal emissions of CH_3Cl in forest ecosystems are considered and where possible quantified. The annual global input to the atmosphere from this source is provisionally estimated at 160000 t of which 75% is released from tropical and subtropical forests and 86% is attributable to *Phellinus*. The possible impact of the contribution from fungi and other biological sources on the atmospheric CH_3Cl burden and stratospheric ozone depletion is assessed.

Until recently organohalogen compounds were considered to be almost entirely anthropogenic in origin. It has become increasingly apparent in the last ten years however, that they are extensively biosynthesized in both marine and terrestrial environments (Asplund & Grimvall, 1991; Gribble, 1996). Within the fungal kingdom organohalogen compounds are widely produced by the ecologically important Basidiomycota. Members of this group form close mycorrhizal relationships with arboreal plants and orchids and many are capable of rotting leaf litter, woody debris and humic materials; some are plant pathogens e.g. *Armillaria mellea* (Vahl: Fr.) Staude. It has long been accepted that basidiomycetes involved in rotting plant debris play a vital role in the decomposition of lignocellulose, a complex of compounds which constitutes the major portion of the total carbon fixed by photosynthesis and makes up most of the terrestrial biomass (Fung, 1993). Considerable progress has been made in elucidating the physiology and biochemistry of this important degradative process. By contrast our knowledge of the production of organohalogens by basidiomycetes and the role of such compounds in fungal metabolism is extremely limited despite the environmental significance of the area.

Halometabolites biosynthesized by basidiomycetes fall, in general, into two classes, halogenated aromatic compounds and halomethanes, although a few miscellaneous chlorinated aliphatic compounds are also formed. A broad range of haloaromatic metabolities have been identified with over 40 different compounds recorded from 26 genera of basidiomycetes (Field, Verhagen & de Jong, 1995; Swarts *et al.*, 1997). Most are simple *O*-methyl derivatives of chlorophenolic compounds. Thus chlorinated anisyl metabolites are synthesized by wood rotting fungi of both bracket (e.g. *Bjerkandera*) and agaric (e.g. *Hypholoma*, *Mycena*, *Pleurotus*) genera and also by genera involved in the decay of litter (e.g. *Lepista*, *Psilocybe*, *Stropharia*). Various *O*-methyl chlorinated hydroquinone derivatives are formed by several soil-inhabiting agarics (e.g. *Agaricus*, *Coprinus*), wood-rotters (e.g. *Phellinus*) and a single ectomycorrhizal fungus (*Russula subnigricans* Hongo). In addition a dozen genera produce a variety of rather more complex chloroaromatic compounds, nearly all of them specific to a single genus. Thus red chlorinated anthraquinone pigments are present in *Dermocybe* spp. (≡ *Cortinarius* subgenus *Dermocybe*) and mycenon, an unusual chlorinated benzoquinone with an acetylenic side chain is found in *Mycena* spp. Most of these compounds are believed to be derived from the simpler chlorinated metabolites by oxidative coupling.

Surprisingly large amounts of chloroaromatics can be excreted by basidiomycetes during growth both in the laboratory and their natural habitat. Chlorinated anisyl compounds are observed at concentrations ranging from 0·5–47 mg l^{-1} in laboratory cultures and at 15–75 mg kg^{-1} in wood or litter colonized by such fungi in forest environments (de Jong *et al.*, 1994; Field *et al.*, 1995; Hjelm, 1996; Hjelm, Borén & Asplund, 1996). Chlorinated hydroquinone metabolites occur at very high levels ranging from 74 to 2400 mg kg^{-1} in basidiomes of both agarics and bracket fungi. Indeed the maximum permissible limits set by several countries for chlorophenols in soil and industrial effluent are in the range 1–10 mg kg^{-1}, significantly less than might be expected to

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occur naturally as a result of fungal activities in forest environments.

Various physiological functions have been attributed to chloroaromatic compounds produced by basidiomycetes. Many have strong antibiotic action, giving the producing fungus a competitive advantage over rival micro-organisms and fungi. Interestingly, chlorinated anisyl alcohols have an important metabolic role in the ligninolytic system of various white rot fungi where they act as recyclable substrates for extracellular aryl alcohol oxidases, important enzymic components of the lignin degrading system which not only generate H_2O_2 but reduce quinones and phenoxy radicals preventing the repolymerization of the immediate products of lignin degradation (de Jong *et al*., 1994).

The other principal group of halogenated compounds synthesized by basidiomycetes is formed by the halomethanes which have received even less attention than the chloroaromatic compounds until quite recently. Chloromethane (CH_3Cl) is by far the most widely occurring compound in this category and is possibly the only halomethane released by basidiomycetes in significant quantities under natural conditions. The fungal production, metabolic role and environmental significance of CH_3Cl and related halomethanes are the subjects of this review.

FUNGAL PRODUCTION OF HALOMETHANES

Hutchinson (1971) first reported the presence of CH_3Cl in the headspace above cultures of several species placed in *Fomes*, a widespread group of bracket fungi, although no quantitative measurements were made at that time. In a later survey Cowan *et al*. (1973) identified CH_3Cl above cultures of six of 32 *Fomes* species examined. Trace quantities of CH_3Cl have also been detected in compost after commercial cultivation of *Agaricus bisporus* (J. E. Lange) Pilát (Turner *et al*., 1975). In the presence of the appropriate halide ions in the culture medium other halomethanes, such as bromomethane (CH_3Br) and iodomethane (CH_3I), can also be formed by *Fomes* (*Phellinus*) spp. (Hutchinson, 1971; Harper & Kennedy 1986), and Spinnler *et al*. (1994) have even reported the identification of polyhalogenated methanes such as chloroiodomethane, diiodomethane and dichloroiodomethane in cultures of *Bjerkandera adusta*. (Willd.: Fr.) P. Karst. Given the relative proportions of Br^- and I^- to Cl^- in the terrestrial environment, however, it seems unlikely that significant quantities of bromine- and iodine-containing halomethanes are released in nature by fungal activities on land.

As CH_3Cl (b.p. — 24 °C) exists as a gas under normal fungal growth conditions, quantitative measurement of CH_3Cl release by cultures during aerobic growth poses certain technical problems. Nevertheless, Harper (1985) succeeded in adapting techniques devised for the determination of volatile bacterial metabolites to quantification of CH_3Cl release by *Phellinus tuberculosus* (syn. *Fomes pomaceus* (Pers.: Gray) Bigeard & H. Guill.), a white rot typically found on trees of the Rosaceae, especially *Prunus*. With glucose as main carbon source the pattern of CH_3Cl release was characteristic of secondary metabolite production with biosynthesis apparently restricted to the period after exponential growth. Uptake of Cl^- from the medium closely paralleled CH_3Cl release. CH_3Cl yield, i.e. CH_3Cl release expressed as a percentage of Cl^- originally present in the medium, varied from $> 90\%$ at Cl^- concentrations less than 4 mM to 20% at 50 mM. During growth on more natural cellulosic substrates, however, CH_3Cl yields remained uniformly very high over the whole of the concentration range tested though production extended over a longer period of time (Harper & Kennedy, 1986). Thus, CH_3Cl release continued over 4 wk with cotton wool, 8 wk with filter paper and 6 mo with sawdust of *Prunus domestica* L. as growth substrates but CH_3Cl yields could exceed 90% at 50 mM Cl^-. When Cl^- was replaced by Br^- or I^- in the culture medium, CH_3Br or CH_3I were formed. Methylation of Br^- was almost as efficient as that of Cl^- but a sharp decline in I^- methylation was recorded at concentrations greater than 1 mM probably attributable to the toxicity of the ion. When equimolar concentrations of the three halide ions were present in the medium I^- was the preferred substrate for the methylation system. As incubation proceeded, however, Br^- and Cl^- were successively methylated as the concentration of first I^- and then Br^- decreased on conversion to halomethane (Harper & Kennedy, 1986).

DISTRIBUTION OF CH_3Cl BIOSYNTHESIS

Although Harper & Kennedy (1986) using gas chromatography/mass spectrometry confirmed release of CH_3Cl by only four of the six species reported by Cowan *et al*. (1973) as producing the compound, a subsequent comprehensive survey of the distribution of the trait amongst 90 species of polypore by Harper, Kennedy & Hamilton (1988) indicated that the ability to convert Cl^- to CH_3Cl is widespread in the Hymenochaetaceae. Of 60 species examined from six genera in this family 33 (55%) released CH_3Cl during growth in the presence of Cl^-. Biosynthesis was particularly well established in *Phellinus* and *Inonotus*, widely distributed genera characterized by bracket-like perennial basidiomes on trees of temperate and tropical origin. Of 38 species of *Phellinus* investigated 24 (63%) exhibited CH_3Cl release on at least one of the three media employed in the survey whilst of the 13 species of *Inonotus* screened nine (69%) were capable of CH_3Cl biosynthesis. The extent of conversion of Cl^- to CH_3Cl during fungal growth was dependent on the species and the growth substrate, biosynthesis being particularly favoured by cellulose-based media. Amongst CH_3Cl-releasing species examined 60% were capable of converting more than 10% of Cl^- present in the medium to CH_3Cl and with many species yields were much higher (Tables 1 and 2). Yields of CH_3Cl from *Inonotus* spp. were in general substantially less than those from *Phellinus* spp. Harper *et al*. (1988) also screened all *Phellinus* and *Inonotus* species investigated for the presence of methyl esters of benzoic, salicylic and furoic acids (see section on 'Metabolic role of CH_3Cl'). About half of the CH_3Cl-releasing species of *Phellinus* were capable of methyl benzoate production and many of these species also biosynthesized methyl esters of salicylic and 2-furoic acids. Ester production was neither observed in any *Phellinus* species lacking the CH_3Cl biosynthesis trait nor in any *Inonotus* sp. The taxonomic

Table 1. Chloromethane production by *Phellinus* species and the volatile aromatic methyl esters identified (modified from Harper *et al.*, 1988)

	Culture collection accession number	Host tree species or genus from which culture isolated	Cl^- converted to CH_3Cl (%) Glucose/mycol. peptone/ agarose 9·5 mM Cl^-	Malt extract/ agarose 9·5 mM Cl^-	Filter paper/mycol. peptone 10 mM Cl^-	Methyl esters of aromatic acids present at concn. > 10 ng $vial^{-1}$
P. badius (Berk.) G. Cunn.	CBS 449.76	*Acacia catechu*	0·8	5·1	0	None
P. caryophylli (Racib.) G. Cunn.	CBS 448.76	*Shorea robusta*	0	0	0	None
P. chrysoloma (Fr.) Donk	CBS 180.24	*Picea*†	0	10	15	None
P. cinchonensis (Murrill) Ryvarden	CBS 447.76	*Quercus dilatata*	0	0	0	None
P. conchatus (Pers.: Fr.) Quél.	NCWRF-FPRL 142	*Fraxinus americana*	0	0	0	None
P. everhartii (Ellis & Galloway) A. Ames	CBS 176.34	*Quercus*†	0	0	0	None
P. fastuosus (Lév.) Ryvarden	CBS 213.36	*Gliricidia sepium*	0	0	0	None
P. ferreus (Pers.) Bourdot & Galzin	CBS 444.48	*Pseudotsuga menziesii*	0	0	0	None
P. ferrugineo-velutinus (Henn.) Ryvarden	CBS 218.48	—	11	20	16	Not examined
P. ferruginosus (Schrad.: Fr.) Pat.	CBS 168.29	*Quercus*	0	0	0	None
P. gilvus (Schwein.: Fr.) Patr.	CBS 387.54	*Quercus*†	0	0	0	None
P. hartigii (Allesch. & Schnabl) Pat.	CBS 382.72	*Abies sachalinensis*	0	0	0	None
P. hippophāecola H. Jahn	CBS 168.31	*Hippophāe rhamnoides*	0	1·5	1·9	Benzoate, salicylate, 2-furoate, 3-furoate
P. igniarius (L.: Fr.) Quél.	CBS 381.72	*Pyrus malus*	16	39	63	Benzoate, salicylate, 2-furoate, cinnamate
P. laevigatus (Fr.) Bourdot & Galzin	CBS 256.30	*Betula verrucosa*	0·2	17	1·0	Benzoate
P. lamaensis (Murrill) Pat.	NCWRF-FPRL 383	*Hevea*	0	0	0*	None
P. linteus (Berk. & M. A. Curtis) Teng	CBS 454.76	*Corylus colurna*	0·5	0·9	0*	None
P. lundellii Niemelä	CBS 540.72	*Betula*	55	54	27	Benzoate, salicylate, 2-furoate
P. nigricans (Fr.: Fr.) P. Karst.	CBS 213.48	*Alnus*†	0·3	9·2	0·3	Benzoate, salicylate
P. nigrolimitatus (Romell) Bourdot & Galzin	CBS 599.82	*Picea*†	1·6	0·8	1·2	None
P. noxius (Corner) G. Cunn.	CBS 170.32	*Elaeis guineensis*	0	0	0	None
P. occidentalis (Overh.) Gilb. & Lombard	CBS 196.55	*Crataegus douglasii*	43	29	79	None
P. pachyphloeus (Pat.) Pat.	CBS 446.76	*Mangifera indica*	42	3·8	18	None
P. pectinatus (Klotzsch) Quél.	CBS 445.76	*Murraya*	0	0	0	None
P. pini (Thore.: Fr.) Pilát	CBS 210.36	*Tsuga heterophylla*	4·9	70	47	None
P. populicola Niemelä	CBS 638.75	*Populus tremula*	6·0	43	22	Benzoate
P. pseudolaevigatus Parmasto	CBS 351.80	*Betula manshurica*	2·2	0	3·2	Benzoate, salicylate
P. punctatus (Fr.) Pilát	CBS 301.33	*Salix*†	0	0	0	None
P. rhabarberinus (Berk.) G. Cunn.	CBS 282.77	*Rhus wallichii*†	3·0	6·1	12	None
P. ribis Karst.	NCWRF-FPRL 42	*Crataegus*	21	50	82	Benzoate, salicylate, 2-furoate
P. robiniae (Murrill) A. Ames	NCWRF-FPRL 180	*Robinia*	0·3	24	0	Benzoate, salicylate
P. robustus (P. Karst.) Bourdot & Galzin	CBS 175.34	*Quercus*†	9·8	14	4·7	None
P. senex (Nees & Mont.) Imazeki	CBS 442.76	*Cedrus toona*	0	0·2	0*	None
P. spiculosus (W. A. Campb. & R. W. Davidson) Niemelä	CBS 345.63	*Carya ovata*	0·1	6·9	1·2	None
P. tremulae (Bondartsev) Bondartsev & Borissov	CBS 123.40	*Populus*†	0·4	6·9	0	Benzoate, salicylate
P. trivialis Bres.	CBS 512.63	*Salix caprea*	22	19	47	Benzoate, salicylate
P. tuberculosus (Baumg.) Niemelä	NCWRF-FPRL 33A	*Prunus*	57	46	82	Benzoate, salicylate, 2-furoate
P. viticola (Schwein. apud Fr.) Donk	CBS 214.36	*Picea*†	0	0	0*	None
P. weirii (Murrill) Gilb.	CBS 163.40	*Thuja plicata*	0	0	0	None

* Little or no growth on medium.

† If host from which culture isolated not recorded, typical host genus or species given where known.

implications and biochemical significance of these observations are considered later in this review.

CH_3Cl production was reported by Harper *et al.* (1988) in four out of 13 hymenochaetaceous species examined not belonging to either *Phellinus* or *Inonotus*, but subsequent taxonomic reassignment of several of the isolates has led to a revision of the frequency of occurrence of the trait in this group to one out of eight species investigated. The sole CH_3Cl-producing species in this category was *Hymenochaete corrugata* which released significant quantities of CH_3Cl on all three media on which it was tested.

To assess whether the CH_3Cl biosynthesis trait existed in polypores outside the Hymenochaetaceae, Harper *et al.* (1988) screened 30 representative species of two other major families of poroid fungi i.e. the Ganodermataceae and the Polyporaceae (Table 3). Only one, *Fomitopsis cystisina* (Berk.) Bondartsev & Singer (≡ *Polyporus fraxineus* Bull.: Fr., now classified as *Perenniporia fraxinea*) placed in Group 8 of the Polyporaceae in Table 3, showed significant production of CH_3Cl on all three media on which it was tested, converting as much as 10% of available Cl^- to CH_3Cl on cellulose-based medium. The release of small quantities of CH_3Cl was also detected when *Phaeolus schweinitzii* (Group 4) was cultured on cellulose-based medium but the overall conversion of Cl^- to CH_3Cl of 0·4% was very low.

TAXONOMIC IMPLICATIONS

The Hymenochaetaceae is a family which has achieved acceptance only recently and contains both poroid and corticoid/stereoid members paralleling the current reinterpretation of many other families of non-agaricoid basidiomycetes. Fifty years ago classification based on such features would not have been envisaged, being firmly grounded at that time on the configuration of the hymenium. Traditionally the poroid forms were placed in the Polyporaceae, the resupinate, non-poroid forms in the Corticiaceae and considered quite distantly related. Thus, many of the traditional groups within the polypores (bracket fungi) are often less closely related to each other than they are to other fungi with different spore-bearing surfaces (Watling, 1996). A more natural classification involves grouping together all those fungi which exhibit a dark brown,

Table 2. Chloromethane production by poroid genera of the Hymenochaetaceae excluding *Phellinus* (modified from Harper *et al.* (1988)

	Culture collecton accession number	Host species or genus from which culture isolated	Cl^- converted to CH_3Cl (%)		
			Glucose/mycol. peptone/ agarose 9·5 mM Cl^-	Malt extract/ agarose 9·5 mM Cl^-	Filter paper/mycol. peptone 10 mM Cl^-
Coltricia perennis (L.: Fr.) Murrill	CBS 372.52	Soil inhabiting†	0	0	0*
C. vaellata (Berk.) Teng	CSB 323.29	Old wood (unidentified)†	0	0	0
Coltriciella dependens (Berk. & M. A. Curtis) Ryvarden	CBS 247.50	Various deciduous spp.†	0	0	0
Cyclomyces tabacinus (Mont.) P. Karst.	CBS 311.39	*Shorea robusta*	0	0	0
Hymenochaete corrugata (Fr.: Fr) Lév.	CBS 133.40	*Corylus*†	1·3	3·4	6·4
H. cruenta (Pers.: Fr.) Donk	CBS 596.87	*Abies*†	0	0	0*
H. rubiginosa (Dicks.: Fr.) Lév.	CBS 237.39	*Quercus*†	0	0	0*
H. tabacina (Sowerby: Fr.) Lév.	CBS 134.40	*Pinus/Abies*†	0	0	0
Inonotus andersonii (Ellis & Everh.) Černý	CBS 312.29	*Quercus*	18	18	8
I. cuticularis (Bull.: Fr.) P. Karst.	CBS 445.50	*Fagus sylvatica*	0	1·7	4·2
I. dryadeus (Pers.: Fr.) Murrill	CBS 948.70	*Quercus*	0	0	0
I. glomeratus (Peck) Murrill	CBS 359.34	*Acer*†	0	14	0
I. hispidus (Bull.: Fr.) P. Karst.	CBS 386.61	*Fraxinus*†	3	43	11
I. leporinus (Fr.) Gilb. & Ryvarden	CBS 246.30	*Pinus*†	12	2·3	22‡
I. nothofagi G. Cunn.	CBS 476.72	*Nothofagus*†	1	0	0*
I. obliquus (Pers.: Fr.) Pilát	CBS 314.39	*Betula*†	0	0	18
I. patouillardii (Rick) Imazeki	CBS 364.34	*Quercus gilva*	0	0	0
I. porrectus Murrill	CBS 296.56	*Gleditschia triacanthos*	0	0	0
I. radiatus (Sowerby: Fr.) P. Karst.	CBS 578.81	*Alnus*	0	3	0*
I. rheades (Pers.) Bondartsev & Singer	CBS 127.71	*Populus alba*	15	1·1	8·3
I. tamaricis (Pat.) Maire apud Pilát	CBS 288.33	*Tamarix*	0	0	0

* Little or no growth on medium.

† If host from which culture isolated not recorded, typical host genus given where known (Breitenbach & Kränzlin, 1986; Teng, 1996).

‡ A second collection CBS 420.48 grew slowly and produced no CH_3Cl indicating variation within a taxon or misidentification.

almost black reaction with aqueous solutions of potash, have thick-walled brown (= xanthochroic) hyphae often tapered at the ends and possess special end-cells called setae or, if star-like, asterosetae.

Patouillard (1900) was the first to seriously consider the natural affinities of these fungi which were subsequently termed the Xanthochroic Series. The Hymenochaetales were delineated by Jülich (1981) but it was thirty-three years earlier (Donk, 1948) that the Hymenochaetaceae was erected and the various elements therein delimited. It was left to Corner (1991) to explore the biology of many of its members, although previous classical authors recognized the significance of certain macrocharacters. Using a combination of macro and microcharacters many new genera of poroid fungi were proposed at the turn of the century by several authors including genera herein considered members of the Hymenochaetaceae. Ryvarden (1991) has recently reviewed these genera considering some of those created by Murrill to be based on very small variations, although some of Murrill's groupings in the xanthochroic polypores have been upheld by Fiasson & Niemelä (1984).

Although all the relationships within the poroid and related basidomycetes have not been delineated, a relatively stable state has been achieved within the last 25 years. Thus the Ganodemataceae (Ganodermatales) with their distinctly channelled basidiospores, the Coriolaceae and Polyporaceae (Polyporales) and the Hymenochaetaceae (Hymenochaetales) are now well defined and can be easily differentiated from each other. There are also a few taxa with unique combinations of characters which find their natural affinities closer to other groups, perhaps hydnaceous or corticiaceous forms, e.g. *Boletopsis* in Thelephoracae (Thelephorales). In parallel to the polypores the resupinate fungi with smooth or irregular hymenial surfaces are now placed in several distinct families, or even orders (Jülich, 1981) although many authorities still resist this radical approach. Nevertheless, there are some distinctive groups, e.g. Stereaceae (Stereales) with generally pileate, thickened basidiomes and a hymenochaetaceous element which is easily identifiable and is, as indicated above, assigned to the Hymenochaetaceae.

Ryvarden (1995) defines the Hymenochaetaceae as possessing the following characteristics: (i) hyphal wall brown giving basidiome a black reaction with KOH (xanthochroic); (ii) simple septate hyphae; (iii) equipped usually with setal organs. The family as currently accepted contains 23 genera and nearly 450 species with basidiomes ranging from flattened (resupinate) structures to pileate and clavate forms. The Hymenochaetaceae joins another small family, Asterostromataceae in the Hymenochaetales (Hawksworth *et al.*, 1995). All species of Hymenochaetaceae are considered to be white rot fungi, the family comprising a large proportion of the *ca* 1600 species of wood-rotting fungi described (Taylor & Taylor, 1997). Ryvarden (1995) considers the Hymenochaetaceae a rather young family which possess a common basic set of characteristics allowing it to be easily recognized even in the field. Evolution is postulated to have occurred from more simple resupinate, smooth basidiomes as in the cosmopolitan *Hymenochaete* to the more complicated hydnoid partly reflexed

Table 3. Poroid fungi excluding the Hymenochaetaceae screened for chloromethane production. Grouping according to Ryvarden (1991)

Polyporaceae	
Group 1	
Polyporus squamosus (Huds.: Fr.) Fr.	CBS 426.48
Group 2	
Coriolopsis polyzona (Pers.) Ryvarden	IMI 79126
Datronia scutellata (Schwein.) Domański	CBS 459.66
Lenzites betulina (L.: Fr.) Fr.	CBS 245.66
Trametes hirsutus (Wulfen: Fr.) Pilát	CBS 320.29
T. versicolor (L.: Fr.) Pilát	IMI 83026
Group 3	
Antrodia carbonica (Overh.) Ryvarden & Gilb.	CBS 440.48
Daedalea incana (Lév.) Ryvarden	CBS 452.76
D. quercina L.: Fr.	CBS 202.50
Fomitopsis pinicola (Sw.: Fr.) P. Karst.	CBS 313.82
Laricifomes officinialis (Vill.: Fr.) Bondartsev & Singer	NCWRF.FPRL 81A
Group 4	
Laetiporus sulphureus (Bull.: Fr.) Murrill	CBS 343.69
Phaeolus schweinitzii (Fr.: Fr.) Pat.	CBS 326.29
Pycnoporellus alboluteus (Ellis & Everh.) Kotl. & Pouzar	CBS 418.48
P. fulgens (Fr.) Donk	CBS 285.78
Group 5	
Heterobasidion annosum (Fr.: Fr.) Bref.	CBS 834.72
H. insulare (Murrill) Ryvarden	CBS 451.76
Hydnopolyporus fimbriatus (Fr.: Fr.) D. A. Reid	CBS 384.51
Physisporinus sanguinolentus (Alb. & Schwein.: Fr.) Pilát	CBS 193.76
Rigidoporus microporus (Fr.: Fr.) Overeem	CBS 173.33
R. ulmarius (Sowerby.: Fr.) Imazeki	NCWRF.FPRL 241A
Group 6	
Grifolia frondosa (Dicks.: Fr.) Gray	CBS 573.65
Oligoporus placenta (Fr.) Gilb. & Ryvarden	CBS 447.48
Group 8	
Perenniporia clelandii (Lloyd) Ryvarden	CBS 208.36
P. fraxinea (Bull.: Fr.) Ryvarden	NCWRF.FPRL 17A
P. subacida (Peck) Ryvarden	CBS 442.48
Group 9	
Fomes fomentarius (L.: Fr.) Fr.	CBS 249.50
Group 10	
Nigroporus vinosus (Berk.) Murrill	CBS 176.29
Ganodermataceae	
Ganoderma applanatum (Pers.) Pat.	CBS 250.61
G. resinaceum Boud. apud Pat.	CBS 194.76

Bold lettering: CH_3Cl producers.

forms, e.g. *Hydnochaete* from Australia and America, and to the smooth, stipitate *Stipitochaete*, endemic to tropical America.

Another proposed line arose from resupinate and sessile genera such as *Cyclomyces*, *Phylloporia* and *Inonotus* to the dimitic *Phellinus* and stipitate *Coltricia*. *Phellinus* apparently is still in a strong evolutionary phase reflected in the many species-complexes. *Phellinus* contains 220 species and may even be polyphyletic (Hawksworth *et al*., 1995). *Inonotus* is less complex and is probably older, with about 75 species (Ryvarden, 1995). It is regarded as the genus most closely related to *Phellinus*, differentiated only by its monomitic hyphal system and hence its more short-lived and softer basidiomes. The widespread occurrence of the CH_3Cl biosynthesis trait in *Inonotus* confirms the close taxonomic relationship between the two genera. The failure to detect the presence of methyl esters in *Inonotus* species implies that, if ester production is considered an evolutionary advance on CH_3Cl biosynthesis (as seems likely on biochemical grounds; see 'Metabolic role of CH_3Cl'), *Inonotus* must be regarded as more primitive biochemically than *Phellinus*; again consistent with contemporary taxonomic thinking. The observations that the majority of species studied within the Hymenochaetaceae (58%) released CH_3Cl and that only two species examined outside the family released CH_3Cl indicate that the trait may well be a useful diagnostic character in the circumscription of the family. Similarly the ability to produce methyl esters of aromatic acids as secondary metabolites may be of value in defining the genus *Phellinus*.

Ryvarden (1978) considered *Phellinus ribis* to belong in *Phylloporia* as it displays all the attributes, *viz*. yellowish thick-walled basidiospores, fundamental monomitic hyphal system with only a few simple septate generative hyphae and a distinct thick spongy tomentum separated from a dense and darker context by a black line. Members of the genus also have the ability to colonize leaves and small diameter branches and twigs. Ryvarden & Gilbertson (1993), nevertheless, regarded 'it a matter of taste whether to include it [*Phellinus ribis*] in *Phellinus* or not but the consistent lack of setae in all species and abundant yellow spores make the genus homogeneous'. The release of CH_3Cl and also the biosynthesis of methyl esters of several aromatic acids would appear to place the species firmly within *Phellinus*. It is worth noting, however, that *Phylloporia* is also accepted by Fiasson & Niemelä (1984) as one of their several segregate genera from *Phellinus*. Interestingly, within these genera CH_3Cl release is most common and in greatest quantity amongst members of *Ochroporus*, e.g. *Phellinus ignarius* complex. In contrast members of *Fuscoporia* e.g. *Phellinus ferreus*, gave no evidence of CH_3Cl production under the experimental conditions adopted in the studies described herein.

The precise significance of the two non-hymenochaetaceous species found to release CH_3Cl is difficult to evaluate as no other species examined belonging to the groups of the Polyporaceae in which they are placed was able to produce CH_3Cl. One, *Phaeolus schweinitzii* has been assigned by some authors to the Hymenochaetaceae (Fiasson & Niemelä, 1984) although this has not been generally accepted. Indeed in the field its basidiomes uncannily resemble those of some species of *Inonotus* but it produces a brown-rot and possesses gloeocystidia in the hymenium, characters not in keeping with the Hymenochaetaceae as presently defined. Fiasson & Niemelä (1984) have, nevertheless, created a new subfamily for this species therein. Ryvarden (1991) relates *Phaeolus* to *Laetiporus* in the Poriales (Group 4 in Table 3) and Hibbet & Donoghue (1995) using mitochondrial DNA techniques confirm this suggestion. The production of CH_3Cl by *Phaeolus schweinitzii* does, however, lend some support to Fiasson & Niemelä (1984).

The possession of the CH_3Cl release trait in *Polyporus fraxineus* raises even larger questions. This species is now placed in *Perenniporia* as *P. fraxinea* (Ryvarden, 1978) where it joins about two dozen other species of polyporaceous white rot fungi. It is related to *Trametes* (see Table 3) but, because of the thick-walled spores with a truncate apex, it may form a

link to the Ganodermataceae even though in this family the spores are channelled (Jülich, 1981). What is certain, however, is that there is no close relationship to *Phellinus*. The two other species of *Perenniporia* screened for CH_3Cl emission gave negative results. It is possible that, like *Trametes versicolor* (see Biosynthesis and Metabolic Role of CH_3Cl), these and related taxa have the ability to biosynthesize and utilize CH_3Cl as a methyl donor but do not in general release it at any stage of the growth cycle.

BIOSYNTHESIS AND METABOLIC ROLE OF CH_3Cl

The biosynthetic route by which CH_3Cl is formed in *Phellinus* spp. has still not been resolved. Investigations by White (1982) and Harper & Hamilton (1988) on the incorporation of $^2H_3$-labelled precursors into CH_3Cl by cultures of *P. tuberculosus* demonstrate that the halomethane can arise from the *S*-methyl groups of both L- and D-methionine. Whilst several workers (White, 1982; Wuosmaa & Hager, 1990) have speculated that *S*-adenosylmethionine (SAM) may be the immediate metabolic progenitor of CH_3Cl, the presence of biochemically significant quantities of SAM/halide ion methyltransferase in cell-free extracts has not yet been substantiated. Indeed the metabolic role of CH_3Cl in fungal metabolism described below renders such an intermediate highly unlikely (Harper, McRoberts & Kennedy, 1996).

Initial investigations of fungal production of CH_3Cl suggested that the compound was a stable end product of metabolism whose biosynthesis was confined to the late growth and stationary phases of the growth cycle, characteristics typical of a secondary metabolite (Harper, 1985; Harper & Kennedy, 1986). Unexpectedly subsequent work has led to the conclusion that CH_3Cl plays a far more fundamental role in fungal metabolism. As previously mentioned, the majority of *Phellinus* spp. which release CH_3Cl also accumulate methyl esters of several aromatic acids particularly methyl benzoate and methyl furoate as natural products (Harper & Kennedy, 1986; Harper *et al*., 1988). The observation provoked speculation that the formation of these esters was linked biochemically to CH_3Cl biosynthesis as both processes were inhibited by the pseudohalide ion, SCN^- (Harper & Kennedy, 1986) and the patterns of incorporation of the *S*-methyl groups of labelled methionine into methyl benzoate and CH_3Cl during growth of *P. tuberculosus* were remarkably similar (Harper & Hamilton, 1988). Additional evidence of a biochemical relationship was furnished by the discovery that the occurrence of methyl benzoate as a natural product in *Phellinus* is restricted solely to CH_3Cl-producing species (Harper *et al*., 1988).

By incubating washed mycelia of *P. tuberculosus* with appropriately ^2H-labelled precursors Harper *et al*. (1989) established that CH_3Cl was acting as a methyl donor in the methylation of benzoic and furoic acids during the growth phase. The transmethylation system exhibited a broad substrate specificity methylating the carboxyl group of a wide range of aromatic acids. Several aliphatic acids such as butyric acid were also excellent substrates. Bromo- and iodo-methane in addition to CH_3Cl could act as methyl donors but

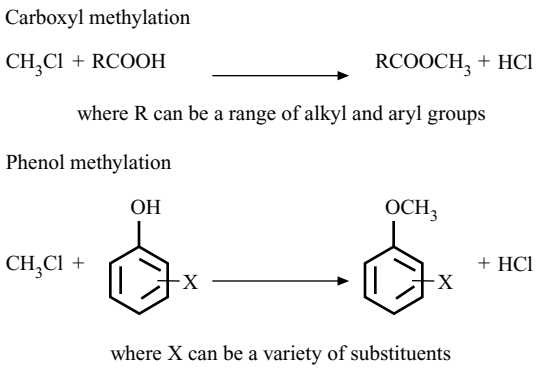

Fig. 1. CH_3Cl-utilizing methylation reactions occurring in *P. tuberculosus* (Harper *et al*., 1989).

chloroethane did not behave as an ethyl donor. A biochemically distinct CH_3Cl-utilizing system capable of methylating phenols and thiophenol was also identified in the fungus. Fig. 1 summarizes the CH_3Cl-utilizing methylation reactions catalysed by intact mycelia of *P. tuberculosus*.

The kinetics of methylation by CH_3Cl were examined in some detail in intact mycelia using C^2H_3Cl and the nature of the relationships found between % C^2H_3-incorporation into ester or anisole and exogenous C^2H_3Cl concentration suggested the existence of a membrane-bound biosynthesis and utilizing complex (McNally, Hamilton & Harper, 1990; McNally & Harper, 1991). A study of the activity of the carboxyl-methylating system during growth in relation to the methyl benzoate accumulation in the culture medium and the incorporation of C^2H_3 groups from exogenous C^2H_3Cl was conducted by Harper *et al*. (1989). The investigation clearly indicated that during the early growth phase CH_3Cl biosynthesis was closely coupled to its utilization in methyl ester biosynthesis in the complex, but that in the late trophophase and early idiophase the system became less tightly channelled allowing leakage of CH_3Cl from the complex and subsequent emission by the fungal culture. Normally a compound released for the first time at this stage of the growth cycle would be regarded as a secondary metabolite. In this instance, emission during the idiophase would seem simply a reflection of the breakdown of the strict coordination of biosynthesis and utilization of the compound which governs its role as a primary metabolite.

An important corollary of this rationale for CH_3Cl release is that, if tight coupling of biosynthesis and utilization is maintained throughout the growth cycle of a species, no emission of CH_3Cl will occur, so raising the possibility that the use of CH_3Cl as a biochemical methyl donor may not be restricted to fungal species which release CH_3Cl. The non-hymenochaetaceous white rot fungus *Phanerochaete chrysosporium* Burds., a species frequently employed as a model organism in studies of lignin degradation, does not release CH_3Cl at any stage of growth but does produce veratryl alcohol (3,4-dimethoxybenzyl alcohol) (Harper *et al*., 1990). Veratryl alcohol is a secondary metabolite which is biosynthesized by many white rot fungi and plays a central role in lignin degradation. The 3- and 4-*O*-methyl groups of this key compound are known to be ultimately derived from methionine. Harper *et al*. (1990) measured the incorporation of

the C^2H_3 group into the *O*-methyl groups of veratryl alcohol isolated from *P. chrysosporium* cultures supplemented with C^2H_3Cl, or L-(*methyl*-$^2H_3$) methionine and showed that CH_3Cl was as effective a precursor as L-methionine. Similar results were obtained with two other white rot fungi producing veratryl alcohol, *Trametes versicolor* (syn. *Coriolus versicolor* (L.: Fr.) Quél) and *Phlebia radiata* Fr. Additional evidence that CH_3Cl is involved in veratryl alcohol biosynthesis was provided by a study of the effect of supplementation of fungal culture medium with CH_3Cl (Harper, Buswell & Kennedy, 1991). In the presence of 0·6 mM CH_3Cl not only was veratryl alcohol biosynthesis induced earlier in the growth cycle but peak concentrations of the idiolyte were significantly increased.

In recent work on *P. chrysosporium* using C^2H_3-labelled precursors efficacies of CH_3Cl and L-methionine as methyl donors in veratryl alcohol biosynthesis have been compared with that of SAM, the conventional biological methylating agent (Harper, McRoberts & Kennedy, 1996). High levels of C^2H_3 incorporation into the *O*-methyl groups were recorded when either labelled L-methionine or C^2H_3Cl were present but no significant labelling was detected when labelled SAM was added, despite the fact that previous experiments had demonstrated that intact mycelia were permeable to the compound. Incorporation of C^2H_3- from C^2H_3Cl was strongly antagonized by unlabelled L-methionine; conversely C^2H_3-incorporation from labelled L-methionine was reduced by CH_3Cl. These results suggest that L-methionine is converted either directly or via an intermediate (which is not SAM) to CH_3Cl which is used as the immediate methyl donor in veratryl alcohol biosynthesis. Notwithstanding these observations methylation of phenolic substrates by *P. chrysosporium* does not appear to be exclusively dependent on CH_3Cl as the organism contains at least two SAM-dependent phenol *O*-methyltransferases (Coulter *et al*., 1993; Jeffers, McRoberts & Harper, 1997). A comparison of the relative utilization of CH_3Cl and SAM in the methylation of acetovanillone by *P. chrysosporium* at different stages of growth was undertaken by Coulter, Hamilton & Harper (1993) by taking advantage of the selective inhibition of SAM-dependent enzyme systems by *S*-adensylhomocysteine (SAH). This study together with later work by Harper, McRoberts & Kennedy (1996) confirmed the existence of two biochemically distinct routes for *O*-methylation:

(i) via SAM-utilizing enzymes which can be inhibited by SAH and are induced early in the growth cycle. Two such enzymes with pH optima of between seven to eight have been purified from cell-free extracts. Their exact metabolic role is unclear but they do not appear to participate in veratryl alcohol biosynthesis.

(ii) via a CH_3Cl-dependent system whose activity is not suppressed by SAH. The enzyme which is at a low level in early growth attains peak activity in mid-growth phase. It has a pH optimum of six but is not detectable in cell-free extracts, signifying that it is either highly labile or membrane-bound. The main metabolic function identified to date for this enzyme or enzyme complex is the methylation of phenolic hydroxyl groups in veratryl alcohol biosynthesis.

The biochemical rationale for the use of CH_3Cl as a methyl donor in veratryl alcohol biosynthesis is not immediately apparent. Further investigations are clearly required to establish whether CH_3Cl plays a role in other methylation reactions in wood-rotting fungi and indeed whether its utilization as a metabolic intermediate is widespread in the fungal kingdom. It is conceivable that CH_3Cl is exploited as a methyl donor in methylation reactions which do not require a methyl donor as highly activated as SAM. Such a strategy may have considerable advantage to an organism producing large amounts of a methylated product with a rapid turnover such as veratryl alcohol since the energy costs associated with the formation of CH_3Cl from methionine or a simple derivative of methionine may be substantially less than those from SAM. It is puzzling that the release of CH_3Cl in significant quantities as a natural product should be almost entirely confined to the Hymenochaetaceae when species of other families of the Poriales (e.g. *Trametes versicolor*) and indeed other families of the basidiomycetes Meruliaceae-Stereales (e.g. *Phanerochaete chrysosporium*) employ CH_3Cl in a similar metabolic role in methyl transfer without detectable release occurring. Emission of CH_3Cl is obviously not an unavoidable consequence of its use as a metabolic intermediate. Presumably release of the halomethane confers some competitive advantage on the organism. Hutchinson (1971) suggested that fungal production of halomethanes, particularly bromomethane, might have an ecological significance in that the compounds have insecticidal activity. It seems unlikely that the rate of production of such compounds would allow the accumulation in nature of concentrations sufficient to permit such a role. Another possibility is that the release of CH_3Cl may be associated with the high methoxyl content of lignin which can be as much as 20% (Chen & Chang, 1985). The diversion of excess one-carbon fragments from this source into volatile CH_3Cl which will rapidly diffuse from the cell may represent a means of overcoming the biochemical handicap likely to be imposed by a very large one-carbon pool in the cell.

ENVIRONMENTAL SIGNIFICANCE OF CH_3Cl

Atmospheric concentrations

The recognition of the role of man-made halogenated gases, in particular the chlorofluorocarbons (CFCs) in catalysing ozone destruction in the upper atmosphere and in enhancing the greenhouse effect in the lower atmosphere, has focussed considerable attention on the identification of natural sources of volatile halocarbons in the atmosphere, and on the assessment of their global fluxes. The gaseous monohalomethanes, in particular CH_3Cl, are the most important of these naturally produced compounds in terms of atmospheric abundance and Table 4 summarizes recent data regarding their environmental concentrations, the generally accepted values for the atmospheric residence time of each and the global inputs to the atmosphere from various natural and, where applicable, anthropogenic sources. It is clear that CH_3Cl is by far the most abundant of these gases, and is overwhelmingly of natural origin, with between 3·5 and 5×10^6 t y^{-1} arising from biological and chemical processes in the marine and terrestrial environments: man-made emissions of 30×10^3 t y^{-1} are negligible in comparison (Edwards, Campbell & Milne,

Table 4. Environmental concentrations and estimated global atmospheric inputs of the naturally-occurring monohalomethanes

	Air concn. (pptv)[a]	Seawater concn. (ng l^{-1})	Atmos. residence time (y)	Global input to atmosphere (10^5 t y^{-1})		References
CH_3Cl	550–630	4–12	1·5	35–50	Natural:	See text
					3–20 Oceanic	
					4–14 Biomass burning	
					0–20 Other Terrestrial	
				0·3	Anthropogenic	
CH_3Br	10	0·4–1·2	0·8	0·8	Natural:	Butler (1995)
					0·3–0·9 Oceanic	Lobert *et al.* (1995)
					0·3 Biomass burning	Mano & Andreae (1994)
				0·6	Anthropogenic:	Shorter *et al.* (1995)
					0·3–0·65 Fumigation	
					0·05–0·22 Vehic. emissions	
CH_3I	2–20	0·6–20	0·02	3–13	Natural (oceanic)	Rasmussen *et al.* (1982)
						Reifenhauser & Heumann (1992)
						Singh, Salas & Stiles (1983)

[a] pptv, parts per 10^{12} by volume.

Table 5. Chlorocarbon emissions to the atmosphere and their contribution to stratospheric chlorine

	Estimated emissions (1990)[a] (10^3 t y^{-1})	Atmospheric concentrations[b] (pptv)[c]	Atmospheric lifetime[b] (y)	Contribution to stratospheric chlorine (%)
Man-made				
$CFCl_3$ (CFC 11)	440	272	50	23
CF_2Cl_2 (CFC 12)	550	532	102	30
$C_2F_3Cl_3$ (CFC 113)	200	84	85	7
CHF_2Cl (HCFC 22)	170	117	13	3
CCl_4	100	103	42	11
CH_3CCl_3	730	109	5	9
Natural				
CH_3Cl	4000	600	1·5	17

[a] Based on data from Prather & Watson (1990).
[b] Montzka *et al.* (1996).
[c] pptv, parts per 10^{12} by volume.

1982; Koppmann *et al.*, 1993). Despite the vast inputs over the last 25 years of long-lived CFCs, naturally produced CH_3Cl is still the most abundant volatile halocarbon in the atmosphere (Table 5), its tropospheric concentration of 600 pptv (parts per 10^{12} by volume) exceeding that of the most significant of the man-made CFCs, CF_2Cl_2, which is present in the atmosphere at 530 pptv (Kaye, Penkett & Ormond, 1994; Montzka *et al.*, 1996). Current atmospheric concentrations of CH_3Cl are such that between 15% and 20% of chlorine-catalysed ozone destruction in the stratosphere is attributable to this naturally-produced halocarbon. In computer models of the atmosphere employed to date in assessing the impact of man-made halocarbons on the ozone layer it is invariably assumed that the rate of natural CH_3Cl production has remained unchanged throughout past millenia at a level similar to that currently observed. Until all the major natural sources of CH_3Cl have, however, been characterized and quantified this premise cannot be regarded as well-founded. If naturally produced CH_3Cl is largely biological in origin it is possible that climatic variation in the past, with the accompanying changes in terrestrial vegetation and marine biota, may have resulted in variations in natural CH_3Cl production of sufficient magnitude to cause fluctuations in ozone levels in the stratosphere comparable to those observed since the introduction of CFCs.

Natural sources of CH_3Cl

A variety of sources both abiotic and biological have been proposed for the production of CH_3Cl in nature, but surprisingly little progress has been made to date in quantifying the fluxes from individual sources. Low temperature combustion of plant material, particularly foliage, results in volatilization as CH_3Cl of a small proportion of the Cl^- normally present and so the halocarbon is released during forest fires and in the course of slash-and-burn agriculture in the tropics (Palmer, 1976; Rasmussen *et al.*, 1980). Crutzen *et al.* (1979), however, have calculated that the release of CH_3Cl by biomass burning is at least an order of magnitude less than its rate of destruction in the atmosphere and Andreae (1991) has estimated that it is unlikely to be responsible for more than 25% of the natural flux. Recent work by Lobert, Keene & Logan (1998) has indicated that emissions by this route are in the region of 1×10^6 t y^{-1}. Although several investigations have suggested that volcanic eruptions are associated with increased atmospheric concentrations of CH_3Cl (Rasmussen *et*

al., 1980; Inn *et al.*, 1981), thermodynamic calculations by Symonds, Rose & Reed (1988) clearly indicate that the annual flux derived by volcanic emission is insignificant and that raised CH_3Cl levels in the plume arising from a volcanic eruption are attributable to the combustion of vegetation associated with lava flows.

Historically the oceans have been regarded as the major source of atmospheric CH_3Cl. Measurement of CH_3Cl concentrations in seawater and air over the Pacific by Rassmussen *et al.* (1980) and Singh, Salas & Stiles (1983) led to computation of a global oceanic flux of approximately 5×10^6 t y^{-1}. Abiotic production by reaction of Cl^- in seawater with CH_3I released by marine algae has been proposed (Zafiriou, 1975), but no correlation has been observed between oceanic CH_3I and CH_3Cl concentrations (Singh *et al.*, 1983). Indeed, the coexistence of high levels of CH_3Cl and relatively low concentrations of CH_3I and *vice versa* betokens an independent origin for each compound. CH_3Cl can be formed in the ocean by direct biosynthesis by marine macroalgae and phytoplankton. On the basis of field measurements Manley & Dastoor (1987) estimated that the giant kelp, *Macrocystis pyrifera* (L.) C. Agardh a dominant primary producer in Californian coastal waters, released CH_3Cl at a rate of 160 ng g^{-1} F.W. d^{-1}. Subsequently Wuosmaa & Hager (1990) isolated and purified an *S*-adenosylmethionine/halide ion methyltransferase from the red alga, *Endocladia muricata* (H. Post & Rupr.) J. Agardh. Nevertheless, even assuming that the CH_3Cl release rate observed by Manley & Dastoor (1987) is typical of all marine macroalgae, the global standing crop of 58×10^6 t can, at the most, be responsible for the production of only 3000 t of CH_3Cl y^{-1}, an insignificant contribution to the total global flux. The first direct evidence of CH_3Cl release by phytoplankton cultures has recently been obtained by Moore and co-workers (Moore *et al.*, 1995; Tait & Moore, 1995) who demonstrated emission by non-axenic cultures of both warm-water and cold-water diatoms. Overall rates of biosynthesis were very low and when scaled to phytoplankton abundance in the oceans cannot account for more than 20000 t y^{-1}, 0·5% of the global flux. Interestingly Moore and co-workers (Tait, Moore & Tokaczyk, 1994; Moore, Groszko & Niven, 1996), on the basis of observations made in the N.W. Atlantic and Pacific oceans and a critical reappraisal of previous data collected by other workers, have now concluded that the flux of CH_3Cl from the oceans is very much less than hitherto estimated. Indeed large areas of the ocean, particularly cooler waters, appear undersaturated with CH_3Cl and may represent a sink for the compound. A net annual flux from the oceans of only about 300000 t was calculated suggesting that, contrary to previous supposition, the terrestrial rather than the marine environment may in fact be the major source of atmospheric CH_3Cl.

Higher plants represents a possible terrestrial source of CH_3Cl. Emission by higher plants was first reported by Varns (1982) who demonstrated that tubers of the potato (*Solanum tuberosum* L.) release CH_3Cl at rates of up to 17 ng g^{-1} F.W. d^{-1} for a short period postharvest. A survey of 60 cultivars of potato by Jeffers & Harper (unpubl. data) has indicated CH_3Cl emissions by tubers of up to 590 ng g^{-1} F.W. d^{-1} within 48 h of harvest. Saini, Attieh & Hanson (1995) conducted a survey of 118 species of herbaceous plant recording halomethane release from leaf discs of a wide variety of plants when floated in solutions containing relatively high concentrations of halide ion. As the rate of methylation of I^- was 2000-fold higher than that of Cl^-, their assay for halomethane emission involved incubation of leaf discs with KI. Detectable amounts of halomethane were released by 87 species representing 44 families from 33 orders. The emission rates ranged over four orders of magnitude from 70 ng g^{-1} F.W. d^{-1} to 650 μg g^{-1} F.W. d^{-1}. Paradoxically, halophytic species had relatively low emission rates, maximum production being recorded with species belonging to the Brassicaceae.

Subsequent investigation of the enzymology of the process in *Brassica oleracea* L. revealed that the enzyme responsible for halide methylation could also attack sulphide ion, HS^- (Attieh, Hanson & Saini, 1995). *In vitro* I^- and HS^- are far better substrates that Cl^- by over three orders of magnitude casting doubt on the role of Cl^- as a substrate for the enzyme *in vivo*. Whilst I^- is unlikely to be a major substrate for the enzyme *in vivo* given its low concentration in plant tissue it seems quite feasible that HS^- is the normal physiological substrate, the major function of the enzyme being detoxification of HS^-. The Brassicaceae are very rich in sulphur-containing secondary metabolites which can undergo hydrolysis releasing highly toxic H_2S on cellular damage. Nevertheless, the concentrations of Cl^- (5–70 mM) normally present in plant sap (Cram, 1976) are such that it is unlikely that methylation is entirely restricted to HS^- and it is conceivable that trace amounts of CH_3Cl are released. Accurate estimation of the global CH_3Cl flux, if any, from higher plant sources must, however, await measurement of rates of CH_3Cl emission by intact plants *in situ* under normal growing conditions.

The most intensively studied terrestrial biological source of CH_3Cl is the emission of the halocarbon by poroid fungi involved in rotting of wood (Harper, 1985; Harper & Kennedy, 1986; Harper *et al.*, 1988). The high affinity of the fungal methylating system for Cl^- ensures that even when growing in a relatively low chloride environment, such as wood, these fungi release significant quantities of CH_3Cl. The rotting of wood must, therefore, be viewed as a potentially important terrestrial source of atmospheric CH_3Cl, but no attempt has yet been made to estimate the magnitude of the annual global flux of CH_3Cl to the atmosphere from this source. In the remainder of this review we consider the parameters affecting fungal emission of CH_3Cl in forest ecosystems and present an estimate of the likely order of magnitude of the flux.

ESTIMATION OF GLOBAL CH_3Cl EMISSIONS BY FUNGI

In order to reach even a relatively crude estimate of the flux of CH_3Cl to the atmosphere attributable to the activities of wood-rotting fungi a number of parameters relating to the growth substrate, physiology and distribution of such fungi must be evaluated, namely:

(i) Woody tissue decomposed annually by basidiomycetes
(ii) Chloride content of wood.
(iii) Global abundance of CH_3Cl-releasing species.

Table 6. Chloride content of wood from temperate species

	Location*	Cl^- content (mg kg^{-1} D.W.)		Location	Cl^- content (mg kg^{-1} D.W.)
Abies amabilis	B	124	*L. speciosa*	G	6
A. nobilis	B	33	*Leptospermum polygallifolia*	E	556
A. veitchii	B	53	*Liriodendron tulipifera*	B	123
Acacia pravissima	E	609	*Lomatia ferruginosa*	A	219
Acer palmatum	A	146	*Magnolia campbelli*	A	21
A. palmatum (1)	F	24	*M. dawsoniana*	A	37
A. palmatum (2)	F	32	*M. grandiflora*	H	48
A. pseudoplatanus	C	160	*Malus sylvestris*	A	48
A. pseudoplatanus	E	115	*M. sylvestris*	C	128
Aesculus hippocastanum	A	92	*Metasequoia glyptostroboides*	A	72
A. hippocastanum	C	394	*Metrosideros umbellata*	E	389
A. indica	A	71	*Myrtus apiculata*	A	246
Alnus glutinosa	A	55	*Nothofagus antarctica*	A	66
A. glutinosa	C	38	*N. dombeyi*	A	302
A. incana	B	179	*N. dombeyi*	D	83
Alnus sp.	G	74	*N. fusca*	A	166
Aralia spinosa	E	153	*N. nervosa*	D	413
Araucaria araucana	A	251	*N. obliqua*	A	185
A. araucana	E	372	*N. obliqua*	E	376
Berberis darwinii	E	84	*N. procera*	B	101
Betula sp.	G	8	*Olearia paniculata*	E	294
Betula pendula	A	62	*Osmanthus fragrans*	H	75
Betula sp.	A	43	*Parrotia persica*	A	8
Buddleia davidii	E	88	*Picea abies*	B	17
Carya ovata	A	161	*P. glauca*	B	22
Castanea sativa	A	93	*P. sitchensis*	B	26
Cercidiphyllum japonicum	A	57	*Pinus contorta* var. *contorta*	B	42
Chamaecyparis lawsoniana	A	291	*P. nigra* var. *maritima*	B	38
C. lawsoniana	D	559	*P. pungens*	B	42
Cladrastis sinensis	A	76	*P. radiata*	B	39
Cornus capitata	A	171	*P. sylvestris*	C	110
Corylus avellana	A	38	*P. sylvestris*	F	15
C. avellana	C	127	*Pittosporium tenuifolium*	E	193
Cotoneaster frigidus	E	122	*P. tenuifolium*	A	45
Cotoneaster sp.	D	14	*P. tobira*	H	43
Crataegus monogyna	C	206	*Podocarpus macrophyllus*	H	128
Crinodendron hookeranum	A	339	*Populus wilsonii*	D	14
Cryptomeria japonica	A	521	*Prunus* sp.	A	54
Cunninghamia lanceolata	A	454	*Prunus* sp.	D	23
Cupressus macrocarpa	A	268	*Prunus spinosa*	C	178
Davidia involucrata	A	33	*Quercus cerris*	B	494
Drimys winterii	A	162	*Q. cerris*	D	80
Embothrium longifolium	A	125	*Q. ilex*	B	220
Escallonia sp.	E	355	*Q. petraea*	A	21
Eucryphia cordifolia	E	443	*Q. petraea*	B	305
E. moorei	A	677	*Q. robur*	B	200
Euonymus europaeus	C	180	*Q. rubra*	B	351
Fagus sylvatica	A	208	*Quercus* sp.	C	260
F. sylvatica	B	24	*Rhododendron davidsonianum*	D	481
F. sylvatica	C	87	*R. oreodoxa*	D	169
F. sylvatica (1)	D	35	*R. ponticum*	D	41
F. sylvatica (2)	D	149	*Sabina chinensis*	H	13
Fraxinus excelsior	A	89	*Salix cinerea*	C	61
F. excelsior	C	37	*Sequoia giganteum*	A	174
Fuchsia magellanica	E	2535	*S. sempervirens*	A	352
Gardenia jasminoides	H	28	*Sorbus aucuparia*	D	115
Gevuina avellana	E	1378	*Sorbus* sp.	A	33
Ilex aquifolium	A	110	*S. teodori*	D	29
I. aquifolium	C	299	*Stewartia pseudocamellia*	A	42
I. aquifolium	D	113	*Styrax japonicum*	A	164
Juglans nigra	B	227	*Taxus baccata*	D	15
J. regia	B	259	*Tilia platphyllos*	A	277
Juniperus recurva	A	502	*Ulmus glabra*	C	73
Larix decidua	F	38	*U. procera*	A	39
L. kaempferi	B	94	*U. procera*	D	107
			Mean (% RSD = 20)		185

* For key to locations see legend on Table 7).

Table 7. Chloride content of wood from tropical species

	Location*	Cl^- content (mg kg^{-1} D.W.)		Location	Cl^- content (mg kg^{-1} D.W.)
Acacia mangium	L1	82	*Nesogorolonia papaverifera*	M	31
Afzelia bipendensis	M	37	*Parashorea lucida*	N	69
Baillonella toxisperma	M	33	*P. venulosa*	N	95
Canarium sp.	L1	113	*Petersianthus macrocarpus*	M	42
Chlorophora excelsa	M	23	*Pterocarpus soyauxii*	M	37
Cratoxylum sp.	L1	25	*Pterocarpus* sp.	L1	46
Decaisnea fargesii	N	15	*Shorea dispar*	N	24
Diospyros crassiflora	M	53	*Shorea* 'Red Meranti'	N	187
Distemonanthus benthamianus	M	21	*S. pauciflora*	N	39
Dyera costulata	L1	75	*Shorea* sp.	L1	39
Elaeis guineensis	L1	5100	*Sindora baccariana*	N	49
Entandrophragma candetti	M	20	Species from *Myristicaceae* (9612)	N	24
E. cylindricum	M	61	Species from *Myristicaceae* (9613)	N	32
E. utile	M	53	Species from *Myristicaceae* (9629)	N	9
Erythrophleum ivorense	M	37	*Terminalia superba*	M	117
Guarea cedrata	M	27	*Triplochiton scleroxylon*	M	1014
Guibourtia tesmanii	M	60	Unidentified sp. from Myristicaceae (Penarahan)	L1	73
Livistona australis	D	2612	Unidentified woody lianas (aboriginal names)		
Lophira elata	M	23	Kayo mahapet	L2	188
Lovoa trichilioides	M	66	Kayo meriki	L2	84
Mansonia atissima	M	18	Kerwig	L2	180
Milletia laurentii	M	55	Senegal	L2	667
Mixed unidentified spp.	L1	38	Tangai	L2	31
Morus mesozygia	M	21	Tenago	L2	169
Nauclea diderrichii	M	17			
			Mean (% R.S.D. = 13)		249

* Key to Locations
A. Castlewellan, Co. Down, Northern Ireland.
B. Tollymore, Co. Down, Northern Ireland.
C. Crossgar, Co. Down, Northern Ireland.
D. Edinburgh, Scotland.
E. Port Logan, Wigtownshire, Scotland.
F. Ben More, Argyllshire, Scotland.
G. Yunnan, China.
H. Nanjing, Jiangsu, China.
J. New South Wales, Australia.
K. Tasmania, Australia.
L1. Malaysia.
L2. Negri Sembilan, Malaysia (collections in Herb. Gardette duplicates (E)).
M. Cameroon, W. Africa.
N. Kalimantan, Borneo (numbered collections in RBG, Edinburgh (E)).

(iv) Mean percentage conversion of Cl^- to CH_3Cl by each group of hymenochaetaceous CH_3Cl-releasing species.

Each of these parameters is considered individually below and the basis on which a value is assigned to it is outlined.

Woody tissue decomposed annually by basidiomycetes

According to Fung (1993) between 10×10^9 and 18×10^9 t of carbon are fixed in the form of woody tissue per year in the terrestrial biosphere. Assuming a mean value of 14×10^9 t and a carbon content for dry wood of 47% (Atjay, Ketner & Duvigneaud, 1979), then 30×10^9 t D.W. of wood are formed globally per year. Tropical/sub-tropical forests comprise 60% of annual global production of dry matter by forest ecosystems (Atjay *et al.*, 1979) so annual woody tissue production in tropical/sub-tropical and temperate forests is of the order of 18×10^9 and 12×10^9 t D.W. respectively. For reasons discussed below it is necessary to consider Australian forests, both tropical/sub-tropical and temperate, as a single separate category. Satellite data (DeFries & Townshend, 1994) indicate that the areas of Australian tropical/sub-tropical and temperate forests represent 1·9 and 1·2% respectively of the total global areas for each of these categories. Hence the annual production of woody biomass by tropical/sub-tropical (excluding Australia), temperate (excluding Australia) and Australian forests are $17{\cdot}7 \times 10^9$, $11{\cdot}9 \times 10^9$ and $0{\cdot}5 \times 10^9$ t respectively.

If the biosphere is regarded as being in a state of approximate equilibrium a similar quantity of wood must be decomposed per annum as is formed. The main agencies responsible for such decay are the aphyllophoroid Basidiomycota comprising both white and brown rot species. A proportion of above-ground litter in forest ecosystems is, however, destroyed by insects, particularly within the tropics. Swift & Boddy (1984) and Collins (1982) report studies indicating that up to a third of the annual production of grass, wood and leaf litter in W. African savannah and 28% of litter in a Malaysian rain forest were destroyed by attack by insects,

Table 8. Chloride content of wood from *Eucalyptus* species

	Location*	Cl^- content (mg kg^{-1} D.W.)		Location	Cl^- content (mg kg^{-1} D.W.)
E. coccifera	B	511	*E. muelleri*	B	415
E. cordata	B	1601	*E. niphophila*	B	504
E. dalrympleana	B	1199	*E. pauciflora*	C	261
E. dalrympleana†	J	113	*E. rubida*	C	919
E. gigantea	B	514	*E. saligna*†	J	40
E. globulus†	K	139	*E. stellulata*	B	300
E. grandis†	J	90	*E. subcrenulata*	B	244
E. gunni	B	937	*E. urnigera*	B	546
E. johnstoni	B	712	E. viminalis	B	519
E. maculata†	J	431	*Eucalyptus* sp.	E	1716
			Mean (% R.S.D. = 10)		606

* For key to locations see legend on Table 7.
† Heartwood only.

especially termites. About 40% of wood is, however, decomposed by fungi before reaching the litter layer of the forest floor (Cooke & Rayner, 1984) so probably not more than 15–20% of wood in the tropics is mineralized by insect attack. Other routes by which wood is destroyed include the decay of water-logged timber by soft rot fungi of the Ascomycota and mineralization by forest fires (Andreae, 1991). Although these pathways may assume some local significance they are of relatively minor overall importance. To allow for all these other routes of decomposition it is, therefore, assumed that only 75% of tropical/sub-tropical, 85% of Australian and 95% of temperate woody tissue is available for attack by polyporaceous fungi.

Chloride content of wood

As the percentage conversion of Cl^- to CH_3Cl by fungi is comparatively insensitive to Cl^- levels in the growth medium, one of the most critical parameters governing the flux of CH_3Cl from wood-rotting fungi is the concentration of Cl^- in the fungal growth substrate. Unfortunately information in the literature on the Cl^- content of wood, particularly that of tropical species, is exceedingly sparse and that on wood of temperate origin is almost entirely restricted to a few North American species of commercial significance.

The mean Cl^- contents of wood of temperate species recorded in the 10 published investigations which measured this parameter ranged from 2·4 to 123 mg kg^{-1} D.W. with individual observations varying between 0·3 and 200 mg kg^{-1} D.W. (See Table 9 for summary and references). Only three studies exist in the literature in which the Cl^- content of wood of tropical species was measured. Mean Cl^- concentrations observed ranged from 50 to 498 mg kg^{-1} D.W. with individual observations ranging between 50 and 1148 mg kg^{-1} D.W. (see Table 10 for summary and references).

In the light of the limited number and unrepresentative nature of species analysed, and also the wide range of values reported for Cl^- contents of wood of both tropical and temperate origin, it was considered essential to conduct a more geographically and taxonomically wide-ranging survey of Cl^- contents of wood in order to supplement existing data prior to assigning a value to this parameter for use in estimates of CH_3Cl flux. Wood of species representative of temperate areas throughout the world were sampled as was wood of tropical species from a number of locations. Duplicate samples were dried at 70°, ashed in the presence of CaO at 550° and extracted into water as described by Gaines, Parker & Gascho (1984). Chloride in the aqueous extract was determined by ion chromatography using a conductivity detector as described by McKenzie, Ward & Hao (1996). A calibration curve was constructed using standard solutions of KCl. The results of this survey for wood of temperate and tropical species is shown in Tables 6 and 7 respectively. As timber from *Eucalyptus* species possessed a markedly higher Cl^- content than timber of other genera, the results for all eucalypts have been placed separately in Table 8. In view of the dominance of eucalypts in Australian forest ecosystems the mean Cl^- content found for eucalypts is presumed to be broadly typical of Australian wood. This conclusion is consistent with previously unexplained findings of large emission factors for CH_3Cl during the burning of biomass in bush fires in Australia (Tassios & Packham, 1985).

Although the information on Cl^- content of wood in Tables 6–8 is far from comprehensive and is open to criticism on grounds of the limited number of geographical locations utilized for sampling it nevertheless represents a much larger data base than previously available. The mean Cl^- content of 130 samples of wood from temperate species analysed was 185 (range 8–2535) mg kg^{-1} D.W., significantly higher than published results for North American species. It is quite obvious that the Cl^- content of a given species can show large variations at different sites. Thus wood of *Quercus petraea* displayed levels of 21 and 305 mg kg^{-1} D.W. at locations 8 km apart in N. Ireland whilst *Fagus sylvatica* exhibited levels of 208 and 24 mg kg^{-1} D.W. respectively at the same sites. The relative importance of genetic factors, soil conditions and climate in such variation is difficult to assess and is beyond the scope of this review. The mean Cl^- content of wood of 48 tropical species examined was 249 (range 9–5100) mg kg^{-1} D.W. A particularly noteworthy feature was the very high Cl^- content of the two palm wood samples analysed i.e. 5100 mg kg^{-1} D.W. for *Elaeis guineensis* Jacq. and 2612 mg kg^{-1} D.W. for *Livistona australis* (R. Br.) C. Mart. These exceptionally high Cl^- levels are explicable in morphological terms as palm wood

Table 9. Summary of data on Cl^- content of wood of temperate species [excluding Australia]

	Number of samples analysed	Mean Cl^- content (mg kg^{-1} D.W.)	Reference
Maine, U.S.A.	4	12	Young & Guinn (1966)
Oklahoma, U.S.A.	4	28	Osterhaus, Langwig & Meyer (1975)
U.S.A.	18	11	Slocum, McGinnes & McKown (1978)
U.S.A.	7	50	Cutter, McGinnes & McKown (1980)
New Jersey, U.S.A.	8	90	Hall & Naumann (1984)
W. Canada	1	50	Legge, Kaufmann & Winchester (1984)
Ohio, U.S.A.	60	2·4	McClenahen, Vimmerstedt & Scherzer (1989)
Washington State, U.S.A.	3	37	Reinhardt & Ward (1995)
North Carolina, U.S.A.	4	123	Yanosky, Hupp & Hackney (1995)
Oregon, U.S.A.	3	9	McKenzie, Ward & Hao (1996)
N. Ireland, Scotland, China	130	185	Watling & Harper (this study, Table 6)
Weighted mean ($n = 242$)		109	

Table 10. Summary of data on Cl^- content of wood of tropical species [excluding Australia]

	Number of samples analysed	Mean Cl^- content (mg kg^{-1} D.W.)	Reference
Venezuela	3	498	Osterhaus, Langwig & Meyer (1975)
Brazil	2	105	McKenzie, Ward & Hao (1996)
Zambia	1	50	McKenzie, Ward & Hao (1996)
Cameroon, Borneo, Malaysia	48	249	Watling & Harper (this study, Table 7)
Weighted mean ($n = 54$)		254	

consists of fused and lignified leaf bases and leaf tissue normally contains between 500 and 5000 mg kg^{-1} D.W. Cl^-.

It might be argued that such species containing atypically high Cl^- levels should be excluded from consideration in deriving a mean from this data. The abundance of palms in tropical forests, however, both in terms of taxa and the number of individuals, is quite high. For example in a 10 ha plot near Manaus in the Amazon 230 adult individuals of one palm species alone were recorded and at least 150 species of palm are recognized as indigenous to the Amazon region (Henderson, 1995). Although the number of taxa in Africa is less than that in the Americas, the palm flora of Asia and Australasia is as diverse as that of the New World and there are over 2500 species worldwide (Corner, 1966). Thus the inclusion of wood from two palms in 48 samples of wood from tropical species analysed for Cl^- does not seem an unreasonable weighting.

Tables 9 and 10 summarize the results reported here and those published in the literature for temperate and tropical wood respectively. An overall mean Cl^- content for each category of wood is derived from this data as an average of the mean value reported in each investigation weighted for the number of observations made in each investigation. Mean Cl^- concentrations calculated for wood of tropical/sub-tropical, temperate and Australian origin were 254, 109 and 606 mg kg^{-1} D.W. respectively and these values are used in CH_3Cl flux calculations below. It must, however, be borne in mind that the use of these values presupposes that the only Cl^- accessible to wood-rotting fungi is that normally present in the unrotted wood. Most estimates of litter composition on the forest floor indicate that about 25% of detritus is woody (Bray & Gorham, 1964). Fungi decomposing wood in such an environment have access to higher concentrations of Cl^- than those attacking wood in standing trees. Not only will Cl^- present in soil water be readily absorbed into such rotting timber but Cl^- released during decompositions of leaf litter by other fungi will also be available. Soil in non-saline areas can contain up to 40 μg g^{-1} extractable Cl^- and freshwater in general has a Cl^- concentration of between 2 and 100 mg l^{-1} (Grimshaw, Allen & Parkinson, 1989). Leaf litter normally contains between 500 and 5000 mg kg^{-1} D.W. Cl^- (Lobert, Keene & Logan, 1998). Many rain forest trees are characterized by drip-tips at the apex of their leaves which facilitate the removal of excess rain falling on the canopy. This water and also that trickling down the bark surfaces are known to be charged with leaf leachates (Pike, 1978). These sources represent a substantial reservoir of Cl^- which is available to CH_3Cl producing fungi attacking woody detritus on the forest floor and could significantly increase the overall flux of CH_3Cl from the forest environment.

Another related area of uncertainty is the extent to which hyphae of white rot fungi ramify beyond the woody substrate with which their basidiomes are associated. It is quite conceivable that hyphae of wood-rotting fungi extensively penetrate the leaf litter layer and indeed the upper layer of the soil in forest ecosystems. Although such colonization of sterile soil and litter by cultures of white rot fungi is observed in the laboratory, competition with fungi and micro-organisms is likely to restrict the process in nature but, nevertheless, some proliferation of white rot fungal hyphae in non-woody substrates is almost certain to occur with consequent increase in the size of the pool of Cl^- available for conversion to

Table 11. Relative abundance of poroid Hymenochaetaceae in total polypore population in different geographical areas

	Number of polypore spp.	Number of poroid hymenochaetaceous spp.[a]	Number of Group I hymenochaetaceous spp.	Number of Group II hymenochaetaceous spp.	References
African (tropical)	244	39 (16)[b]	28 (72)[c]	11 (28)[d]	Hjortstam, Ryvarden & Watling (1993) Laessøe *et al.* (1996) Ryvarden & Johansen (1980) Renvall & Niemelä (1993)
Australia & N. Zealand	242	51 (21)	33 (65)	8 (35)	Cunningham (1965)
Australia	204	39 (19)	27 (69)	12 (31)	Cunningham (1965)
Canary Islands	20	4 (20)	3 (75)	1 (25)	Watling (unpublished)
China	320	66 (21)	32 (48)	34 (51)	Teng (1996)
Europe	324	59 (18)	36 (61)	23 (39)	Ryvarden & Gilbertson (1986, 1987)
India	321	55 (17)	33 (60)	22 (40)	Larsen & Cobb-Poulle (1990) Modified from Bilgrami, Jamaluddin & Rizwi (1991)
North America (N of 30° N)	349	66 (19)	38 (58)	28 (42)	Ryvarden & Gilbertson (1993, 1994)
North America (S of 30° N)	166	50 (30)	29 (58)	21 (42)	Ryvarden & Gilbertson (1993, 1994)
South America and Carribean	—	66	—	—	Larsen & Cobb-Poulle (1990) Nunez (1996)
South East Asia	—	32	—	—	Larsen & Cobb-Poulle (1990) Hattori (pers. comm.) Hywel-Jones (unpubl.)
United Kingdom	139	26 (19)	15 (58)	11 (42)	Watling (unpubl.) Pegler (1973)
World	1524	371 (24)	220 (59)	151 (41)	Hawksworth *et al.* (1995)

[a] See Larsen & Cobb-Poulle, 1990
[b] Poroid hymenochaetaceous spp. as % of total polypore spp.
[c] Group I hymenochaetaceous spp. as % of total poroid hymenochaetaceous spp.
[d] Group II hymenochaetaceous spp. as % of total poroid hymenochaetaceous spp.

CH_3Cl. Thus calculations of the flux of CH_3Cl by fungi based on the Cl^- content of unrotted wood are likely to be conservative.

Global abundance of CH_3Cl-releasing fungal species

Despite the observation that two non-hymenochaetaceous species can release significant quantities of CH_3Cl it appears from the information currently available that the ability to volatilize Cl^- as CH_3Cl is largely confined to the poroid genera of Hymenochaetaceae. Accordingly in calculations of CH_3Cl emissions it is necessary to take into account the proportion of poroid hymenochaetaceous species relative to the total number of aphyllophoroid basidiomycete species in each class of forest ecosystem under consideration. The poroid Hymenochaetaceae fall into two general groups as regards the percentage of Cl^- converted to CH_3Cl during growth namely *Phellinus* (Group I) and *Inonotus* and other genera (Group II). Information is, therefore, required on the relative proportion which species of each group comprise of the total poroid Hymenochaetaceae species present in each forest ecosystem under consideration. Table 11 summarizes data gathered from the literature on the relative abundance of the poroid Hymenochaetaceae in different geographical regions. As a proportion of the total polypore species the percentage of poroid hymenochaetaceous species varies from 16% in tropical Africa to 30% in North America S of 30° N but generally averages about 20%. As there appears to be no systematic difference between the percentages recorded for tropical/subtropical forest and temperate forest a value of 20% has been employed as a global mean. This proportion is in general agreement with the ratio of poroid hymenochaetaceous species to total aphyllophoroid basidiomycete species recorded during field collections and in qualitative field data in the various regions (Hjortstam, Ryvarden & Watling, 1993; Laessøe *et al.*, 1996).

Group I and Group II species do, however, show some significant differences in distribution. *Phellinus* (Group I) with 220 taxa tends to be the dominant hymenochaetaceous species in tropical forests (60–70% of known *Phellinus* species are tropical). In contrast of the 40 taxa of *Inonotus*, the principal genus of Group II, about 75% are temperate in origin. This difference is reflected in the ratio of the number of species of Group I to that in Group II in the different geographical regions which approximates 70:30 for tropical/subtropical and Australian forests but 60:40 for temperate forests. Again these ratios are consistent with field records for the various regions where they are available, substantiated for instance by analysis of the records of fungus forays held by the British Mycological Society for areas within the British Isles and elsewhere in Europe (see Minter, 1996). In the light of these observations we have utilized in calculations of CH_3Cl flux a 70:30 Group I/Group II ratio for tropical/subtropical and Australian forests and a 60:40 ratio for temperate forests.

Whilst the relative proportion of CH_3Cl producing species to non-CH_3Cl producing species is clearly a useful and comparatively accessible indicator for determining the flux of

CH_3Cl from a given forest environment, it is important when employing it to appreciate its limitations. For it to provide an accurate reflection of CH_3Cl fluxes there must be broad comparability in terms of the number, vegetative mass and longevity of individual colonies between the two groups of species. Obviously a CH_3Cl-producing species with a comparatively low density of short-lived individual colonies of small vegetative mass restricted to a single ecological niche will not contribute, *ceteris paribus*, as much to the CH_3Cl flux as a CH_3Cl producing species with a high density of individual colonies of large vegetative mass and considerable longevity with a wide variety of habitats. A brief consideration of these parameters with respect to the Hymenochaetaceae is, therefore, pertinent.

Some indication of the vegetative mass of a fungal colony or at least the extent of the substrate resource colonized can be gauged from the size and nature of the basidiome. Those of *Phellinus* are perennial and possess a mixture of structural and generative hyphal elements (dimitic); they persist for many years, suggesting the vegetative state has even greater longevity. In some species the basidiome is bracket-shaped, in others hoof-shaped (ungulate), the latter type generally connected with living, damaged or dying trees. Mature basidiomes of *P. alliardii* (Bres.) S. Ahmad, *P. lamaensis* and *P. apiahynus* (Speg.) Rajchenb. & J. E. Wright may weigh upwards of 2·5 kg F.W. and a colony may reach 10 kg. Other species form basidiomes consisting of a poroid sheet (resupinate) and are more frequently associated with small attached twigs or small diameter branches which have been shed and are lying on the ground in contact with the soil surface. Species of *Inonotus* are generally smaller than those of *Phellinus* and are softer and shorter lived with a relatively simple (monomitic) hyphal construction. Group II hymenochaetaceous species also include members of such genera as *Coltricia* and *Cylcomyces* which are stipitate; species of the former arise directly from the soil and some are ectomycorrhizal under certain circumstances.

Phellinus species fruit in all recognizable successional stages of wood decay; some are major colonizers of standing trees, e.g. *P. ferrugineofuscus* (P. Karst.) Bourdot and *P. viticola* on spruce, whilst others continue to fruit even after the tree has fallen e.g. *P. laevigatus* and other members of the *P. ignarius* group (Niemelä *et al*., 1995). *P. nigrolimitatus* is one of seven taxa recorded which can utilize timber in its last stages of decay (Renvall & Niemelä, 1993). A number of hymenochaetaceous taxa are present in the canopy of both temperate and tropical forests, where some are known to link canopy branches together before they finally fall (Rayner & Boddy, 1988). These canopy fungi are undoubtedly important in the wood-rotting process by modifying the structure of the wood before it reaches the forest floor. Nunez & Ryvarden (1993) record *P. callimorpha* (Lév). Ryvarden and *P. inermis* (Ellis & Everh.) G. Cunn. as major components of this habitat in Southern Cameroon. In Malaysia basidiomes of various *Phellinus* spp. have been observed associated with the strangle damage caused by lianas or with termite activity in trees (Watling, unpubl.). Members of the Hymenochaetaceae are, therefore, found throughout the whole successional spectrum of wood-decay processes.

Several species of *Phellinus*, including the destructive root pathogen *P. noxius*, are known on palms, important components of the rainforest particularly in S. America and S. E. Asia. The economically and ecologically important bamboos are attacked by several *Phellinus* spp. e.g. *P. spiniscus* J. E. Wright.

Many *Phellinus* spp. with their thick massive basidiomes, consisting mainly of vegetative tissue, can be regarded as *K*-strategists; they rarely have problems of water shortage and therefore grow actively for long periods. They develop new fertile surfaces after periodic showers and do not produce a new basidiome annually. Other *K*-strategists possess annual but leathery basidiomes that remain sterile at low humidities, especially when growing along trails or in clearings in the forest; they resume sporulation during the rainy season but active growth is maintained for long periods. There appear to be no *r*-strategists in the Hymenochaetaceae. Such species have succulent basidiomes dependent on water turgidity; after sporulation such basidiomes collapse and new ones are produced when humidity is again favourable. Such growth is spasmodic.

To summarize, species of both *Phellinus* and *Inonotus*, the dominant CH_3Cl releasing genera, are, compared with many other aphyllophoroid basidiomycete species, relatively long lived, have comparatively large vegetative mass and a wide variety of ecological niches. Hence any computation of CH_3Cl fluxes based on the relative proportion of CH_3Cl producing species to other aphyllophoroid basidiomycete species is very unlikely to be an overestimate.

Mean percentage conversion of Cl^- to CH_3Cl by each group of hymenochaetaceous CH_3Cl-releasing species

A survey by Harper *et al*. (1988) of CH_3Cl emission in the Hymenochaetaceae involved culture of 39 species of *Phellinus*, 13 species of *Inonotus* and eight species of other poroid genera in the family on three different media containing 10 mM Cl^- and measurement of the overall conversion of Cl^- to CH_3Cl during the growth cycle (see Tables 1 and 2). Of the media employed which were based respectively on glucose, malt extract and cellulose, this last is most akin to the growth substrate of these species in nature and accordingly CH_3Cl yields on this medium have been used in calculation of mean conversion efficiencies. The Cl^- concentration employed in the survey (10 mM) was close to the middle of the range of mean Cl^- concentrations assigned above to different categories of wood, i.e. 109 mg kg^{-1} (3 mM) – 606 mg kg^{-1} D.W. (17 mM). Furthermore, conversion efficiencies on cellulose-based medium are largely independent of Cl^- concentration in the range 0·5–10 mM (Harper & Kennedy, 1986) so no correction of yields is considered necessary to allow for differences in Cl^- concentrations in wood when extrapolating these yields to those in nature. The species screened fell into two distinct groups as regards conversion efficiency. The 35 species of *Phellinus* which exhibited growth on the cellulose based medium displayed a range of conversion efficiencies from 0% to 82% with a mean of 15% (Table 1). By contrast the 16 species of *Inonotus* and other hymenochaetaceous genera which exhibited growth on cellulosic medium showed much

Table 12. Emission of CH_3Cl by fungi in different forest types

	CH_3Cl emission (t y^{-1})
Temperate (excluding Australia)	
Phellinus	31600
Inonotus + other genera	7100
Total	38700
Tropical/sub-tropical (excluding Australia)	
Phellinus	100800
Inonotus + other genera	14400
Total	115200
Australia	
Phellinus	7700
Inonotus + other genera	1100
Total	8800
All forest types	
Total	162700

lower CH_3Cl yields with a range of conversion efficiencies from 0% to 22% and a mean of 5% (Table 2). Conversion efficiencies of 15% and 5% are therefore employed respectively for *Phellinus* and *Inonotus* + other genera in CH_3Cl flux calculations.

Flux calculations

The magnitude of CH_3Cl emissions by each of the two groups of hymenochaetaceous fungi in each forest type were estimated in t y^{-1} using the following formula:

$$w \times \frac{a}{100} \times \frac{c}{10^6} \times \frac{h}{100} \times \frac{g}{100} \times \frac{e}{100} \times 1{\cdot}42,$$

where w is the annual global woody biomass production (t y^{-1} D.W.), a is the percentage of wood decomposed by aphyllophoroid basidiomycetes in the forest type under consideration, c is the concentration of Cl^- in wood of the forest type (mg kg^{-1} D.W), h is the number of poroid hymenochaetaceous species present as a percentage of the total number of polypore species recorded for the area, g is the number of species of the hymenochaetaceous group under consideration (i.e. *Phellinus* or *Inonotus* + other genera) in the area as a percentage of all poroid hymenochaetaceous species present, e is the mean percentage conversion of Cl^- to CH_3Cl by species of the hymenochaetaceous group under consideration, and 1·42 is the mol. wt. ratio to convert amounts of Cl^- to amounts of CH_3Cl.

Estimated global CH_3Cl emissions

Estimates of global emissions by fungi from each forest category are presented in Table 12. Total global emissions are calculated at 160000 t, of which about 75% emanate from tropical/subtropical forest. *Phellinus* is responsible for over 86% of fungal emissions as a result, not only of its abundance in all forest categories, but also the high efficiency with which Cl^- is converted to CH_3Cl by many species in the genus. Australian forest (both tropical/subtropical and temperate) although comprising only 1·5% of the total global forest cover is responsible for over 5% of global fungal emissions of CH_3Cl. This disproportionately large contribution is attributable to the relatively high Cl^- content of wood of *Eucalyptus* spp.

Total fungal emissions are of the same order as the net annual flux from the oceans calculated by Moore *et al.* (1995) at between 200000 and 400000 t and represent by far the largest biological source of CH_3Cl identified to date. Whilst both marine phytoplankton and marine macroalgae have been implicated in CH_3Cl biosynthesis (see 'Natural sources of CH_3Cl') total production from both of these sources combined cannot exceed 25000 t according to estimates based on both laboratory and field measurements of release rates (Manley & Dastoor, 1987; Tait & Moore, 1995).

Hymenochaetaceous fungal emissions would appear to constitute the second largest terrestrial source of CH_3Cl discovered to date only surpassed in magnitude by that produced abiotically during biomass burning which is estimated at $0{\cdot}4–1{\cdot}4 \times 10^6$ t y^{-1}. Although estimated fungal emissions may at first sight appear insignificant ($\sim$ 4%) in the context of an annual atmospheric CH_3Cl input of 4×10^6 t from natural sources, the effect on the magnitude of the flux of changes in the assumptions employed in computation of the estimate must not be overlooked. Calculations of fungal emissions presuppose that the only Cl^- accessible to wood-rotting fungi is that normally present in unrotted wood. If, as suggested previously, Cl^- present in leaf litter and soil water is available for uptake by hyphae of wood-rotting species the estimates presented in Table 12 must be considered the lower limit of a range of possible CH_3Cl emission fluxes. The upper limit, defined by the concentrations of Cl^- present in leaf litter and soil water and the efficiency of uptake by hyphae, may be as much as ten fold higher. A further possible cause of underestimation of fungal CH_3Cl release has been highlighted earlier in this review. The longevity, large vegetative biomass and wide range of habitats colonized by the main CH_3Cl producing genera may give them an ecological significance considerably greater than that inferred from the relative proportion of CH_3Cl producing species to total polypore species used in CH_3Cl flux calculations.

Another area of uncertainty of critical importance is the number of fungal species which are capable of CH_3Cl release. The premise employed in deriving emission fluxes was that CH_3Cl release is confined to species of the Hymenochaetaceae. Whilst only two of 30 non-hymenochaetaceous polypore species screened by Harper *et al.* (1988), *Perenniporia fraxinea* in Ryvarden's Group 8 and *Phaeolus schweinitzii* in his Group 4 discussed earlier (see Table 3), displayed CH_3Cl emission, at least 1250 non-hymenochaetaceous polypore species exist worldwide and it is quite conceivable that there are other major genera of CH_3Cl-releasing polypores yet to be discovered. It is significant in this context that a number of non-hymenochaetaceous white rot species in the Poriales and Stereales e.g. *Phanerochaete chrysosporium*, *Trametes versicolor*, *Phlebia radiata*, are capable of biosynthesizing CH_3Cl for utilization as a biochemical methyl donor (Harper *et al.*, 1990) although the compound does not appear to be released at any stage of fungal growth during laboratory culture of these

species. This observation should not be regarded as necessarily signifying that CH_3Cl release from such species cannot occur in nature as the conditions and substrates normally employed for culture of these fungi in the laboratory bear little relation to those pertaining in their natural habitat.

The flux of CH_3Cl from wood-rooting fungi is, therefore, potentially considerably higher than that indicated in Table 12. The fact that other sources identified to date do not appear to be capable of sustaining the annual atmospheric CH_3Cl input of 4×10^6 t certainly provides an incentive for further investigation of CH_3Cl release by fungi and refinement of estimates of emissions. Only when emissions of CH_3Cl are measured *in situ* in a variety of forest habitats will it be possible to arrive at a definitive conclusion as to the magnitude of CH_3Cl production by fungi. Such studies would also be extremely valuable in determining whether higher plants release significant quantities of CH_3Cl under normal growing conditions. If biological emissions, whether from fungi, higher plants or marine algae, comprise a substantial proportion of CH_3Cl input to the atmosphere it becomes quite probable that natural climatic variation in the past and the consequent changes in vegetational cover (or the more recent global deforestation as a result of human activities) will have a significant impact on the atmospheric CH_3Cl burden. Current global atmospheric models employed to predict the effect of man-made halocarbons on ozone destruction in the stratosphere assume that the rate of natural CH_3Cl production has remained constant throughout previous millennia. Whilst the effects of natural changes in CH_3Cl concentration in the atmosphere are unlikely to rival those of man-made halocarbons it is, nevertheless, important that the possibility of natural fluctuations in stratospheric chlorine attributable to biological changes is not neglected in future atmospheric models. Stubblefield & Taylor (1986) demonstrated rot in samples of fossil wood of Permian age that was identical to the white pocket rot caused by contemporary *Phellinus pini* (Taylor & Taylor, 1997). Whilst this finding cannot be construed as indicating that *Phellinus* existed 250 million years ago it shows that patterns of decay associated with what are believed to be basidiomycetous hyphae were present at that time and could conceivably have released stratospherically active halocarbon even in that far distant age.

We would like to thank all those who, when we realized how little was known about the chlorine content of wood, came to our assistance in supplying samples. These include P. Baxter, RBG Benmore; Cheah Uan Boh, Malaysian Agricultural Research and Development Unit, Kuala Lumpur; Bahiru Duguma, International Centre for Research in Agroforestry, Cameroon; Eric Gardette, Paris; G. Kile, CSIRO, Canberra; D. Knott, RBG Dawyck; Sam Harrison, Forestry Division, Department of Agriculture for Northern Ireland; Mark Newman & Peter Wilkie, Tropical Botany Unit, RBG Edinburgh; David Patterson and his colleagues at the Horticulture Department at RBG Edinburgh including Matty Dickson; B. Unwin, RBG Logan. We also wish to thank Evelyn Turnbull of RBG Edinburgh for assistance in assembling data on species numbers. Thanks are also due to T. Kennedy of Food Science Division, Department of Agriculture for N. Ireland for determining chlorine in the wood samples received and Roy Anderson of the Agricultural and Environmental Sciences Division of the Department of Agriculture for N. Ireland for many helpful discussions.

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(*Accepted 15 December 1997*)