

## COMMENT

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### The red alga *Asparagopsis taxiformis*/*Falkenbergia hillebrandii*—a possible source of trichloroethylene and perchloroethylene?

Trichloroethylene (C<sub>2</sub>HCl<sub>3</sub>) and perchloroethylene (C<sub>2</sub>Cl<sub>4</sub>) are widely used industrially as solvents, dry cleaning, and degreasing agents, and the annual release from such sources is estimated to be 200,000 tons for the former and 300,000 tons for the latter. In European waters typical background concentrations range from 0.01 to 5 ng L<sup>-1</sup>, with higher levels in ground waters of some areas (Herbert et al. 1986). The environmental concentrations of both compounds have been subject to close regulatory scrutiny, and the maximum allowable concentrations in effluent have been based on the principle that these compounds are entirely anthropogenic in origin. Interestingly, McCulloch and Midgley (1996), using the best available estimates of the atmospheric lifetime and atmospheric concentrations of trichloroethylene (Koppmann et al. 1993), have found a large discrepancy between the observed concentrations of trichloroethylene in each hemisphere and the concentrations calculated from the estimated anthropogenic inputs. A total source strength of the order of 1.5 million tons per annum appears to be necessary to explain ambient concentrations of the compound in the atmosphere, an amount sevenfold higher than that calculated as emanating from anthropogenic sources. In this context, the report by Abrahamsson et al. (1995a) that both compounds are produced by a wide range of macroalgal species of both temperate and tropical origin assumes considerable significance. These workers also reported release of both chlorocarbons by the red microalga *Porphyridium purpureum*. In their investigation, Abrahamsson et al. (1995a) employed purge-and-trap techniques to concentrate volatiles and subsequently determined halocarbons by gas chromatography with electron capture detection, but no unequivocal chemical identification of the compounds was performed. Release rates from macroalgal species were mostly in the range of 0.020 to 7 ng g<sup>-1</sup> FW h<sup>-1</sup> for trichloroethylene and 0.0026 to 0.3 ng g<sup>-1</sup> FW h<sup>-1</sup> for perchloroethylene. However, two subtropical red algae *Asparagopsis taxiformis* (Delile) Trev. and *Falkenbergia hillebrandii* (Born.) Falkenb. displayed very much higher rates of trichloroethylene production, 36 and 3,400 ng g<sup>-1</sup> FW h<sup>-1</sup>, respectively. Both algae also released significant amounts of perchloroethylene, with production reported at 0.0021 and 8.2 ng g<sup>-1</sup> FW h<sup>-1</sup>, respectively. *F. hillebrandii* is now considered to be the tetrasporophyte phase in the life history of *A. taxiformis* so that the two species are actually conspecific (Dixon and Irvine 1977). However, the only other *Asparagopsis* species currently recognized, the cold water *A. armata*, has a *Falkenbergia* phase (*F. rufolanosa*), which is considered morphologically indistinguishable from *F. hillebrandii*, so some

confusion has arisen in the literature as to the exact taxonomic identity of various *Falkenbergia* isolates collected. Indeed, recent investigations of *Falkenbergia* isolates collected from locations worldwide in which total DNA restriction fragment length polymorphism was probed using organellar DNA suggests that a third species of the genus may exist (Ní Chualáin 1997; Marshall et al. 1999).

We have attempted to confirm the observations of Abrahamsson et al. (1995) on *F. hillebrandii* using gas chromatography/mass spectrometry to provide unequivocal chemical identification of halocarbons. We have determined rates of production of trichloroethylene and perchloroethylene by laboratory grown cultures of 12 isolates of *Falkenbergia* phases originating from warm and cold water locations throughout the world, including Gran Canaria, where Abrahamsson et al. (1995) collected the *Falkenbergia* sample that they examined. We also collected fresh samples of the alga from Gran Canaria and Ireland. Measurements have been conducted during exposure to both normal and high irradiances, as it is known that increased irradiance can stimulate production of volatile halocarbons by marine algae (Mtolera et al. 1996).

*Isolates and cultural conditions*—Isolates of the *Falkenbergia* phase of *Asparagopsis* spp. were obtained from the collection of Professor Michael Guiry of the Department of Botany, National University of Ireland, Galway, Ireland. The culture collection accession numbers of each isolate, together with the geographical location where they were collected, are as follows: 0510 (Co Clare, Ireland); 0604 (Victoria, Australia); 0985 (Key Largo, Florida); 0996 (San Diego, California); 0997 (Hawaii); 0999 (Yucatan, Mexico); 1026 (Asterias, Spain); 1030 (Messina, Sicily); 1033 (Okinawa Island, Japan); 1075 (Gran Canaria, Spain); 1076 (La Spezia, Italy); 1077 (Civitavecchia, Italy). Fresh *Falkenbergia* samples were also obtained from Quintarilla, Gran Canaria, Spain in April 1997 and from Portaferry, Co. Down, Northern Ireland in February 1998. Isolates were maintained in seawater enriched with 20% von Stosch medium (von Stosch 1964). Algal cultures were established initially in petri dishes (50 mm diameter × 20 mm) containing culture medium (15 ml) and incubated at 20°C illuminated from above with Thorn Grolux and Sylvana Lifeline fluorescent lights at an irradiance of 30 μmol photons m<sup>-2</sup> s<sup>-1</sup> (16:8 h light:dark cycle). The algae were later subcultured into fresh medium (100 ml) in 250-ml conical flasks shaken at 100 rpm at 20°C under a similar light regimen.

To determine the rate of trichloroethylene and perchloro-

ethylene production, algae (1 g FW) were suspended in culture medium (10 ml) under an atmosphere of air, in each of a series of screw-capped vials (25 ml) fitted with polytetrafluoroethylene (PTFE) septa and incubated at 20°C at an irradiance of 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  for periods from 6 to 24 h under fluorescent lights. After incubation for the appropriate time period, duplicate vials were removed from the incubation chamber, and medium (5 ml) was withdrawn from each for analysis as described below. The remaining medium was either discarded or stored at -20°C for future reference. Similar experiments were conducted using high irradiance (330  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) from a quartz halogen lamp to stress the alga and replicate the conditions employed by Abrahamsson et al. (1995a). Control experiments in which culture medium was incubated in the absence of algae were also performed. The algal incubation conditions described by Abrahamsson et al. (1995a) differed slightly from those of this investigation in that no headspace existed above cultures in the former study. However, as the Henry's law constants (concentration in gaseous phase/concentration in aqueous phase) for trichloroethylene and perchloroethylene at 20°C are low, respectively 0.20 and 0.28, this difference had only a minor effect on the sensitivity of the method.

**Halocarbon analysis**—For analysis, a DANI 37.50 purge-and-trap sampler attached to a Hewlett Packard 5890 gas chromatograph directly linked to a Hewlett Packard mass selective detector (MSD) was employed. Incubation medium (5 ml) was placed in a vial (20 ml) and 1,2-dibromoethane (1 ml of a 10  $\mu\text{g L}^{-1}$  solution) was added as an internal standard. Previous work in the laboratory had established that the release of this halocarbon did not occur under the conditions pertaining in these experiments. Vials were then sealed with aluminum crimp caps fitted with PTFE-coated butyl rubber seals, placed in the purge-and-trap sampler, and allowed to equilibrate at 50°C for 1 h prior to purging. Helium was employed as the purge gas at a flow rate of 30  $\text{ml min}^{-1}$  for a purge time of 30 s.

Volatiles were trapped on Tenax TA and thermally desorbed at 250°C for 1 min onto a Chrompak Poraplot Q capillary column (10 m  $\times$  0.32 mm internal diameter with 10  $\mu\text{m}$  of divinylbenzene/styrene polymer as bonded phase). The injector port temperature and MSD transfer line were maintained at a temperature of 250°C, and the gas chromatograph oven was held initially at 40°C for 1 min, then programmed to 230°C at 10°C  $\text{min}^{-1}$  and maintained at this temperature for 5 min. The MSD was operated in the selective ion-monitoring mode with ions monitored as follows: trichloroethylene  $m/z$  95, 97, 130, and 134; perchloroethylene  $m/z$  164, 166, 168; and dibromoethane  $m/z$  107 and 109. The limits of detection for trichloroethylene and perchloroethylene in solution under the conditions employed in the assay were 0.1  $\mu\text{g L}^{-1}$ .

For determination of rates of chemical degradation of tetrachloroethane to trichloroethylene and of pentachloroethane to perchloroethylene, the following experimental protocol was employed. Sterile seawater (14.5 ml) containing tetrachloroethane or pentachloroethane (1  $\text{mg L}^{-1}$ ) was placed in a vial (20 ml) under an atmosphere of air and carbon tetrachloride (0.5 ml of a 15  $\text{mg L}^{-1}$  solution) added

as an internal standard. Vials after sealing with aluminum crimp caps fitted with PTFE-coated silicone septa were placed in the purge-and-trap sampler and equilibrated at 30°C for 31 min. Consecutive vials were sampled every 60 min for trichloroethylene and every 20 min for perchloroethylene, and concentrations were measured by gas chromatography/mass spectrometry using the conditions described above, except that a purge time of 3 s was employed. The concentration of the carbon tetrachloride internal standard was measured by monitoring ions at  $m/z$  117, 119, and 121.

**Trichloroethylene and perchloroethylene production**—For periods of incubation from 6 to 24 h under either normal (30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) or high (330  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) irradiance, none of the 13 *Falkenbergia* isolates showed production of trichloroethylene or perchloroethylene. The lowest rate of release of each compound that would have been detectable in a 24 h incubation under these conditions was 0.04  $\text{ng g}^{-1} \text{FW h}^{-1}$ . These results provide no support for the contention of Abrahamsson et al. (1995a) that *F. hillebrandii* has the ability to biosynthesize environmentally significant quantities of trichloroethylene or perchloroethylene. *F. hillebrandii* was reported to be the most prolific producer of these compounds of all the algal species examined by the group. The isolates in our study were originally collected from widely scattered locations in the Northern Hemisphere, including Gran Canaria where the sample examined by Abrahamsson et al. (1995a) was obtained. The lowest level of release detectable in the present study was approximately 5 orders of magnitude less than the production rate reported for trichloroethylene by Abrahamsson et al. (1995a) and over 2 orders of magnitude less than that reported for perchloroethylene. There are several possible explanations for these conflicting results. Marshall et al. (1999) have established that many *Falkenbergia* isolates release other halogenated compounds, particularly under high irradiances. It is conceivable, in the absence of rigorous chemical identification of gas chromatographic peaks by Abrahamsson et al. (1995a), that another compound with a similar retention time was mistaken for trichloroethylene. Scarratt and Moore (1999) have also failed to confirm the results of Abrahamsson et al. (1995a) as regards the formation of trichloroethylene and perchloroethylene by the red microalga *Porphyridium purpureum*, although the release of other halocarbons was detected. Furthermore, Nightingale et al. (1995), in a study of halocarbon release by macroalgae in beds and rock pools on the coast of Scotland, could find no evidence of trichloroethylene release by several species reported as producing the compound by Abrahamsson et al. (1995a).

A difference between the Abrahamsson et al. (1995a) study and that reported here is that in the current investigation the majority of the *Falkenbergia* isolates investigated were laboratory-cultivated, whereas Abrahamsson et al. (1995a) employed a field-collected sample from Gran Canaria. Some quantitative differences as regards rates of halocarbon release might be anticipated between field-collected and laboratory-cultured isolates. However, if the biosynthetic trait for trichloroethylene and perchloroethylene production

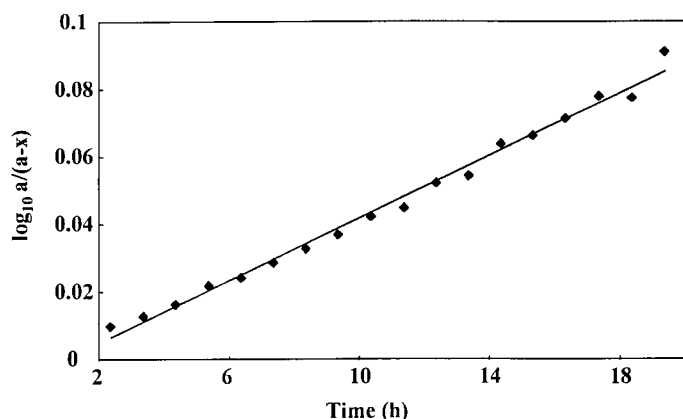


Fig. 1. First-order dehydrohalogenation of tetrachloroethane to trichloroethylene measured at 30°C in sterile seawater (where  $a$  is the initial molar concentration of tetrachloroethane at  $t = 0$ , and  $x$  is the molar concentration of trichloroethylene at time  $t$ ).

is established in this species to the extent suggested by the high rates of release recorded by Abrahamsson et al. (1995a), it is difficult to explain why production of neither compound was detectable in any of the genetically diverse collection of laboratory-cultured isolates examined at rates several orders of magnitude less than those reported by Abrahamsson et al. (1995a). Moreover, no release of either compound from the two field-collected samples examined was observed. It is pertinent to note that isolates of *Falkenbergia* cultured in the laboratory under similar conditions to those used in this investigation displayed high release rates for several volatile bromocarbons (Marshall et al. 1999).

An alternative explanation of the inconsistency between the results of the present investigation and those of Abrahamsson et al. (1995a) is that the single sample of *F. hillebrandii* employed by the latter workers may have been contaminated in situ prior to collection by chlorinated ethylenes derived from an anthropogenic source or an unidentified natural source. Both trichloroethylene and perchloroethylene can be formed abiotically quite readily in aqueous solution by dehydrohalogenation of 1,1,2,2-tetrachloroethane and pentachloroethane, respectively (Cooper et al. 1987; Mross and Konietzko 1991; Joens et al. 1995). The half-life for this first-order elimination reaction for 1,1,2,2-tetrachloroethane at 25°C in buffer has been variously reported at from 94 to 172 d at pH 7.0 and at about 1 d at pH 9.0. The rate of the reaction was found to be independent of both ionic strength and buffer composition (Joens et al. 1995). Seawater possesses a pH of approximately 8.2, so that, on the basis of the above data, the half-life of tetrachloroethane might be anticipated to be of the order of 5 to 10 d at 30°C in seawater.

In experiments in this laboratory, we have demonstrated that the conversion of 1,1,2,2-tetrachloroethane to trichloroethylene is in fact more rapid, with a half-life of 2.9 d in seawater at 30°C (see Fig. 1). Pentachloroethane undergoes even faster transformation to perchloroethylene, with a half-life of 2 h in seawater at 30°C being observed. It may well be that certain components of seawater significantly accelerate the rate of these elimination reactions. These obser-

vations raise the possibility that trichloroethylene and perchloroethylene found in the marine environment may not be of direct anthropogenic or biological origin, but may be derived by abiotic dehydrohalogenation of tetrachloroethane and pentachloroethane, respectively. Although both of the latter compounds have significant usage in industry (Mross and Konietzko 1991), they are employed mainly as intermediates with little subsequent release to the environment. However, it seems quite feasible for both compounds to arise biologically in the marine environment. While neither has been identified as a natural product to date, the formation of another highly chlorinated ethane, hexachloroethane, by the red alga, *Euclima platycladum*, has been established using gas chromatography/mass spectrometry by Abrahamsson et al. (1995b), so that the biosynthesis of tetrachloroethane and pentachloroethane by algae does not seem implausible.

In conclusion, our negative findings regarding the production of trichloroethylene and perchloroethylene by a number of *Falkenbergia* isolates obtained from warm and cold water environments, including Gran Canaria, do not support the claims of Abrahamsson et al. (1995a) that these halocarbons are released by a variety of macroalgal species. However, a possible source of trichloroethylene in the marine environment is abiotic dehydrohalogenation of tetrachloroethane. Nevertheless, the direct biosynthesis of the compounds in the marine environment cannot be excluded until a comprehensive survey of a broader range of marine biota, in particular pelagic microalgae and microorganisms, has been conducted.

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