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Evidence of the natural production of trichloroethylene (Reply to the comment by Marshall et al.)

Since the publication of the article dealing with the natural formation of trichloroethylene and perchloroethylene (Abrahamsson et al. 1995), at least one investigation has been made in order to confirm our findings. Marshall et al. (2000) conducted a number of experiments with several laboratory grown *Falkenbergia* phases and found no production of trichloroethylene and perchloroethylene. In this comment, we would like to point out three major differences between the investigations made by us and those by Marshall et al. (2000) and show evidence indicating a marine source of trichloroethylene. We should emphasize to the readers that the ocean is of minor importance as a producer of perchloroethylene compared to anthropogenic sources, whereas the contribution of anthropogenic and marine sources of trichloroethylene are probably of equal magnitude.

The following discussion will deal with the differences between the investigations regarding analytical procedure, cultivation and physiology of algae, and incubation of algae. The fourth issue will be to present some supportive results.

Analytical procedure—The method for the determination of halocarbons that has been used by Abrahamsson et al. (1995) is described in detail by Ekdahl and Abrahamsson (1997). In the latter paper comparisons were made between the method used by Abrahamsson et al. (1995) that uses a relatively large trap and a megabore gas chromatographic column, versus an improved method with a microtrap and a capillary column. The main differences between the two methods are due to basic chromatographic theory. The detection in Ekdahl and Abrahamsson (1997) was made both with electron capture detection and mass spectrometry. Ekdahl and Abrahamsson (1997) found a shift in relative retention times between separations performed with a megabore column and the capillary column due to differences in polarity between the two stationary phases. On the other hand, the elution order of trichloroethylene or perchloroethylene was not changed.

The method used by Abrahamsson et al. (1995) and Ekdahl and Abrahamsson (1997) was designed to minimize sample handling, and thereby the risk of contamination of the samples. The analytical method has been thoroughly evaluated according to traditional analytical procedures. As described in Abrahamsson et al. (1995), seawater blanks were monitored continuously during the investigation, and no contamination from the incubation media or the laboratory could be observed. The overall detection limit, the precision of the incubation procedure, and the analytical procedure were calculated for data presented in Abrahamsson et al. (1995). The detection limit for trichloroethylene and perchloroethylene was 8.4 pmol L^{-1} and 1.2 pmol L^{-1}, respectively, and the relative standard deviations were 14% for trichloroethylene and 26% for perchloroethylene. These values are calculated from 11 incubated seawater blanks (incubation period of 6 to 12 h), and the detection limit was calculated as three times the standard deviation of the blanks.

All of the subtropical algae investigated in Abrahamsson et al. (1995) were collected at the same occasion, and the experiments were performed within 2 h after collection. No signs of contamination could be seen in any of the experiments. Therefore, we do not agree with Marshall et al. (2000) that contamination is the cause of the disagreeing results.

In the investigation by Abrahamsson et al. (1995), the identity of the individual compounds was ascertained through spiking of samples to determine the relative retention times of the compounds. Even if degassed seawater was used to prepare the standard solutions, trace amounts of trichloroethylene could be found, and consequently a standard addition was performed. Marshall et al. (2000) claimed that since the mass spectrometer was not used in the investigation presented by Abrahamsson et al. (1995), the identity of trichloroethylene could not be established. In order to optimize resolution, Abrahamsson et al. (1995) used a dual-column system. Two capillary columns with different polarities were connected with glass-fit connectors, thus improving the selectivity of the chromatographic system (Abrahamsson and Klick (1990). Even if the identification of the individual compounds was done based on careful determination of relative retention times only, we have found no reason to doubt the identity of trichloroethylene based on the relative retention times found during the investigation.

Cultivation and physiology of algae—In Abrahamsson et al. (1995) the analytical equipment was brought to the Canary Islands in order to be able to investigate freshly collected algae within 2 h after collection. The approach of Marshall et al. (2000) was to investigate cultivated strains. Even if they collected species of *Falkenbergia* at the Canary Islands, these species were transported to Ireland and kept in culture for an unknown period of time before the incubation experiments were performed.

Cultivated algae differ from freshly collected ones in that they are adapted to laboratory conditions, as has been described in a textbook by Cole and Sheath (1990). It is therefore not surprising that they also differ in production of halocarbons.

Falkenbergia hillebrandii and *Asparagopsis taxiformis* have so-called vesicle cells. These cells contain high amounts of bromine and iodine, which have been localized by X-ray microanalysis (Wolk 1968), and they are very fragile. If the vesicle cells break, free iodine is released (Kylin 1928). Iodine, together with hydrogen peroxide and the peroxidases of the alga, gives rise to many possible chemical interactions and consequently different halogenated organic

compounds, compared to reactions made only by peroxidases and hydrogen peroxide.

Marshall et al. (2000) stated: ''However, if the biosynthetic trait for trichloroethylene and perchloroethylene production is established in this species to the extent suggested by the high rates of release recorded by Abrahamsson et al. (1995), 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. (1995).'' We do not agree with this statement. The algae change their metabolism according to the environmental conditions and their life cycle. It is therefore incorrect to claim that if the algae under certain conditions produce toxic volatile halocarbons, they should always do so under any environmental condition.

Marshall et al. (2000) suggest that trichloroethylene and perchloroethylene could possibly be derived from biosynthesis of tetrachloroethane or pentachloroethane by algae, or by abiotic dehydrohalogenation. In bacteria, fungi, algae, and plants, ethylene is produced and readily released from all tissues to the surroundings. The concentration of ethylene is generally estimated to 4.4 nmoles L^{-1} in the organisms. The rate of production depends on the age of the organism and the environmental conditions. Ethylene production is usually related to physiological stresses. Any type of wounding will induce ethylene biosynthesis, as well as all kinds of stress. Ethylene is synthesized by plants from S-adenosylmethionine, which is synthesized from methionine and adenosine 5′-triphosphate (ATP) (ATP is mainly formed in light), and is an intermediate in the ethylene biosynthetic pathway. The immediate precursor of ethylene is 1-aminocyclopropane-1-carboxylic acid (ACC), which is converted to ethylene by the enzyme ACC oxidase (Taiz and Zeiger 1998). It is possible that both trichloroethylene and perchloroethylene are formed by halogenating reactions from ethylene produced during stress by the algae, and not only from the conversion of tetrachloroethanes and pentachloroethanes.

Incubation of algae—Another difference between the two investigations is that the incubation setup used by Marshall et al. (2000) is not identical to ours. Marshall et al. (2000) performed their experiments with a headspace of air, whereas we took care to avoid headspace. There are several reasons why such a volume of air should be avoided. Marshall et al. (2000) comment on the losses of halocarbons to the air inside the incubation chamber, and suggest that the losses should be of minor importance due to the relatively low Henry's law constants. However, other parameters might be changed using headspace, owing to the presence of organic compounds, as well as an alga.

The headspace present in the incubation experiments performed by Marshall et al. (2000) may well have changed factors initiating oxidative stress, thereby changing the production of hydrogen peroxide, and consequently the production of trichloroethylene and perchloroethylene by the algae. During the incubation, we have a high production of hydrogen peroxide in the closed vials and other stress-related compounds, as the algae become deficient in total inorganic carbon and the pH increases to high levels (pH $\sim$ 10) due to photosynthesis. The uptake of inorganic carbon at pH 8.2 or higher depends on the ability of the algae to convert HCO_3^- to CO_2. Carbon acquisition mechanisms of marine macroalgae are described in the textbook by Falkowski and Raven (1997).

Table 1. The halocarbon production rates of two macroalgae determined by GC-MS. The rates are given in ng per g fresh weight (FW) and hour (ng g FW^{-1} h^{-1}). (Abrahamsson and Ekdahl, unpubl. data.)

Compound	*Meristiella gelidium*	*Gracilariopsis lemaniformis*
Methyliodide	2	0.8
Ethyliodide	2	0.2
Tetrachloromethane	*	0.4
Trichloroethene	20	0.1
Dibromomethane	30	2
Chloroiodomethane	3	*
2-iodobuthane	*	1
Dibromochloromethane	40	2
1-iodobuthane	*	0.1
Bromoform	2 500	100
Diiodomethane	40	*

* Not detected.

We believe that a large headspace such as that used by Marshall et al. (2000) will change the chemical environment in an unpredictable way, unless otherwise explained.

The different results shown by the two investigations raise the question of which method mimics best the natural environment. We have compared the production rates measured in our incubation experiments with production rates measured in a natural environment at the Canary Islands (Ekdahl et al. 1998). The production rates derived in the laboratory experiments are in agreement to the in situ measurements. Unfortunately, *Falkenbergia hillebrandii* was not present in the investigated pool.

Supportive results—Identification of naturally produced halocarbons by two red macroalgae, *Meristiella gelidium* and *Gracilariopsis lemaniformis,* was achieved by using the method described in Ekdahl and Abrahamsson (1997), and the results are presented in Table 1. The incubations were made in duplicates, with a corresponding blank consisting of only seawater. The algae (0.6 g fresh weight) were incubated in 60 ml of seawater at a light intensity of 300 μmol photons m^{-2} s^{-1} for 3 h. The identification and quantification were performed with a mass spectrometer as detector. The measured production rates are comparable with the results presented by Abrahamsson et al. (1995), and Ekdahl et al. (1998). Also, tetrachloromethane, an even more controversial compound, was produced by one of the algae.

Earlier we have shown that there are large diurnal variations in the production of halocarbons (Ekdahl et al. 1998) in a tidal pool. Not only the traditionally expected halocarbons showed diurnal variations, but also trichloroethylene and perchloroethylene. Comparisons were made with the anthropogenic 1,1,1-trichloroethane, which did not vary over a period of 24 h. The highest concentrations were measured at the time of day with maximum photosynthesis.

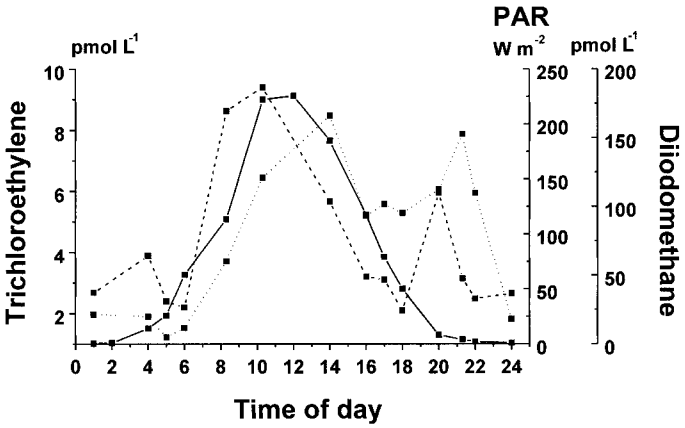

Fig. 1. Diurnal variation of the concentrations of trichloroethylene (dashed line, left axis), diiodomethane (dotted line, right axis), and PAR (straight line, right axis). Samples were taken every hour or every 2 h during a 24-h period at a station located at 60°23′S, 6°00′E in the Southern Atlantic Ocean (Abrahamsson and Lorén, unpubl. data). The determinations were made according to Ekdahl and Abrahamsson (1997).

Similar results were obtained in the open ocean. The determination was made according to Ekdahl and Abrahamsson (1997). The low concentrations in oceanic seawater do not permit the use of a mass spectrometer as detector. However, a gas chromatography–mass spectrometry system, with an identical chromatographic capillary column, confirmed the retention times and the elution order. As can be seen in Fig. 1, the concentrations of trichloroethylene and diiodomethane, measured at chlorophyll maximum at 25 m, reach their maxima during midday and again indicate the relationship between the production of trichloroethylene and photosynthesis. A second maximum was observed in the evening, which probably is caused by hydrogen peroxide produced during respiration. The levels of tetrachloromethane and 1,1,1-trichloroethane did not vary over this 24-h period.

Conclusions—The results obtained by Marshall et al. (2000) do not contradict the findings of Abrahamsson et al. (1995), but they clearly demonstrate that the history of an algae, and the pretreatment of the algae, are crucial factors for the ability of algae to produce halocarbons. The results from these two studies prove the need to standardize the methods used for production studies of halocarbons, in order to be able to make relevant comparisons.

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