Environment Agency

Evidence

A Literature review of the potential health effects of marine microalgae and macroalgae

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Evidence Directorate

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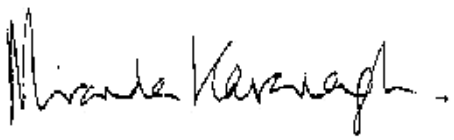

Miranda Kavanagh

Director of Evidence

Executive summary

The purpose of this report is to review the evidence in the literature regarding potential human health risks in bathing waters in the UK posed by phytoplankton, cyanobacteria and macroalgae in relation to the Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC:

Article 8

Cyanobacterial risks

1. When the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks.

Article 9

Other parameters

1. When the bathing water profile indicates a tendency for proliferation of macro-algae and/or marine phytoplankton, investigations shall be undertaken to determine their acceptability and health risks and adequate management measures shall be taken, including information to the public.

Terms of reference:

To review existing scientific knowledge of marine macro and micro algae, and the potential health risks posed by direct contact, in relation to bathing waters and recreation.

To identify serious gaps in existing knowledge which would compromise the EA's ability to comply with Articles 8 and 9 of the 2006 Directive.

If gaps are identified to outline the work necessary to rectify this.

The work is not to include an evaluation of -

the aesthetic impacts of blooms at bathing waters;

the risks caused through the vectoring of shellfish toxins via fish or shellfish.

Conclusions

Based on a review of the extensive literature on potentially harmful algae, risk assessments have been made primarily with reference to the Environment Agency's list of 'notifiable' and nuisance bloom-forming phytoplankton and cyanobacteria. An assessment has also been made of possible hazards presented by marine macroalgae (seaweeds). These assessments are summarized in the table below.

The risks to human health due to short-term contact, aspiration (ingestion) or inhalation (of aerosols or dry particles) with marine phytoplankton, including toxic genera, that presently occur in UK waters are considered to be generally low, as is contact with seaweeds. In contrast, the risks to human health, either via contact, aspiration or inhalation, presented by blooms of freshwater cyanobacteria is assessed to be high. Research requirements as regards human exposure to cyanobacteria in recreational and other waters as identified by the international community have been highlighted.

Summary of assessments of risk to human health posed by alge in recreational waters, based on reports in the literature			
Alga or group of algae	**Contact**	**Aspiration**	**Inhalation[1]**
Non-toxic marine phytoplankton causing nuisance blooms			
Diatoms	**low**	**low**	**low**
Emiliania huxleyi	**low**	**low**	**low**
Noctiluca scintillans	**low**	**low**	**low**
Phaeocystis spp.	**low**	**low**	**low**
Toxic marine phytoplankton			
Amphidinium carterae	**low**	**low**	**low**
Chrysochromulina polylepis	**low**	**low**	**low**
Fibrocapsa japonica	**low**	**low**	**low**
Heterosigma akashiwo	**low**	**low**	**low**
Karenia mikimotoi (*Gyrodinium aureolum*).	**low**	**low**	**low**
Nodularia spumigena (cyanobacterium) Not considered a major threat to health in that it does not bloom extensively in UK waters. Not mentioned in WHO Guidelines. The nodularin toxin is regarded as equivalent to microcystin LR and an action level of $5x10^4$ cells.mL^{-1} has been recommended	**high**	**high**	**high**
Pfiesteria spp.	**low**	**low**	**low**
Prorocentrum lima	**low**	**low**	**low**
Prymnesium parvum	**low**	**low**	**low**
Diatoms and dinoflagellates normally toxic to humans via shellfish vectors			
Alexandrium spp. (paralytic shellfish poisoning - PSP) *Dinophysis acuminata* (diahorretic shellfish poisoning - DSP) *Prorocentrum lima* (DSP) *Pseudonitzschia* spp. (amnesic shellfish poisoning - ASP)	**low**	**low**	**low**
Freshwater cyanobacteria			
The WHO Guidelines state that, "For practical purposes, the present state of knowledge implies that health authorities should regard any mass development of cyanobacteria as a potential health hazard."	**high**	**high**	**high**
Seaweeds			
No genera occurring in UK waters presently appear to present a high risk.	**low**	**-**	**-**
Bryozoans			
The bryozoan *Alcyonidium diaphanum* ('sea chervil') causes contact dermatitis in fishermen. It could be mistaken for a seaweed on the strand line. Beach monitors should be able to identify it and be aware of its potential effects.	**low**	**-**	**-**
Note: [1] Inhalation via aerosols or wind dispersed particles of dried algal material, e.g. originating from dried scums			

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1 Introduction

In 1991 an editorial in the *British Journal of Industrial Medicine* (Baxter, 1991) headed "Toxic marine and freshwater algae: an occupational hazard?" concluded that

"At present our knowledge of the hazards to those whose jobs or recreations bring them into contact with algal blooms is very limited. So far hardly any epidemiological studies of such groups have been performed, or even contemplated, but these will be essential if risk assessments and criteria for advising workers and the public are to be developed."

A decade later, in a wide-ranging report entitled *Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters*, Anderson *et al*. (2001) discussed the recreational use of beaches and coastal waters and commented:

"A request to the worldwide Internet mailing list for workers on red tides and HABs called PHYCOTOXINS, asking for examples of HAB species which cause problems related to recreational use of beaches and nearshore marine waters gave only few responses with exact names of HAB species and the references to problems they have caused. There were, however, many requests from list subscribers who wanted information on this topic. These responses clearly show that official guidelines and/or regulations on beach safety during HABs are scarce and only exist in a few countries. This section is based upon information to be published by World Health Organization (WHO) (compiled by Sörensen, pers. comm.)."

The WHO information referred to was subsequently published in 2003 as part of the *Guidelines for Safe Recreational Water Environments. Volume 1: Coastal and Fresh Waters*, chapter 7 'Algae and cyanobacteria in coastal and estuarine waters' and chapter 8 'Algae and cyanobacteria in fresh water', which are referred to throughout this review.

Anderson *et al*. (2001) go on to identify -

- species toxic to humans through inhalation of sea spray (aerosols)
- species toxic to humans through dermal contact
- species toxic to animals (including humans) through oral intake while swimming

- and the WHO Guidelines (Anon., 2003) also identify the same possibilities for exposure: dermal contact, ingestion (aspiration) of water or scum and exposure through inhalation. These publications form the baseline of this report.

The Environment Agency maintains a list of 'notifiable species' of marine phytoplankton (Table 1) for which it routinely monitors, and also monitors the occurrence of 'nuisance blooms' of algae such as *Noctiluca* and *Phaeocystis* and also various diatoms and this review concentrates on these. In addition, the evidence for possible harmful effects of freshwater cyanobacteria on human health is considered.

Table 1. EA 'notifiable' toxic marine microalgal species.

Class	Species	Toxic Effect [1]	Action Levels
Bacillariophyceae	*Pseudo-nitzschia* spp.	ASP	>150,000 cells/litre
Dinophyceae	*Alexandrium* spp. *Gymnodinium catenatum* *Dinophysis acuminata* *D. acuta* *D. norvegica* *Prorocentrum lima* *Amphidinium carterae* *Karenia mikimotoi* *(G. aureolum)*	PSP PSP DSP DSP DSP DSP Ichthyotoxic Ichthyotoxic	Presence of species Presence of species >100 cells/litre >100 cells/litre >100 cells /litre >100 cells /litre Presence of species Presence of species
Haptophyceae	*Chrysochromulina polylepis* *Prymnesium parvum*	Ichthyotoxic Ichthyotoxic	Presence of species Presence of species
Raphidophyceae	*Fibrocapsa japonica* *Heterosigma akashiwo*	Ichthyotoxic Ichthyotoxic	Presence of species Presence of species
Cyanophyceae	*Nodularia spumigena*	Hepatotoxic	1,000 filaments/mL

Note: *[1] ASP = amnesic shellfish poisoning; DSP = diahorretic shellfish poisoning; PSP = paralytic shellfish poisoning*

2 Non-toxic phytoplankton blooms

2.1 *Noctiluca scintillans*

Noctiluca scintillans is a large cosmopolitan athecate (unarmoured) dinoflagellate species that is distributed world wide in cold and warm waters. It is a non-photosynthetic phagotroph, feeding on phytoplankton (mainly diatoms and other dinoflagellates), protozoans, detritus, and fish eggs.

Toxic blooms of *N. scintillans* have been linked to massive fish and marine invertebrate kills. Although this species does not produce a toxin, it has been found to accumulate toxic levels of ammonia in food vacuoles (possibly a buoyancy mechanism), which is then excreted into the surrounding waters possibly acting as the killing agent in blooms (Okaichi & Nishio, 1976). Landsberg (2002) has catalogued a number of deleterious effects caused by *Noctiluca* blooms including the production of mucus that caused mechanical damage to fish gills and interfered with respiration, and a bloom in Venezuela that covered 3 km^2 and resulted in the mass mortality of the mussel *Perna perna*. The mouse bioassay indicated that the mussels were non-toxic. The gills of the mussels were found to be covered in a mucilaginous substance that probably caused death by suffocation.

It has been reported that *Noctiluca* can carry both potentially toxic (but not necessarily pathogenic) bacteria (Kirchner *et al*., 2001) and toxic algae (Escalera *et al*., 2007) and may act as a vector for the distribution of these organisms.

There are no reports of this alga having adverse effects on human health.

2.2 Blooms of diatoms

Diatoms are in ecological terms one of the most important groups of microalgae. They are cosmopolitan and found in all aquatic environments. When diatoms reach cell densities typically encountered in blooms they may be considered potentially harmful. Smayda (2006) lists a number of diatoms (Table 2) that are reported to be harmful.

One of the main harmful effects of blooms of non-toxic diatoms is the creation of hypoxic and anoxic conditions as the bloom senesces and dies and there is increased microbial oxygen demand resulting in large-scale mortalities of fauna in the water column and the benthos as the bloom sediments.

Other harmful effects are due to what Smayda (2006) terms ‘non-toxicological stressors’ due to their abundance or morphology; for example the production of copious amounts of mucilage by *Coscinodiscus wailesii*, which reaches nuisance bloom proportions in the North Sea and can clog fishing nets, and by *Thalassiosira mala*, which clogged the gills of cultured shellfish in Tokyo bay thereby causing large scale mortality.

Table 2. Diatoms reported to have had harmful effects on aquaculture and natural fisheries. From Smayda (2006) where specific effects are detailed. None of these is known to affect human health due to direct contact or via aerosols.

Cerataulina pelagica	*Leptocylindrus minimus*
Chaetoceros concavicornis	*Rhizosolenia chunii*
Chaetoceros convolutus	*Skeletonema costatum*
Chaetoceros debilis	*Thalassiosira aestivalis*
Chaetoceros socialis	*Thalassiosira diporocyclus*
Chaetoceros wighami	*Thalassiosira mala*
Corethron criophilus	*Thalassiosira rotula*
Coscinodiscus wailesii	

Chaetoceros concavicornis and *Chaetoceros convolutus* have adverse effects on finfish in the NE Pacific, causing distress or death. These diatoms have serrated setae (spines), which cause mechanical damage to the gills. For example, in salmonids the diatoms become retained in the inter-lamellar spaces of the gills, where they irritate the goblet cells. This stimulates the over-production and accumulation of mucus on the respiratory epithlium, which in turn reduces the efficiency of oxygen exchange, resulting in hypoxia. As few as 5 cells mL^{-1} seawater may impair oxygen uptake sufficiently in salmonids to cause mortalities and lower concentrations rendered some species of salmon more susceptible to bacterial pathogens (Yang and Albright, 1992; Albright *et al*., 1993). Other incidents related to the effects of other diatoms on fish are reviewed in Landsberg (2002).

2.2.1 Effects on human health

There is a report of the diatom *Fragilaria striatula* possibly causing dermatitis in professional fishermen in Scotland (Fraser and Lyell, 1963). The skin reaction was associated with handling ropes and nets, which had become coated with a mucilaginous layer of the diatom. Bacteria were associated with the diatom growth but their possible involvement was not investigated. There was a subsequent report of evidence of *F. striatula* causing dermatitis in lobster fishermen in the Bardsey sound area of the Lleyn peninsula, N. Wales (Beer *et al*., 1968) and this diagnosis was supported in a follow-up note describing work with a unialgal culture of the diatom (Beer and Jones, 1969).

This could be classified as ‘occupational exposure’, the symptoms occurring after frequent and prolonged contact. The possible effect of short-term exposure remains unknown.

As regards reports of other non-toxic diatoms causing symptoms in humans, the website of the Environmental Protection Authority of the State of Victoria, Australia, http://www.epa.vic.gov.au/water/coasts/surf_diatoms.asp advises bathers to be on the lookout for patches of surf zone diatoms on certain beaches:

“The diatoms which usually make up these patches are not toxic, although they may cause some irritation to the human body. It is advisable to avoid swimming in dense patches or at least shower after swimming or surfing”.

Cell densities in seawater are reported to reach 12 x 10^6 cells. mL^{-1}. The surf-zone diatom common on the surf beaches of Victoria is *Anaulus australis*. Surf-zone diatoms occur in various locations around the world, such as *Attheya armatus* on the NE Pacific coast and *Asterionellopsis glacialis* in Brazil.

It is possible, therefore, that if bathers in UK waters were to swim in blooms of non-toxic diatoms, especially those algae possessing setae, they might experience some iritation of eyes, mucus membranes or skin.

The same website reported the investigation of reports of discolouration of water and foam at Mornington Peninsula surf beaches in the vicinity of the Melbourne Water ocean outfall at Boags Rocks.

http://www.epa.vic.gov.au/water/coasts/boags_rocks.asp

Among the algae detected were the diatoms *Cylindrotheca closterium* and *Asterionellopsis glacialis*, unicellular green algae, non-toxic cyanobacteria and low numbers of a filamentous actinomycete of the *Nocardia* group (which, it was suggested, was a contributing factor in excessive foaming). The conclusion by the EPA was, "There is no evidence to suggest the algal blooms near Boags Rocks were toxic to people, however, it is advisable to avoid swimming in dense patches of discolouration."

2.2.2 Polyunsaturated aldehydes in diatoms

These compounds have been reported to be present in diatoms such as *Thalassiosira rotula*, *Skeletonema marinoi* (formerly *costatum*) and *Pseudo-nitzschia delicatissima* (Hansen *et al*., 2004a,b, 2007; Ribalet *et al*., 2007) and are discussed in more detail in sect. 2.3.5.

2.3 *Phaeocystis* spp.

Phaeocystis, a member of the class Prymnesiophyceae, is a cosmoplitan genus, consisting of six species. It is polymorphic and has a complex life cycle where it may alternate between single cells 3–9 μM in size, which may be motile or non motile, and a colonial form up to several mm in diameter where the cells are embedded in a polysaccharide matrix, and it is this form that can occur in huge blooms. The mucilaginous matrix can constitute 80% of the total biomass of a bloom at maximum development (Thingstad and Billen, 1994).

Only three species are known to form blooms: *P. globosa* in temperate regions (North Sea coasts, the English Channel and China), *P. pouchetii* in temperate to polar waters of the northern hemisphere, and *P. antarctica* in polar and sub-polar waters of the southern hemisphere (Alderkamp *et al.* 2007 and references therein; Rousseau *et al*., 2007).

The distinctive characteristic of the huge *Phaeocystis* blooms is the production of foam (Lancelot, 1995) when the cells have become nutrient limited and senescent; the cells start to lyse and the colony matrix begins to degrade. The result of this breakdown is the release of huge amounts of dissolved organic matter rich in poly-saccharides, which over time tend to form stable and persistent hydrogels (definition of hydrogel: a colloid in which the particles are in the external or dispersion phase and water in the internal or dispersed phase). It is this foam (hydrogel) that causes a nuisance when driven by wind and tide onto bathing beaches, often forming windrows a metre or more high. This complex process and the fate of carbohydrates originating from *Phaeocystis*

has been described in detail by Thingstad and Billen (1994) and by Alderkamp *et al*. (2007).

Although producing nuisance blooms resulting in anoxia and fish kills (see Landsberg, 2002), *Phaeocystis* has historically not been regarded as toxic until quite recently. Smayda (2006) briefly reviewed the literature and concluded

"Notwithstanding the diverse nuisance and toxic impacts reported for *Phaeocystis,* its blooms are usually without the apparent negative impacts, particularly fish kills, reported for the related haptophyte species *C. polylepis* and *P. parvum* f. *parvum* and f. *patelliferum*."

As noted in Hansen *et al*. (2003) the International Oceanographic Commission has placed *P. globosa* and *P. pouchetii* on its list of harmful algae (see http://www.bi.ku.dk/ioc/) and the entry states: "*P. globosa* was reported to form

toxins in China (Qi *et al*., 2002). However, the short abstract published only mentions that hemolysin(s) was formed", but these records are now several years old.

A more recent review by Verity *et al*. (2007) discusses the toxic properties of *Phaeocystis* spp. in the context of allelopathy. Those properties, taken from the literature cited, are summarised in Table 3. The concept of allelopathy in phytoplankton - the production of compounds by an alga that inhibit the growth of competing algae - is discussed by Legrand *et al.* (2003). It goes some way to explaining the reasons for the toxic characteristics of many algae.

Table 3. Compounds with allelopathic and possibly toxic properties produced by *Phaeocystis* spp.

Compound	Biological effect	Methodology	Reference
dimethyl sulphide (DMS)		Both compounds produced as a result of exo-enzyme action on dimethyl-sulphoniopropionate (DMSP)	Liss *et al.* (1994); Verity *et al*. (2007) and refs. therein
acrylic acid	antibiotic? allelopathic		
?	Low haemolytic activity. Anaesthetic effect by fly bioassay	Extracted from *P. pouchetii* cells and associated seawater medium with organic solvent and sorbent fractionation	Stabell *et al.* (1999)
? polyunsaturated aldehyde	Antimitotic by sea urchin embryo bioassay	Extracted from *P. pouchetii* cells and associated seawater medium with organic solvent. Cell extract mildly toxic, seawater extract completely prevented cell division	Hansen *et al.* (2003)
polyunsaturated aldehyde	Cytotoxic by yeast bioassay Antimitotic by sea urchin embryo bioassay	Extracted from *P. pouchetii* cells and associated seawater medium with organic solvent. Cell extract mildly toxic, seawater extract significantly toxic	Hansen *et al*. (2004a,b)
?	Haemolysis – human erythrocyte lysis bioassay	Intact, live *P. pouchetii* cells showed the highest activity. Filtered sea water and cell extracts were less haemolytic or without effect	van Rijssel *et al.* (2007)
galactolipid	Haemolytic activity	Extracted from *P. globosa*	van Rijssel *et al.* (2007) and refs. therein.

2.3.1 Dimethyl sulphide (DMS) and acrylic acid

DMS and acrylic acid are produced by many algae, including *Phaeocystis* spp. and *Emiliania huxleyi*, possibly as a result of physiological stress. These compounds are produced in equimolar amounts from the cleavage of dimethylsulphoniopropionate (DMSP) by a membrane-bound extracellular enzyme. (Liss *et al*., 1994; Verity *et al.*, 2007 and refs. therein).

2.3.2 DMS

The role and significance of *Phaeocystis* as a producer of DMS has been reviewed by Liss *et al*. (1994). DMS not only occurs in the oceans and atmosphere, it is responsible for the characteristic flavor of many foods and beverages consumed by humans, such as asparagus, potatoes, ripened cheeses and beer (Pérez-Gilabert and García-Carmona, 2001). The production of DMS has also been detected in several fungi and correlated with melanogenesis. The enzyme tyrosinase (polyphenol oxidase) is involved in the biochemical pathway of melanin production. It has been shown in studies on the effect of DMS on fungal tyrosinase that it acts as a slow-binding competitive inhibitor and may have a metabolic regulatory role (Pérez-Gilabert and García-Carmona, 2001).

In a study on rats, groups of 15 males and 15 females were given doses of 0 (control), 2·5, 25 or 250 mg DMS. kg^{-1}. day^{-1} for 14 weeks. No effects on the rate of body-weight gain, intake of food and water, results of haematological examinations, serum-enzyme levels, urinary cell excretion, renal concentration tests, organ weights or histo-pathological changes were attributable to the treatment. A no-untoward-effect level of 250 $mg.kg^{-1}$ body $weight.day^{-1}$ was established (Butterworth *et al*., 1975).

van Duyl *et al*. (1998) measured the concentrations of DMS in the water during a spring bloom of *Phaeocystis*. DMS production and consumption were roughly in balance, with production only slightly exceeding consumption at the start of the bloom. Rates of production and consumption were highest during the exponential growth phase of *Phaeocystis* and declined in the course of the bloom (from 300–375 to less than 5 $nmol.dm^{-3}.d^{-1}$. Given the toxicological data for rats it seems unlikely that short-term exposure to these low concentrations would have any significant effect on humans, including tyrosinase involved in skin pigmentation.

2.3.3 Acrylic acid

Noordkamp *et al*. (1998) studied the production of acrylic acid during growth of axenic laboratory cultures and samples of *Phaeocystis* collected from the field. In the exponential phase of growth only 12.5% of the total acrylate was found in the culture medium as opposed to 98% in the stationary phase.

The concentration of acrylate in exponentially growing cultures was 0.1–1 µM, similar to that observed in the field, but in the stationary phase the concentration ranged from 1–4 µM and was due both to cellular excretion and lysis. In the exponential phase of growth acrylate was associated with the colonies and appeared to be concentrated in the 7µm layer of mucilage on the colony surface. The deduction that the acrylate was concentrated in the surface layer was based on the relationship between colony volume and acrylate content. The concentration of acrylate appeared to correlate with colony surface area not volume. It was calculated that the concentration of acrylate was quite high, 1.5–4.2 mM for one axenic isolate, 2.8–6.5 mM for another and 1.3 mM in a bloom sample. They concluded that the high concentration on this ‘microscale’, as they termed it, might be sufficient to inhibit bacteria, but as it appeared tightly bound in the exponential phase and it was suggested that it might not be accessible to bacteria. When colonies *in vitro* started to decay then acrylate was released into the surrounding medium. However, in bloom samples acrylate was not detected so and was assumed to be consumed by bacteria. Subsequent studies showed that the acrylate does not appear to have an adverse effect on bacteria surrounding *Phaeocystis* (Noordkamp *et al*., 1998).

It has been suggested (Verity *et al*., 2007) that acrylic acid in *Phaeocystis* may play a role in deterring predators as has been shown for the related genus *Emiliania huxleyi*

(Wolfe *et al*., 1997) where there was an enzyme-mediated release of high concentrations of acrylate in grazer food vacuoles.

2.3.4 Toxicity of acrylic acid to humans

The International Programme on Chemical Safety (IPCS) website

http://www.inchem.org/documents/hsg/hsg/v104hsg.htm#SectionNumber:2.1

Health and Safety Guide No. 104 ACRYLIC ACID provides the following information: "It is recommended that exposure of the general public to acrylic acid in the ambient air and drinking-water does not exceed the guidance values given in the Environmental Health Criteria No. 19 Acrylic Acid (IPCS, 1997). These are as follows: Inhalation exposure: 54 μg/m^3; Oral exposure via drinking water: 9.9 mg/ litre." Other toxicological data are provided in this paper, including animal tests.

Given the concentrations of acrylate reported in *Phaeocystis* colonies and in foam and the above recommended limits this compound probably does not present a significant hazard to bathers via inhalation (aerosols) or by ingestion of seawater.

2.3.5 Polyunsaturated aldehydes

Polyunsaturated aldehydes (PUAs), which are highly reactive compounds, were originally reported to be present in diatoms such as *Thalassiosira rotula*, *Skeletonema marinoi* (formerly *costatum*) and *Pseudo-nitzschia delicatissima* (Hansen *et al*., 2004a,b, 2007; Ribalet *et al*., 2007).

The production and release of PUAs by the diatoms arises from the decomposition of unsaturated fatty acids in an enzymatic cascade, thought to be induced as a response to mechanical stress. Unsaturated fatty acids liberated from membrane storage sites by phospholipases are converted to lipid hydroperoxides by lipoxygenases, which again are transformed into unsaturated aldehydes by lyases. PUAs have cytotoxic effects on several cell types, such as inhibition of hatching and cell division in crustaceans, echinoderms and polychaetes and cell proliferation in human cells, diatoms and bacteria (Ribalet *et al*., 2007 and refs. therein) and see Table 3. The most toxic PUA released by *P. pouchetii* has been identified as the α, β, γ, δ- unsaturated aldehyde 2-*trans*-4-*trans*-decadienal (Hansen *et al*., 2004).

There appear to be no reports of deleterious effects of PUAs on humans apart from *in vitro* studies on human cells.

2.3.6 Haemolytic galactolipid

In 1997 a massive bloom of *Phaeocystis globosa* in coastal waters off S China caused extensive fish mortalities. A glycolipid with digalactose and polyunsaturated fatty acid (heptadecadienoyl) moieties was shown to be responsible for the cause of the fish mortality by induction of pores in the cell membrane of target cells. Both the isolated toxin and supernatant of the *P. globosa* cultures inhibited cultures of other microalgae (van Rijssel *et al*., 2007 and refs. therein).

2.3.7 Other organisms associated with *Phaeocystis* colonies

There are reports of other organisms associated with *Phaeocystis* colonies and foam and it is possible that these could give rise to irritation by dermal contact.

Verity *et al*. (2007) highlight the work of Sahzin *et al.* (2007) who enumerated organisms associated with *Phaeocystis pouchetii* colonies growing in mesocosms in Norway and in blooms of *P. globosa* in the English Channel.

The surface of large (>250 µm) *P. pouchetii* colonies were populated with *Pseudo-nitzschia* cf. *granii* var. *curvata*, which did not exceed 40 cells per colony, and to a lesser extent by other phytoplankton and protists. *Pseudo-nitzschia delicatissima* colonized the surface of large (>100 µm) *Phaeocystis globosa* colonies from the English Channel. The abundance of these diatoms reached 130 cells per colony and formed up to 70% of the total carbon associated with *Phaeocystis* cells during late bloom stages and it was suggested that the diatoms were able to utilize the polysaccharides in the colony matrix for growth.

Armonies (1989) found that *Phaeocystis* foam in the Dutch Wadden Sea contained significant numbers of harpacticoid copepods, which are normally found as meiofauna in the upper sediment layers. It was not clear whether they had been passively entrapped or actively migrated. It is possible but unlikely that these could act as allergens.

2.3.8 Effects on human health

Phaeocystis is not generally considered a hazard to health: “These blooms are not directly detrimental to human health as are other toxic blooming algae” - a quotation from a Belgian scientific report (Anon., 2002a). References to any symptoms caused by *Phaeocystis* foam appear to be rare and anecdotal, for example, this statement from the Friends of the Earth Marinet website: www.marinet.org.uk/glossary.html

“Children love to play in it. But beware - it can produce an irritating rash in allergic youngsters.”

The national report for the Netherlands regarding HAB incidents in 2001, submitted to the ICES-IOC Working Group on Harmful Algal Bloom Dynamics (Anon., 2002b) includes this statement: “In August, swimmers at the ‘Hoek van Holland’ beach (in the Rhine outflow) reported skin irritations. Microscopic counts of a sample collected at this time indicated a *Phaeocystis* concentration of 4.0×10^9 cells.L^{-1}.”

2.4 *Emiliania huxleyi*

This cosmopolitan alga often forms massive blooms in many of the world’s seas and oceans, including the waters around the UK, producing spectacular satellite images due to the optical properties of its calcareous scales (coccoliths). It produces dimethyl sulphide (see Malin *et al*., 1993) as outlined in 2.3.2.

Blooms are reported to be non-toxic (Jahnke, 1992) and the alga has been shown to be non-toxic in the *Artemia* brine-shrimp lethality bioassay (Rhodes *et al*., 1995, cited in Houdan *et al*., 2004). There appear be no reports in the scientific literature regarding known toxicity or other deleterious effects on humans via aspiration, dermal contact or aerosols.

2.4.1 Other coccolithophore algae

Houdan *et al*. (2004) studied the potential toxicity of eleven members of the order Coccolithales, nine coastal and two oceanic, as regards toxicity as measured by the brine shrimp (*Artemia salina*) lethality bioassay. Five coastal coccolithophores, *Pleurochrysis carterae*, *P. elongata*, *P. placolithoides*, *P. roscoffensis* and *Jomonlithus littoralis*, were observed to be toxic to *Artemia*. *Pleurochrysis elongata*, *P. placolithoides* and *P. roscoffensis*, were more toxic than *P. parvum*, the toxic control and *Jomonlithus littoralis* was more toxic than *Prymnesium parvum* during the growth phase. The oceanic species were not toxic as measured by this bioassay.

Other prymnesiophytes known to be toxic to other organisms, such as fish and invertebrates, are listed in Landsberg (2002) and Houdan *et al*. (2004). There are no references to toxic effects on humans.

3 Toxic Phytoplankton

3.1 Toxic diatoms and dinoflagellates causing shellfish poisoning

Various species of the diatom *Pseudonitzschia* produce the toxin domoic acid that is the cause of amnesic shellfish poisoning (ASP). Certain species of the dinoflagellate genus *Alexandrium*, e.g. *A. minutum* and *A. tamarense*, produce various toxins belonging to the saxitoxin group that are the cause of paralytic shellfish poisoning (PSP), as does *Gymnodinium catenatum*, and the dinoflagellates *Dinophysis acuminate*, *acuta* and *norvegica* produce toxins in the okadaic acid group, that are responsible for diarrhetic shellfish poisoning (van Egmond *et al*., 2004; Leftley and Hannah, 2008). The shellfish that feed on these algae concentrate the toxins in their tissues and because they filter large volumes of water, e.g. mussels may filter several litres per hour, the algae do not need to be present in high concentrations. For example, one to two hundred cells per litre of toxic *Alexandrium* may be sufficient to engender toxicity in the shellfish sufficient to cause poisoning if eaten (Townsend *et al*., 2001 and refs. therein) and blooms of *Alexandrium* and *Dinophysis* do not approach the densities of 'nuisance algae' blooms such as *Phaeocystis* spp. - see notes to Table 6.

The WHO guidelines (Anon., 2003) note the absence of evidence of adverse effects due to ingestion of seawater containing seawater containing species of algae responsible for ASP, PSP and DSP as well as those responsible for neurotoxic shellfish poisoning (NSP). This is discussed further in sect. 4. It was also noted also that there is little information on adverse effects of dermal contact with marine waters containing these groups of toxic algae and no incidents appear to have been reported in the scientific literature since 2003.

3.2 *Prorocentrum lima*

The epibenthic and epiphytic dinoflagellate *Prorocentrum lima* is widely distributed in coastal seas and estuaries in both temperate and tropical regions around the world and is common in UK waters. It produces diarrhetic shellfish poisoning (DSP) toxins, primarily okadaic acid and dinophysistoxin-1 (Quilliam, 2004 and refs. therein) and also the toxic macrolide prorocentrolide (Leftley and Hannah, 2008).

P. lima has been found associated with seaweed and seagrass in Fleet Lagoon, Dorset. This is a coastal area where shellfishery closures have occasionally been enforced due to elevated levels of DSP toxins in shellfish (Foden *et al*., 2005) and *P. lima* is probably the cause. (See also notes (5) and (6) in Table 6).

Although normally epiphytic or epibenthic in habit, planktonic blooms of *P. lima* have been described, for example in locations in the W Adriatic (Ingarao *et al*., 2007) reaching densities of ca. 4.7×10^5 cells.L^{-1}.

There is no evidence from the literature that. *P. lima* is a danger to human health other than due to consumption of DSP toxins via shellfish vectors. One of the authors of this report (JWL) can add this anecdotal evidence: He has cultured hundreds of litres of DSP toxic *Prorocentrum lima* as part of experiments to toxify shellfish. He has been exposed to cultures for many hours and has even immersed his hands in dense cultures and can report no short-term ill-effects.

3.3 *Amphidinium carterae*

A. carterae is cosmopolitan and is found in estuarine and coastal areas of both tropical and temperate waters. It occurs all round the British Isles. Like *Prorocentrum lima* it is predominantly sessile, being epibenthic on seaweeds and benthic in sand, where it buries itself in the surface layer. Bioassays have shown cell extracts to be lethal to mice, ichthyotoxic and haemolytic (Yasumoto *et al*., 1987; Landsberg, 2002). Its toxicity to humans is via the food chain as it is one of the dinoflagellates implicated in ciguatera fish poisoning in tropical and sub-tropical regions (Anderson *et al*., 1987). There are no reports that it causes harm to humans via inhalation, aspiration or dermal contact. Regarding the latter, because of its benthic lifestyle the possibility that it might come into contact with human skin cannot be ruled out.

3.4 *Karenia mikimotoi* (formerly *Gyrodinium aureolum*)

Gyrodinium aureolum, *G*. cf. *aureolum*, *G. nagasakiense* and *G*. *mikimotoi* are now widely accepted as being synonymous with *K. mikimotoi*, see - http://www.algaebase.org/search/species/detail/?species_id=44334 http://www.bi.ku.dk/ioc/details.asp?Algae_ID=73

Because of the fact that there have been major blooms of *K. mikimotoi* around the British Isles in recent years - in the western English Channel in 2003 (Vanhoutte-Brunier *et al*., 2008), along the W coast of Ireland in 2005 (Silke *et al*., 2005) and the Scottish coast in 2006 (Davidson *et al*., 2007) - this dinoflagellate is discussed in some detail.

K. mikimotoi is cosmopolitan and it is one of the commonest dinoflagellates occurring in northern European waters, being found throughout the North Sea, eastern Irish Sea, Celtic Sea, western English Channel and Scottish coastal waters (Smayda, 2006). Harmful blooms of *K. mikimotoi* occur worldwide and their history has been reviewed briefly by Silke *et al*. (2005) and Smayda (2006) with the latter reviewing harmful incidents in UK waters.

Although responsible for extensive mortalities both in natural ecosystems and aquaculture systems the mechanism of toxicity is not fully understood. Silke *et al*. (2005) suggested that the harmful effect may be due to a combination of toxicity and reduction in dissolved oxygen in the water column and the benthos caused by increased oxygen demand both from algal respiration from bacteria as a dense bloom senesces and decays. In situations where the water column becomes stratified and there is no mixing, the bottom water becomes deficient in oxygen or even completely anoxic due to algal and/or bacterial consumption, leading to extensive mortalities of benthic fauna.

3.4.1 Toxins in *K. mikimotoi*

Two toxins, gymnocin-A and -B, have been isolated from *K. mikimotoi* (Satake *et al*., 2002, 2005; Tsukano *et al*., 2006) and these compounds resemble some of the brevetoxins in the related alga *Karenia brevis*, the dinoflagellate that produces potent polyether toxins, the brevetoxins (BTXs), which are responsible for neurotoxic shellfish poisoning (Leftley and Hannah, 2008) and respiratory symptoms in humans (see sect. 5.). However, the toxicity of a mixture of gymnocins-A and -B was 250 times less than 42-dihydro BTXb when these compounds were bioassayed using a freshwater fish,

Tanichthys albonubes (Igarishi *et al*., 1999, cited in Satake *et al*., 2005). The disparity between the apparent weak toxicity of the gymnocins as measured by laboratory bioassays and obvious effects of *K. mikimotoi* blooms in the natural environment was attributed to low solubility in water of the pure compounds, a factor that may have reduced contact with the fish gills. It was suggested that under bloom conditions the cells of *K. mikimotoi* clog the gills of fish and are in direct contact with the lamellae and thereby might enhance transfer of the gymnocins.

A study of the structure-activity relationship of gymnocin-A indicated that the α, β-unsaturated aldehyde functionality of the side chain caused cytotoxicity, which was also related to the length of the molecule (Tsukano *et al*., 2006).

Toxic effects due to other compounds have been reported and are summarized in Table 4.

Table 4. Reports of toxic or allelopathic effects of *K. mikimotoi.*

Effect	Compound	Detail	Reference
production of free radicals	superoxide anion and hydrogen peroxide	*K. mikimotoi* produced superoxide in the culture medium but to a lesser extent than *Chattonella marina* (Raphidophyceae). Also fish mucus and other compounds stimulated O_2^- production in *C. marina* but not *K. mikimotoi*.	Yamasaki *et al.* (2004) and see Marshall *et al*. (2005)
toxicity of lipids	glycoglycerolipids	*Gymnodinium mikimotoi* contained 17% of mono-galactosyl diacylglycerol and digalactosyl diacylglycerol, which had haemolytic activity.The major unsaturated fatty acid of the glycolipids was octadecapentaenoic acid (18:5n-3)	Parrish *et al*. (1998)
	fatty acids	Pure (synthesised) octadeca-pentaenoic acid (18:5n-3) stimulated mucus production and affected morphology of ionocytes and inhibited ATPases in gills of sea bass *Dicentrarchus labrax*. Possible effects on osmoregulation	Sola *et al*. (1999)
	fatty acids	Pure (synthesised) octadeca-pentaenoic acid (18:5n-3) and other fatty acids known to be present in *G*. cf. *mikimotoi* altered intracellular pH of isolated trout hepatocytes and decreased K^+ uptake into the hepatocytes, indicating ATPase inhibition, High concentrations (10^{-5}–10^{-3}	Fossat *et al*. (1999)

		M) of fatty acid were necessary to induce these effects.	
	fatty acids	Pure (synthesised) octadeca-pentaenoic acid (18:5n-3) delayed or inhibited first cleavage of sea urchin *Para-centrotus lividus* eggs and caused abnormalities in embryo development.	Sellem *et al*. (2000)
haemolysis*	whole algal cells	Using an improved assay, clones of *K*. *mikimotoi* were found to have unexpectedly high haemolytic activity compared to isolates *of K. brevis*.	Neely and Campbell (2006)
effects on shellfish	whole algal cells	Eight commercially important juvenile shellfish of various genera were exposed to *G. aureolum* (*K. mikimotoi*). The effect appeared to be selective. Lesions and other pathological effects were observed in some of the genera.	Smolowitz and Shumway (1997)
effect on immune system	whole algal cells	Manila clams (*Ruditapes philippinarum*) were exposed to *K. mikimotoi* at bloom concentrations for varying periods of time. Sub-lethal and pathological changes were observed. Total haemocyte counts increased in clams exposed to the harmful alga, while the percentage of dead haemocytes, as well as haemocyte size and complexity, decreased.	Hégaret *et al.* (2007)
lysis of phyto-plankton cells	volatile sesqui-terpenes - cubenol epicubenol and α-cadinol	Sesquiterpenes were found to be excreted by *G. nagasakiense* (= *K. mikimotoi*) both in culture and natural blooms. Cubenol at 5 ppm. caused lysis of *Heterosigma akashiwo*, *Chattonella antiqua*, and *C. marina* as well a *G. nagasakiense* itself.	Kajiwara *et al.* (1992)
growth inhibition	exotoxins	Inhibit growth of other microalgae (allelopathy)	Gentien *et al.* (1990). See also Legrand *et al*. (2003)

**** Note:** Using a carp erythrocyte assay Eschbach et al. (2001) did not detect haemolytic activity in a cultured strain of G. aureolum originally known to be toxic when first isolated. This was attributed to loss of toxicity during prolonged culture.*

3.4.2 Effects on human health

There are no reports that *K. mikimotoi* has affected human health via seafood poisoning. In reviewing the detrimental effects of *G. aureolum* (= *K. mikimotoi*) blooms Mahoney *et al*. (1990) commented:

"The potential threats of *G. aureolum* blooms are not clear. An absence of documented poisoning of humans associated with the species suggests that a serious public health problem is unlikely".

Despite the wide range of toxic effects of whole cells and isolated compounds only two references in the literature to possible symptoms in humans caused by *K. mikimotoi* have come to light. Blooms of *Gyrodinium aureolum* (= *K. mikimotoi*) occurred in New Jersey (USA) coastal waters in the summers of 1984 and 1985 (Mahoney *et al*., 1990). Cell concentrations in the bloom zones during the period of observation ranged from 1.5–36 x 10^6 cells.L^{-1} (geometric mean 3.4 x 10^6 cells.L^{-1}). These adverse effects on humans were noted:

"Symptoms in humans coincidental with bloom exposure to the bloom water included nausea, sore throat, eye irritation and lung congestion (Marino, personal communication). Complaints were primarily from individuals, such as lifeguards, who had relatively extensive contact with the bloom water, or who were on the beaches for long periods when it was present."

The second reference was in the WHO guidelines (Anon., 2003) and is dealt with in sect. 5.

An Environment Agency report from SW region, Cornwall division/area, dated 26 July 2002, recorded a relatively low density bloom at Whitsand Bay, Tregonhawke, in which the major taxa were identified as *Gyrodinium aureolum* (11 x 10^3 cells.L^{-1}) and *Pseudonitzschia* spp. (55 x 10^3 cells.L^{-1}) and noted on the report form against 'Evidence of toxic effect', simply, "Yes, lifeguards falling ill."

A protracted bloom of *Karenia mikimotoi* occurred in summer 2005 along the northern half of the western Irish coastline. This bloom subsequently dissipated during the month of July and was then succeeded by a bloom of the same alga in the southwest in late July. The bloom was very intense and resulted in discolouration of seawater and foaming in coastal embayments. Major mortalities of benthic and pelagic marine organisms were observed and devastation of marine faunal communities was reported and observed in a number locations. Deaths of echinoderms, polychaetes and bivalve molluscs were observed in County Donegal and Mayo, while farmed shellfish and hatchery raised juvenile bivalve spat suffered significant mortalities along the Galway and Mayo coasts. Reports of dead fish and crustaceans were received from Donegal, Galway, West Cork and Kerry.

The progress and effects of this exceptional bloom were observed and reported in great detail (Silke *et al*., 2005). No adverse effects on humans were reported (J. Silke, personal communication).

During the persistent and extensive bloom of *K. mikimotoi* that occurred in Scottish waters in 2006, mortalities of marine fauna were attributed to the dinoflagellate, but there were no reports of effects on human health (Davidson *et al*., 2007; K. Davidson, personal communication).

3.4.3 Conclusions

Given the paucity of hard evidence it is not possible to say whether *K. mikimotoi* blooms would present a serious health hazard to humans either by inhalation of aerosols, aspiration or by direct contact with skin or conjunctiva.

Gentien *et al.* (2007) studied the motility and autotoxicity of *K. mikimotoi* in laboratory cultures. The dinoflagellate excretes a toxic glycoglycerolipid and/or fatty acid (Parrish *et al*., 1998), which may become toxic to the alga itself (autotoxicity) if a certain cell density is exceeded. They postulated that the range of action of the toxin is the result of a balance between the production flux at the cell membrane, the molecular diffusion and the rate of decay of the unstable toxin. Local saturation of the medium does not occur since the toxin is unstable and the toxic effect acts only at a short distance. They concluded that, under the conditions of their experiments, each algal cell is surrounded by a microzone of toxin no greater than 175 µm in diameter. The toxin would only be effective as regards allelopathy and autotoxicity within that radius. They cited evidence that the toxicity of *K. mikimotoi* to another alga, *Heterocapsa circularisquam*, was due to direct contact. The tentative conclusions to be drawn from this as regards human contact is that the effect of the exotoxin lipid(s) is very localized and that there would probably have to be direct contact with some sensitive tissue. Any possible effect would, of course, be related to cell density – Davidson *et al.* (2007) reported a peak bloom density of *K. mikimotoi* of 3.7 x 10^6 cells.L^{-1} in Scapa Flow, Orkney.

K. mikimotoi is athecate, i.e. it is a naked dinoflagellate, and more susceptible to lysis than thecate (armoured) dinoflagellates such as *Alexandrium* spp. When subjected to turbulence created by waves or by a swimmer, lysis would release the gymnocin endotoxins. This is an explanation that has been proposed with regard to the irritant effects of blooms of *K. brevis* (see Kirkpatrick *et al*., 2004). Compounds such as gymnocins or lipids might be released from *K. mikimotoi*, which, if present in sufficient concentration, could cause adverse effects in humans, either by inhalation of aerosols or contact with water containing those compounds. Analysis of samples of *K. mikimotoi* from the Irish bloom for presence of brevetoxins showed none to be present (Silke *et al*., 2005).

On balance, given the available evidence, *K. mikimotoi* blooms are not presently seen as representing a serious threat to human health via direct contact, aspiration or inhalation of aerosols.

Annual blooms of *Karenia brevis*, a closely related and more toxic species, occur in Florida and elsewhere in the USA, such as Texas, and engender respiratory and other symptoms in humans. The health authorities do not ban swimming during such blooms but advise caution (see sect. 5.).

3.5 *Pfiesteria piscicida* and putative estuary associated syndrome (PEAS)

Putative estuary associated syndrome (PEAS), also referred to as possible estuary associated syndrome, is one of six recognised human poisoning syndromes resulting from algal toxins that impact on human health due to consumption of contaminated seafood, direct contact with bloom water or inhalation of toxin in aerosol form (Van Dolah *et al*., 2001). The second and third modes of exposure are relevant to this review.

Currently, it is a health problem so far only associated with highly eutrophic estuaries on the eastern seaboard of the United States, which are subject to toxic blooms of *Pfiesteria*-like organisms.

3.5.1 *Pfiesteria*-like organisms

P. piscicida was first discovered in 1988 in an aquarium at North Carolina State University and was thought to be responsible for incidents of fish deaths in local waters. It was subsequently found associated with fish kills in the Pamlico and Neuse estuaries of North Carolina (Burkholder *et al*., 1992). The 'phantom' dinoflagellate as it was called due its 'ambush-predator' behaviour was claimed to release potent neurotoxin(s) and was found to have a complex life cycle involving amoeboid as well as dinoflagellate zoospores and cysts (Burkholder and Glasgow, 1997). A second member of the toxic *Pfiesteria* complex, *Pfiesteria shumwayae*, was later identified (Glasgow *et al*., 2001). This species has since been reclassified as *Pseudopfiesteria shumwayae* (Litaker *et al.*, 2005).

Pfiesteria-like organisms are heterotrophic dinoflagellates that are able to become mixotrophic (the ability to assimilate organic compounds as carbon sources but not as energy sources) through kleptoplasty (retention of chloroplasts from prey). They therefore have great versatility in acquiring nutrients by assimilating both dissolved organic and inorganic compounds, as well as ingesting particulate food.

Because of the growth response of *Pfiesteria* to anthropogenic nutrients and its potential impact on marine resources and human health, it has maintained a high public as well as scientific profile, especially within the USA, and has engendered sensational newspaper headlines such as "The cell from hell". Research on the subject is extensive but also controversial (for overviews see Burkholder *et al*., 2001; Kaiser, 2002; Miller and Belas, 2003).

A brief overview of the ecology of *Pfiesteria* has been provided by Glibert and Burkholder (2006) in an issue of *Harmful Algae* dedicated entirely to the ecology of the organism. They say, "One of the important facts to emerge in recent years about *Pfiesteria* spp. is that they are common estuarine organisms with widespread distribution. They generally occur in low abundance in the water column and often are found in or near the sediments." - see Bowers *et al.* (2006). The effect of various biotic and abiotic factors on the growth, encystment, and excystment of *Pfiesteria piscicida* in laboratory cultures has been described by Saito *et al*. (2007).

3.5.2 Effects of *Pfiesteria on* human health

The first indication that exposure to *Pfiesteria* posed a risk to human health came from reports of illness among investigators working with *Pfiesteria* spp. in the laboratory. The exposure of laboratory personnel to aerosols from ichthyotoxic *Pfiesteria* cultures was associated with a range of symptoms from nausea, respiratory problems, eye irritations and skin lesions, to numbness and memory loss (Glasgow *et al*., 1995). Similar symptoms were also then reported by watermen working in areas associated with *Pfiesteria*-related fish kills (Grattan *et al*., 1998). The adverse health effects observed in people associated with estuaries containing toxic *Pfiesteria*-like organisms were collectively referred to as 'putative estuary associated syndrome (PEAS') by the US Centers for Disease Control and Prevention (Anon., 1999).

The most recent update on *Pfiesteria* from the CDC (Anon., 2004) states:

"Scientists do not yet know if *P. piscicida* affects human health. However, anecdotal reports of symptoms such as headache, confusion, skin rash, and eye irritation in

laboratory workers exposed to water containing high concentrations of *P. piscicida,* along with reports of similar symptoms in people living near waters where *P. piscicida* has been found, have caused concerns among the public."

Research by Patterson *et al.* (2007) suggested that, although transient irritancy induced by exposure to *Pfiesteria*-laden water might exacerbate the response to a true sensitizer, thereby enhancing the dermatitis, inflammation, and lesions caused by other micro-organisms, allergens, and occupational activities, the *Pfiesteria* extract which was tested was not capable of inducing dermal sensitization at the concentrations evaluated.

Their findings were consistent with other recent reviews and CDC sponsored epidemiology studies (Moe *et al*., 2001; Morris *et al*., 2006) that attribute reported symptoms to other common causes and concluded that exposure to *Pfiesteria*-containing estuarine environments does not pose a significant health risk. However these conclusions have been disputed (Shoemaker and Lawson, 2007).

A definitive association between exposure to toxin(s) from *Pfiesteria* and human illness has not been possible because of the lack of characterisation and identification of the putative toxin(s). Moeller *et al.* (2007) characterised metal-containing organic toxins produced by *P. piscicida.* It is proposed that the toxicity of these metal containing organic compounds is due to carbon-sulphur metal (copper and iron) based radical production. The toxin is produced rapidly and then vanishes quickly upon exposure to sunlight and other environmental factors. The role of these newly discovered toxins in fish kills and/or PEAS remains to be tested so the toxicity of these organisms still remains anecdotal.

Vogelbein *et al.* (2008) have produced a comprehensive review of all aspects of the present state of knowledge about *Pfiestetria* including a critical evaluation of the evidence regarding PEAS, occupational and recreational exposure and toxicity.

3.5.3 Global distribution

P. piscicida has now been confirmed over a wide geographical range (Lin *et al.*, 2006; Rublee *et al.*, 2005). Of interest is the record from Ace Lake, Antarctica; this is a saline lake which has not contained fish for thousands of years (Park *et al*., 2007). The presence of *Pfiesteria piscicida* and *P. shumwayae* from sediment samples from Oslofjord, Norway, has also been confirmed by identification using microscopy and molecular methods (Jakobsen *et al*., 2002).

3.5.4 *Pfiesteria* in the UK

P. shumwayae was recorded in the UK 'in low numbers' during an investigation in 2001 into the cause of atypical DSP results in cockles in the Bury Inlet, SW Wales (Anon., 2002c). This report states:

"There is no known occurrence of *P. shumwayae* being associated with toxins in shellfish. It is normally associated with fish kills (of which there were none in the Inlet), and therefore this is not considered to be a likely cause of the toxicity seen in the cockles."

There is no indication in the report as to what methods, e.g. molecular probes or electron microscopy, were used in identifying *P. shumwayae* (see Anon., 1997; Jakobsen *et al*., 2002; Bowers *et al*., 2006).

This report does, however, highlight the potential need for vigilance in monitoring reports of the unusual occurrence of dead fish on beaches, or reported incidents of skin irritation and/or lesions in water users in eutrophic estuaries in the UK as these would probably be the first indications of any harmful effects due to *Pfiesteria* spp.

3.5.5 Monitoring strategies

Because of the possible health implications and economic impact on coastal areas in several US states, there is a considerable amount of information available on monitoring strategies. For example, the US Centers for Disease Control and Prevention (CDC) organised a conference on the theme '*Pfiesteria*: From Biology to Public Health' (see Anon., 2001b).

Protocols for monitoring *Pfiesteria* and related fish health and environmental conditions in US Coastal Waters (Turgeon *et al*., 2001) deal specifically with fish kills, but these would be among the first signs of harmful effects due to the dinoflagellate. The proceedings of the National Oceanographic and Atmospheric Agency workshops to standardize protocols for monitoring toxic *Pfiesteria* species and associated environmental conditions were reported by Luttenberg *et al*. (2001). A brief summary of monitoring for *Pfiesteria*-like organisms in coastal waters is given in Anderson *et al*. (2001).

As regards public information, a good is example the State of Maryland Department of Natural Resources website, which has a page entitled 'What you should know about *Pfiesteria*' - http://www.dnr.state.md.us/bay/cblife/algae/dino/pfiesteria/facts.html , which provides very comprehensive information. It includes this advice:

Is it safe to swim and boat in coastal waters?

- Swimming, boating, and other recreational activities in coastal waters are generally safe. To be on the safe side, the following common-sense precautions are recommended:
- Comply with state closures of water bodies and public health advisories. Do not go into or near the water in areas that are closed by the state.
- If you notice significant numbers of fish that are dead or that exhibit lesions or other signs of disease, avoid contact with the fish and water, and promptly report the incident to your state's environment or natural resource agency.

3.6 *Chrysochromulina polylepis*

The toxic haptophyte *C. polylepis* is found in the waters around Norway, Sweden and Denmark, and also in the Irish Sea. It first attracted attention following a massive bloom in Norway in 1988, which resulted in extensive mortalities to fish, invertebrates and other aquatic organisms (Landsberg, 2002; Smayda, 2006). Its toxic properties have been reviewed by Landsberg (2002). It produces haemolysins, which have been identified as mono-galactosyl diacylglycerol and octadecapentaenoic acid (18:5n-3).

The *Chrysochromulina polylepis* toxin appears to interfere with cell membrane functions and ionic balance (Underdal *et al*., 1989; Meldahl *et al*., 1993 cited in Landsberg, 2002). Because of this nonspecific effect *C. polylepis* affects a wide range of aquatic organisms from protists to fish (Edvardsen and Paasche, 1998, cited in Landsberg, 2002). On the basis of evidence of toxic compounds extracted from mussels affected by a bloom of *C. polylepis* it has been suggested that the alga may produce a group of ichthyotoxins similar to those of *Prymnesium parvum* (Stabell *et al*., 1993).

3.6.1 Effects on human health

There are no reports in the literature regarding harmful effects on humans caused by *C. polylepis* via inhalation, aspiration or dermal contact.

3.7 *Prymnesium parvum*

P. parvum is a cosmopolitan member of the Prymnesiophyceae which, unlike most other toxic microalgae, excretes a complex of toxins into the surrounding medium. These toxins have ichthyotoxic, cytotoxic and haemolytic activity but appear to share a common mechanism in that they cause increased cell membrane permeability, the effect of which is particularly lethal to gill breathing animals such as fish and bivalve molluscs and massive kills due to *Prymnesium* blooms are quite common (Johansson and Granéli, 1999 and refs. therein). A comprehensive review of *P. parvum* biology and toxicity has been produced by Watson (2001) and see also Landsberg (2002) and Smayda (2006).

The occurrence of fish kills is worldwide and, although *P. parvum* is euryhaline, these incidents usually occur in brackish waters such as estuaries and freshwater bodies that are saline or have a high mineral content. The alga occurs in inland waters in the State of Texas, USA, where it was first identified in 1985 and the following US states have also reported its occurrence and impact: Alabama, Arizona, Arkansas, California, Florida, Hawaii, Louisiana, Maine, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Texas, Washington and Wyoming (information from the TWPD website mentioned below).

In Texas, where it is commonly known as 'golden alga', *P. parvum* is a major environmental problem; blooms have been responsible for fish kills in a number of river basins.
The State of Texas Parks and Wildlife Department (TPWD) web page http://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/ga/ states for information –

What is Golden Alga (*Prymnesium parvum*)?

- A naturally occurring microscopic flagellated alga that typically occurs in brackish waters
- Under certain environmental stresses, this alga can produce toxins which can cause massive fish and bivalve (i.e. clams and mussels) kills
- There is no evidence these toxins harm other wildlife, livestock or humans

On the page headed "Biology of Golden Alga, *Prymnesium parvum*" http://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/ga/bio.phtml the TPWD states:

"Unlike toxic red tide blooms on the coast, golden alga toxins have no apparent lethal effect on non-gill breathing organisms. Cattle, predators, scavengers, birds and other animals have been observed drinking water during a bloom, and many eat the dead fish during on-going golden alga fish kills with no apparent effects. The golden alga toxins are acid-liable, meaning they breakdown in acidic conditions, such as in the stomach. Researchers believe this is why animals are able to drink the water and eat affected fish without having toxic effects. Also, terrestrial animals have skin layers to protect them; these same layers protect them from the toxins. Officials from the Texas Department of State Health Services have stated that the golden alga is not known to be a human health problem, but people should not pick up dead, or dying, fish for eating."

And in a public 'information card' produced by the State of Texas Commission for Environmental Quality - http://www.tceq.state.tx.us/files/gi-378.pdf_4146980.pdf the same information is reiterated:

"*P. parvum* produces toxins that can affect gill-breathing organisms including fishes and freshwater molluscs. The most visible result of a fish kill caused by golden alga is dead and dying fishes of all species and sizes. Aquatic insects do not appear to be affected and may be alive during a toxic event. Large numbers of birds (including pelicans, cormorants, gulls, herons, and vultures) may be present and actively feeding on the dead and dying fishes.

The Texas Department of State Health Services has stated that golden alga is not known to cause human health problems, but people should not pick up dead or dying fishes for consumption.

- Mammals and birds have been observed eating dead fishes and drinking water within areas experiencing toxic golden alga blooms; no immediate harmful effects have been recorded. Complications, secondary infections or other effects may occur. Be careful of spines and bones from dead fishes on the shoreline. Puncture wounds can get infected.
- Swimming near dead fishes is not recommended since bacteria levels associated with decomposition may be high."

Moustaka-Gouni *et al*. (2004) reported the coincidence of a *Prymnesium parvum* bloom and the mass kill of birds and fish in Lake Koronia, Greece, in 2004. The bird kill was later reported by the World Organization for Animal Health (OIE) to be due to avian botulism - see ftp://ftp.oie.int/SAM/2004/FAUNE_A.pdf .

3.8 *Fibrocapsa japonica*

Harmful blooms of *F. japonica* causing extensive kills of farmed fish have occurred in various parts of the world (Smayda, 2006 - Figure 9), mainly in temperate regions (see de Boer *et al*., 2005). It was originally described in Japanese waters in 1973 and was first observed in European coastal waters in 1991, including those of France and the Netherlands, and two years later in the German Bight (Smayda, 2006 and refs. therein). *F. japonica* has become well established in the North Sea and was reported to have reached a population maximum of 2.4 million cells.L^{-1} during 1997 in the German Bight (Rademaker *et al.*, 1998).

As with other raphidophytes, the specific mechanisms of toxicity remain to be elucidated. Khan *et al*. (1996a) isolated five neurotoxic components from a European isolate of *F. japonica*, designated FjTx-I, FjTx-II, FjTx-IIIa, FjTx-IIIb and FjTx-IV, which were tentatively identified as brevetoxin compounds, PbTx-1, PbTx-2, PbTx-9, PbTx-3 and oxidized PbTx-2 on the basis of TLC and HPLC analysis. Brevetoxins have also been identified in an American strain of *F. japonica* by means of an ELISA technique (Bridgers *et al*., 2004).

Using various sophisticated analytical techniques, including LC-MS, GC-MS and NMR, Fu *et al*., (2004) elucidated the molecular structures of the three main haemolytic compounds (Fj1, Fj2 and Fj3) isolated from a European strain of *F. japonica.* They were identified as polyunsaturated fatty acids (PUFA): 6,9,12,15-octadecatetraenoic acid (C18:4n-3, OTA), 5,8,11,14,17-eicosapentaenoic acid (C20:5n-3, EPA) and 5,8,11,14-eicosatetraenoic acid (C20:4n-6 = arachidonic acid, AA). Pure commercial samples of EPA and AA were found to show the same spectroscopic and chromatographic characteristics as Fj2 and Fj3 and had a similar strong haemolytic effect. It was postulated that, when *F. japonica* cells accumulate in fish gills during blooms, these PUFA could be the cause of ichthyotoxicity.

3.8.1 Possible toxicity to sea mammals

Fibrocapsa has been implicated in the death of seals but hard evidence has proved elusive. A report of the European Environment Agency (Walday and Kroglund, 2002) states -
"In the summer of 1997 the alga *Fibrocapsa japonica* was found in almost all samples from the Dutch algal bloom programme. The toxin fibrocapsine produced by this alga has been found in dead seals in Germany, and accumulation of fibrocapsine through the food chain may have contributed to the large numbers of ill and underfed young seals in the Dutch Wadden Sea during the summer of 1998."

- but no reference is cited. A report by the German Federal Environmental Agency (Brockmann *et al*., 2003) cites only an unpublished source -

"Besides, there has been increasing evidence for the recent intrusion of other non-indigenous phytoplankton species, among these several toxic forms (*Chattonella* spp., *Fibrocapsa japonica*, *Heterosigma akashiwo*), which contribute to the total number of potentially harmful species in German coastal waters. These species may constitute a permanent risk to aquaculture, fisheries, tourism and the marine biota in case of occasional mass development. The brevetoxin-like toxin of *Fibrocapsa* has been found in high concentrations in the tissue of dead seals at the German West Coast (Siebert, unpubl.)."

Similarly, references to (variously) 'fibrocapsin' or 'fibrocapsine' in the scientific literature are scarce. Rademaker *et al.* (1998) reported that fibrocapsin had been shown to be a cyclic polyether with a lactone moiety with a higher specific toxicity than brevetoxin and near to that of tetrodotoxin, but no supporting literature citations were given.

On the website of Expressed Sequence Tag (EST) Analysis of Toxic Algae (ESTTAL, an EC Framework programme)
http://genome.imb-jena.de/ESTTAL/cgi-bin/Fibrocapsa.pl it is stated:

"**Fibrocapsin**
The "toxicity scenario" behind the fish kills is under debate. Raphidophycean flagellates produce neurotoxic (fibrocapsin), hemolytic, haemo-agglutinating compounds and oxygen radicals. The high toxicity of *F. japonica* is proposed to result from the production of a secondary metabolite named fibrocapsin. The toxicity of this phycotoxin is between tetrodotoxin and dioxin. The chemical structure, a cyclic lipophilic polyether, is similar to brevetoxins of the dinoflagellate *Karenia brevis*. Fibrocapsin is a neurotoxin targeting specifically sodium channels. Fixation of the toxin is thought to provoke hyperexcitability of neuronal cells, and the toxin appears capable of targeting the peripheral and central nervous system."

3.8.2 Effects on human health

No effects of whole cells or cellular components *Fibrocapsa japonica* on humans by dermal contact, aspiration or inhalation appear to have been reported. Given that the flagellate is fragile and easily lysed and that it appears to contain a brevetoxin-like compound, it is conceivable that if a bloom were to occur and this compound was dispersed, for example as an aerosol, then symptoms might result following inhalation.

In contrast to the report of Rademaker *et al.* (1998) regarding raphidophytes in the North Sea, the relative cellular toxicity of various raphidophytes, including *F. japonica*, originating from Japan, New Zealand and various coastal sites in the USA, was

estimated to be 0.1 to 0.5 that of a *Karenia brevis* cell (Bridgers *et al*., 2004). It was suggested by these workers that blooms of raphidophytes would have to be of correspondingly higher densities to have the same effect as that of a *K. brevis* bloom. Also, as discussed by Smayda (2006), there is a higher probability that blooms will occur in chemically modified habitats and at fish-farming sites.

It is interesting to note that two of the fatty acids from *Fibrocapsa* reported by Fu *et al*. (2004) as having strong haemolytic activity are widely promoted as human dietary supplements. Eicosapentaenoic acid (EPA) is a component of 'omega-3'-rich fish oils, and non-toxic microalgae such as *Porphyridium cruentum* have been investigated as a possible source of arachidonic acid for use in infant feeding formulations. This highlights the fact that the toxic effect of such compounds probably operates at a microsopic level where there may be microzones of very high concentrations around individual cells (see sect. 3.4.3).

3.9 *Heterosigma akashiwo*

This alga is cosmopolitan and is widely distributed in the Atlantic and Pacific oceans and is found in European waters from Norway in the north to Spain and Portugal in the south (Smayda, 2006). Its toxic properties have been reviewed by Landsberg, (2002) and Smayda (2006). To quote the latter: "*Heterosigma akashiwo* is probably the most versatile harmful algal species known: it is antagonistic to organisms ranging in size from bacteria to invertebrates to fish. Its multiple modes of antagonism range from nutritional inadequacy, to suppression of feeding and growth, and to toxicity causing mortality." These antagonistic properties towards various organisms have been tabulated in detail by Landsberg (2002).

The exact nature of the toxins and mechanisms of toxicity in *H. akashiwo* and other raphidophytes remains unclear. Some mechanisms of ichthyotoxicity, which may also be involved with allelopathy, that have been postulated are production of reactive oxygen species and the production of toxins that have yet to be fully characterized (Twiner *et al*., 2004; Smayda, 2006; Kempton *et al*., 2008).

Khan *et al*. (1997) reported that they had isolated from *H. akashiwo* four toxic components which were, on the basis of the analytical methods used, tentatively identified as brevetoxins, neurotoxins that have been well characterized and are also produced by the dinoflagellate *Karenia brevis* (see sect. 5.).

Uncharacterised extracellular compounds excreted by *H. akashiwo* were found to have an irreversible effect on respiratory activity in human and mammalian cells *in vitro* (Twiner *et al.*, 2004). These compounds were also found to affect cytosolic calcium concentrations in cultured sf19 insect cells leading to apoptosis (cell death). The effect was related to the concentration of extracellular Ca^{2+}. It was concluded that the extracellular compounds were inhibiting the plasma membrane Ca^{2+}-ATPase transporter (Twiner *et al*., 2005). It was suggested that the compounds may play a role in the ichthyotoxicity and allelopathy of the alga.

In laboratory studies Keppler *et al*. (2005) demonstrated sublethal effects (significantly increased oyster hepatopancreas lysosomal destabilization rates) of cells of *H. akashiwo* on the southeastern oyster, *Crassostrea virginica*.

The most notable impact of *H. akashiwo* has been on aquaculture where it has caused massive kills of farmed fish at various locations round the world and has also affected shellfish (Landsberg, 2002; Smayda, 2006; Kempton *et al*., 2008).

3.9.1 Effect on humans

Smayda (2006) cites a report of a *H. akashiwo* bloom in New Zealand in 1992 and its effects on shellfish (Rhodes *et al*., 1993) as stating that fishermen operating in the area reported skin irritation. What Rhodes *et al*. (1993) actually reported in the original reference was:

"Before sampling at Coromandel, brown-coloured water had been observed and mussels were reported as having a peppery taste. In tests carried out by laboratory staff, mussels caused a tingling sensation on the tongue lasting several minutes. Skin irritations were also reported by fishers (sic) in the bloom area. The causative species was not established in either case."

The World Health Organization Guidelines for Safe Recreational Water Environments (Anon, 2003: Vol. 1, chapter 7) cite a personal communication from I. Falconer that 'skin irritation problems' were reported by swimmers exposed to dense blooms of *H. akashiwo.* These tenuous attributions to *H. akashiwo* as being the possible cause of symptoms in humans are the only references that have come to light.

Brevetoxin-like compounds released from lysed cells of *H. akashiwo* might cause symptoms in humans if dispersed in the same way as brevetoxins released from *K. brevis* (see sect. 5.) but in the absence of hard evidence this remains speculation.

3.10 Other toxic raphidophytes

The IOC harmful algal database lists seven toxic raphidophytes: *F*. *japonica*, *H. akashiwo* and five species of *Chattonella*. The latter genus is not on the Environment Agency's list of notifiable algae, although it has been a major cause of fish kills in various parts of the world (Bourdelais *et al*., 2002; Landsberg, 2002; Smayda, 2006) and is known to contain brevetoxin-like compounds (Khan *et al*., 1996b; Bourdelais *et al*., 2002 and refs. therein; Bridgers *et al*., 2004 and refs. therein).

Smayda (2006) has discussed the occurrence and possible causes of raphidophyte blooms in detail and has coined the term 'the *Chattonella-Fibrocapsa-Heterosigma* triad'. He says:

"The conspicuous emergence and bloom dynamics of the *Chattonella-Fibrocapsa-Heterosigma* triad in continental European coastal waters during the past decade has provoked interest as to their origins. Elbrächter's (1999) analysis of the various claims of origin led him to conclude that *F. japonic*a is an introduced species, while the *Chattonella* spp. and *H. akashiwo* are indigenous species often misidentified because of their difficult taxonomy. Should this be the case, the *Chattonella* spp. would then have been components of the hidden flora. This gives rise to a different question: what has stimulated their growth during the past decade making them more evident? There is reason to believe that this query has to be addressed at the raphidophyte group level, rather than at the species level. In European waters and other regions, where new occurrences of *Chattonella* spp. and *F. japonica* are increasingly being reported, *H. akashiwo* is usually present. These three raphidophyte genera appear to co-occur, detection of one of the three genera is symptomatic of the presence of the other two genera. That is, a raphidophyte niche may be opening up globally, particularly in chemically modified habitats and at fish-farming sites, and possibly in "competition" with the dinoflagellate life-form niche. Peperzak (2002) has mapped the regional expansion of raphidophyte blooms and locations of associated fish kills in European coastal waters. Most of the known, major raphidophyte species are now reported to be present in European coastal waters: *Ch. antiqua, Ch. marina, Ch.* aff. *minima, Ch. subsalsa, Ch.* aff. *verruculosa, F. japonica and H. akashiwo*."

Smayda also makes the observation that there is an unexpectedly low incidence of fish-killing blooms of *H. akashiwo* at farmed fish sites in Scotland, although *H. akashiwo* is apparently present in Scottish waters and he identifies temperature as possibly being one limiting factor. The temperature tolerance of isolates of *F. japonica* from various geographic regions has been studied by de Boer *et al.* (2005) who observed that an isolate from the German Wadden Sea encounters lethal temperatures in winter and must have a resting stage, able to survive temperatures <4°C, to explain its occurrence in this region.

3.11 *Nodularia spumigena*

N. spumigena is a filamentous cyanobacterium found in brackish waters. It occurs commonly in the Baltic Sea and in Australia and New Zealand. In the UK it has been recorded as occurring in a brackish lake linked to the Humber estuary (Lewis, 1997). In the Baltic particularly it regularly forms dense blooms during the summer (Toruńska *et al*., 2008).

N. spumigena produces the hepatotoxin nodularin, a cyclic pentapeptide, which is water soluble. The LD_{50} of nodularin is ca. 50 $\mu g.kg^{-1}$ body weight when administrated intraperitoneally in mice and is, therefore, one of the more potent of the known natural toxins. According to Toruńska *et al*. (2008) during blooms of *N. spumigena* most of the toxin is found within the cyanobacterial cells, with only a small amount released into the environment. When the bloom collapses and the cyanobacterial cells lyse, the concentration of dissolved nodularin in the water increases. In the Gulf of Finland in August 1999 the average cell-bound concentration of nodularin in the water varied between 150–220 $\mu g.L^{-1}$, whereas in filtered water samples, i.e. free dissolved toxin, it was from 0.01 to 18.7 $\mu g.L^{-1}$ (Kankaanpää *et al*. 2001, cited in Mazur and Pliński, 2003). Nodularin is a very stable molecule in its free state in the aquatic environment and its loss mainly occurs due to dilution, adsorption onto suspended particles and onto sediments and by microbial and photolytic degradation (Toruńska *et al*., 2008 and refs. therein).

Although much of the cyanobacterial biomass and toxin after a bloom is degraded in the water column about 10% reaches the sediments where it is broken down by microbial action (Toruńska *et al*., 2008).

According to Mazur and Pliński (2003), elevated concentrations of toxin, a thousand- to a million-fold higher than in water, can be found in surface scums – accumulations of cyanobacteria. These are wind driven on to the shore where they accumulate, presenting a threat to humans and animals.

No human deaths have been reported due to ingestion of *Nodularia*. There is a report of a party of adults and children in Australia having developed skin rashes after being in contact with water containing toxins from *Nodularia* and *Microcystis* (van Apeldoorn *et al.*, 2007) but this could have been due to either or both of the cyanobacteria.

No toxicological data, such as a no observable effect limit (see Table 7), exist for nodularin but, since it is structurally similar to the microcystins and they have the same effect (both are hepatotoxins) it is assumed to have the same value as microcystin-LR (Funari and Testai, 2008). No guidelines have been set for *Nodularia* and nodularin in the World Health Organization guidelines (Anon., 2003), but, assuming that it represents a risk equivalent to microcytin-LR, it has been proposed that an action level should be set at 50,000 $cells.mL^{-1}$, equivalent to 10 $\mu g.L^{-1}$ of nodularin (Fitzgerald *et al*., 1999, cited in van Apeldoorn *et al*., 2007).

4 Aspiration (ingestion) of water containing toxic phytoplankton

The consumption of seafood contaminated with algal toxins is one of the main causes of human illness caused by toxic marine phytoplankton. There is a plethora of literature on these phycotoxins; see, for example, van Egmond *et al.* (2004), Hallegraeff *et al*. (2004) and Leftley and Hannah (2008) and their chemistry, biochemistry and toxicology is well understood. There is, however, little reported in the literature concerning direct exposure of humans to these algae by the route of aspiration (swallowing water containing the algae) other than of chronic exposure to cyanobacteria and cyanobacterial toxins in drinking water.

It is necessary, therefore, to carry out a very basic risk assessment on a similar basis to that for cyanobacterial toxins (see sect. 6.) the starting point for which is the acute reference dose (RfD) for the consumption of individual phycotoxins in seafood, which has been set provisionally by the Joint FAO and WHO Codex Alimentarius Commission of the United Nations (Anon., 2005 and see Leftley and Hannah, 2008). The RfDs for the three major phycotoxin groups, are based on the lowest observable effect levels and were calculated including a safety factor of 3 or 10, are given in Table 5. The question then is, how many toxic cells would have to be swallowed and, since they are suspended in water, what volume of water? The latter, of course, depends on the concentration of the cells, i.e. the higher the number of cells per unit volume, as in a dense bloom, the smaller the volume that would have to be consumed to achieve the RfD.

Table 5. Summary of data used in the derivation of acute reference doses. Abstracted from a report of the FAO/WHO Codex Alimentarius Commission (Anon., 2005).

Toxin Group	**LOAEL §** **µg.kg^{-1} body weight**	**Safety Factor [Human data (H)]**	**Provisional Acute Reference Dose RfD ***
Domoic Acid	1,000	10(H)	100 µg/kg **6mg/adult ***
Okadaic Acid	1	3(H)	0.33µg/kg **20 µg/adult ***
Saxitoxin	2	3(H)	0.7 µg/kg **42 µg/adult ***
§ * LOAEL = lowest observable adverse effect level			

Table 6 shows examples of estimated volumes of water from a bloom that would have to be swallowed, using examples from the literature. In these examples the toxin concentrations per cell are the highest quoted in the publications cited. As regards the cell densities, in the case of *Dinophysis* and *Alexandrium*, the cell densities are exceptional. It is also assumed that all the toxin(s) would pass from the algae in the

process of digestion and be absorbed by the gut - there appears to be no empirical data.

The calculations are based on the RfD for adults (60 kg body weight) so the alternative in terms of µg/kg could be used in calculations for children.

Table 6: Calculated intake by swallowing various algae, in terms of cell numbers and volumes, necessary to obtain the acute reference dose, based on examples taken from the literature.

Toxin	Alga	Toxin content per cell (pg) [range observed]	Highest recorded cell density in bloom (cells.L^{-1})	Location	Toxin acute reference dose RfD (µg per adult) (1)	No. of cells equivalent to RfD	Equivalent volume of the bloom water that a bather would have to swallow (litres)
Domoic Acid	*Pseudo-nitzschia* spp.	117 [0 –117] (**2**)	5.3 x 10^4	Southern California Bight	6,000	5.1 x 10^7	962.0
Okadaic acid (OA)	*Dinophysis acuminata*	57.7 [0–57.7] (**3**)	5 x 10^4 (**4**)	Bay of Seine N. France	20	3.46 x 10^5	6.9
OA	*Prorocentrum lima* strain 3g from UK	17.13 [0.42–17.13] (**5**)	see note (**6**)	Fleet Lagoon, UK	20	1.16 x 10^6	see note (**6**)
	Prorocentrum lima from W Adriatic (**7**)	Not reported -assume toxin content as above	4.7 x 10^5	Oranta Harbour (**7**)		1.16 x 10^6	2.4
Saxitoxin	*Alexandrium tamarense*	10.7 (STX-eq) [Mean]	7 x 10^5 (**8**)	SE Nova Scotia	42	3.92 x 10^6	5.6

Notes:

(1) *See Table 5.*

(2) *Schnetzer et al. (2007) reported domoic acid (DA) concentrations per cell at several sampling stations in the S Californian Bight during 2004 exceeded previously reported maxima for natural populations of Pseudo-nitzschia (mean = 24 pg DA.cell^{-1}, range = 0–117 pg DA.cell^{-1}).*

(3) *Smayda (2006, Table 3) cites reports of maximal concentrations of 100 pg OA.cell^{-1} in D. acuta, 25 pg OA.cell^{-1} in D. acuminata, 23 pg OA + 192 pg DTX-1.cell^{-1} in D. fortii and 101 pg DTX-1.cell^{-1} in D. rotundata.*

(4) *An exceptionally high concentration (Marcaillou et al., 2001). Lindahl et al. (2007) observed D. acuminata in various fjords. Cell numbers varied throughout the year at various locations from a few hundred per litre at various depths to a maximum of 38,000 per litre concentrated at the pycnocline. Cellular content of OA in cells collected at various times, locations and depths ranged from 0.11(±0.02) to16.63(±4.35) pg.cell^{-1}; cells also contained dinophysistoxin-1 (DTX-1). If DTX-1 is assumed to be of equivalent toxicity to OA then the calculations above are an underestimate where both toxins are present.*

(5) *OA was measured in a number of isolates from Fleet Lagoon, UK, and the range indicates individual concentrations found in these isolates, the highest concentration being in isolate 3g. All strains also contained DTX-1 (Nascimento et al., 2005).*

(6) *Although P. lima may be detected in the water column it is an epiphyte and is usually attached to a surface and difficult to quantify. Foden et al. (2005) looked at the distribution of P. lima on a variety of macroalgae and macrophytes (seagrass) collected from Fleet Lagoon. P.lima was present on most substratum species over the sampling period from 10^2 to 10^3 cells.g-^{1} fresh weight of plant biomass.*

(7) *Bloom concentrations at 0.5 m in Oronto Harbour were recorded varying from 0.5 to 4.7 x 10^5 cells.L^{-1} (Ingarao et al., 2007).*

(8) *An exceptionally dense bloom, which killed caged salmon (Cembella et al., 2002). Often Alexandrium blooms are usually found at much lower cell densities, for example in Scapa Flow, Orkney, where there have been a number of PSP toxic Alexandrium blooms: In 1998-99 the highest concentration recorded was 1,600 cells l^{-1} but concentrations of 200–400 cells.L^{-1} were more typical (Joyce, 2005). The cell density of an offshore bloom of Alexandrium* in the Gulf of Maine, USA, was 5.5 × 10^3 cells.L^{-1} (Townsend *et al*., 2001).

4.1 Possible exposure of bathers to free toxins dissolved in the water

The possibility of bathers swallowing water containing dissolved phycotoxins must be considered. Other than cyanobacterial toxins in fresh water (sect. 6.) there is a dearth of information regarding the occurrence and stability of dissolved phycotoxins in natural waters.

4.1.1 Domoic acid

Domoic acid (DA) is a water-soluble neurotoxin produced and released by several species of the diatom *Pseudo-nitzschia* and by *Nitzschia navis-varingica* (Leftley and Hannah, 2008). DA accumulates in the culture medium in dense laboratory cultures of limited volume but there is no evidence that the free form of the toxin accumulates to any significant concentrations in the ocean as it is photolabile (Bates *et al*., 2003; Bouillon *et al*., 2006).

4.1.2 Okadaic acid and its congeners

Okadaic acid and its congeners are lipophilic compounds and of low solubility in water. Their chemistry and stability has been discussed by Quilliam (2004). There appear to be no reports of okadaic acid and related compounds being detected in significant quantities in natural waters.

The extracellular production of okadaic acid by *Prorocentrum lima* in laboratory cultures was observed by Rausch de Traubenberg *et al*. (1995). It was subsequently shown that *Prorocentrum* spp. produce soluble sulphated diol esters of the parent acid (Quilliam, 2004 and refs. therein).

4.1.3 The saxitoxins

No reports of the detection of the free toxin in natural waters were found.

4.1.4 Nodularin

Little toxicological data are available. It is considered equivalent in toxicity to microcystin-LR produced by freshwater cyanobacteria (see Table 7). It is water soluble and stable in solution (see 3.11).

5 Dispersal of microalgae and their toxins as aerosols or airborne particles

There are a number of reports of microalgae, all dinoflagellates, causing repiratory distress in humans. In this section the literature is reviewed and also information is provided regarding advice given to the general public provided by various state and government authorities in the United States where the problem is endemic.

5.1 *Karenia brevis*

Harmful blooms of the dinoflagellate *K. brevis* are a regular occurrence around the coast of Florida and in the Gulf of Mexico. The alga produces polyether toxins, the brevetoxins, which are the cause of neurotoxic shellfish poisoning (NSP) (Kirkpatrick *et al*., 2004). One aspect of these blooms that directly affects humans that is well documented is respiratory irritation due to inhalation of aerosols containing toxic cells or toxins released from lysed cells (Cheng *et al.*, 2005a,b,c; Fleming *et al*., 2005; Pierce *et al*., 2005). The aerosols are generated when bubbles burst in white-capped waves and are carried onshore by the wind (Cheng *et al.*, 2005a,b,c and refs. therein). Those with pre-existing respiratory conditions, such as asthma, are particularly susceptible (Abraham *et al*., 2005; Fleming *et al*., 2007; Steensma, 2007) as brevetoxins, which are sodium channel blockers, are also thought to be histamine activators (Fleming *et al*., 2007).

The mass median aerodynamic diameters of the aerosols were measured by Cheng *et al.* (2005b) and found to be in the range 7–9 µm, which they considered a relatively large size for inhaled ambient particles. They concluded that inhaled particles of that size would be predominantly deposited in the upper respiratory tract (nasal, oral, and pharyngeal area), and subsequent respiratory irritation could result from the presence of the particles themselves or from toxins associated with the particles.

There is much information regarding the possible respiratory effects of *K. brevis* blooms provided to the general public by state and federal authorities via official websites -

From the US Centers for Disease Control: http://www.cdc.gov/hab/redtide/about.htm

“The human health effects associated with eating brevetoxin-tainted shellfish are well documented. However, scientists know little about how other types of environmental exposures to brevetoxin - such as breathing the air near red tides or swimming in red tides -may affect humans. Anecdotal evidence suggests that people who swim among brevetoxins or inhale brevetoxins dispersed in the air may experience irritation of the eyes, nose, and throat, as well as coughing, wheezing, and shortness of breath. Additional evidence suggests that people with existing respiratory illness, such as asthma, may experience these symptoms more severely.”

From the Florida Fish & Wildlife Research Institute:
http://research.myfwc.com/support/view_faqs.asp?id=13

FAQs

How is red tide related to respiratory irritation?

People experience respiratory irritation (coughing, sneezing, and tearing) when the red tide organism (*K. brevis*) is present along a coast and winds blow its toxic aerosol onshore.

CAUTION: People with severe or chronic respiratory conditions (such as emphysema or asthma) are advised to avoid red tide areas. Generally, symptoms are temporary. Once exposure is discontinued, symptoms usually disappear within hours.

Is it safe to swim during a red tide?

Yes, swimming is safe for most people. However, red tide can cause some people to suffer from skin irritation and burning eyes. Use common sense. If you are particularly susceptible to irritation from plant products, avoid red tide water. If you experience irritation, get out of the water and thoroughly wash. Do not swim among dead fish because they can be associated with harmful bacteria.

From the Texas Department of State Health Services (TDSHS) Seafood and Aquatic Life Group http://www.dshs.state.tx.us/seafood/RedTide.shtm :

Other symptoms associated with *K. brevis* blooms

Classically, NSP produces gastrointestinal and neurologic symptoms associated with ingestion of shellfish containing high levels of brevetoxins. However, some individuals have experienced respiratory, mucous membrane, and skin irritation simply by walking on the beach during this and other *K. brevis* red tides. Unlike the *Alexandrium* species of red tide organisms, which affect the North Atlantic and North Pacific coasts, *Karenia* species possess an outer shell that is very fragile and easily disrupted by the vigorous mechanical action of the surf. On disruption, *Karenia* organisms release cellular endotoxins into the surrounding water. Wind and surf action produce a fine aerosol that generally travels only a short distance from the beach. Thus, in areas with a great deal of surf action, airborne exposure to the toxins can be a problem for some individuals. *Alexandrium* species have a hard outer shell that is not easily disrupted, so airborne exposure is generally not a problem in red tides along the Pacific coast or the northern Atlantic coast.

Exposed individuals frequently report an acute but rapidly reversible syndrome consisting of conjunctival irritation, rhinorrhea, sneezing, cough and (rarely) respiratory distress similar to an asthma attack. Persons swimming or wading in *K. brevis* red tides may experience eye and skin irritation accompanied by redness and itching. Following short-term exposures, the symptoms usually subside when the individual leaves the immediate vicinity of the beach. Prolonged exposure, however, can cause symptoms that linger for hours or days after the person leaves the affected area. Consequently, DSHS recommends that visitors to the affected areas avoid swimming or wading in red tide waters.

And the information card *Red Tide Facts* issued by the TDSHS states:
http://www.tceq.state.tx.us/files/gi-367.pdf_4003674.pdf

Health Tips

1. Red tide can cause burning eyes and skin irritation. If your skin is easily irritated, avoid red tide-affected water. If you experience irritation, get out of the water and thoroughly wash with fresh water.
2. Symptoms common when breathing red tide toxins include coughing, sneezing, and teary eyes. Symptoms are usually temporary. Wearing a particle filter mask may lessen the effects, and research shows that using over-the-counter antihistamines may decrease your symptoms. Check the marine forecast. Fewer toxins are in the air when the wind is blowing offshore.
3. For people with respiratory problems: avoid red tide affected areas, especially when winds are blowing toxins near shore, and take your short-acting inhaler with you. If you have symptoms, leave the beach and seek air conditioning.

Having provided the information, the various state authorities appear to leave it up to the individual to decide, perhaps not surprising since tourism is an important industry. For example -

From the Texas Parks & Wildlife Department
http://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/redtide/

> Although some travelers may be concerned with how the red tide may affect their vacation plans, there are miles of clean beaches to enjoy on the Texas coast. When making travel plans, heed the advice of the Texas Department of State Health Services: **get the current facts and draw your own conclusions** (report authors'emphasis).

5.2 *Karenia brevisulcata (Gymnodinium brevisulcatum)*

Anon. (2001a) provided this account of a harmful bloom of *K. brevisulcata*, which caused a number of incidents involving respiratory distress in New Zealand.

"Off Kaikoura and Wairarapa along the central east coast of New Zealand reports of sporadic massive mortality of fish and other marine fauna started in mid-January during the summer (S hemisphere) of 1997-98. During this period large numbers of dead tuna, striped marlin, broad bill swordfish, sea urchins, starfish, and abalone washed up on the shore between Castle Point and Palliser Bay. More than 200 people at Riversdale, Castle Point, and Mataikona were reported to suffer from respiratory distress. Further outbreaks of human respiratory syndromes occurred two weeks later in several areas in Hawke Bay, north of Wairarapa coast. In all cases, swimmers, surfers, and beach-goers complained of a dry cough, sore throat, running nose, and eye and skin irritations. Most people recovered upon leaving the affected area and no lasting effects were noted.

Wellington is located to the southwest of Wairarapa coast. An unprecedented algal bloom from February to March 1998 decimated almost all marine organisms in the harbor. During this period 87 people were reported to have suffered from respiratory distress. 'Sun-burn' facial sensation was also experienced by some divers, and headaches were reported by several hatchery workers in the NIWA's Mahanga Bay laboratory in Wellington Harbour."

The toxin(s) of *K. brevisulcata* have not yet been fully characterized but crude extracts have been shown to interact with mammalian sodium channels (Truman, 2007). The brevetoxins from the related *K. brevis* act on voltage-gated sodium channels and the *K. brevisulcata* toxins may prove to be brevetoxins or similar molecules.

5.2.1 *Ostreopsis ovata*

This dinoflagellate is known to produce palytoxin and its congeners, the ostreocins. Palytoxin, a large and complex molecule, is one of the most potent non-peptide toxins known, its mode of action being to inactivate Na^+/K^+ ATPases. It has been implicated in seafood poisoning in tropical and sub-tropical areas (Katikou, 2007; Vale *et al*., 2007).

In the summer of 2005, about 200 people who spent time on or near beaches in a stretch of the northwest Italian coast in the area of the city of Genova, sought medical treatment for symptoms such as rhinorrhoea, cough, fever, broncho-constriction with mild breathing difficulties, wheezing, and, in a few cases, conjunctivitis. For almost all these people, the symptoms stopped after a few hours, although 20 people required admission to hospital.

The cause of these symptoms was suspected to be *Ostreopsis* spp. as in previous years, similar events were reported in other regions of Italy: Toscana (W coast of Italy), Puglia (SE coast of Italy) and Sicily, although these were not as widespread and as severe.

In the first days few days of the incident *Ostreopsis ovata* was found to be present in seawater at cell densities up to several thousands cells.L^{-1} and hundreds of thousands of cells.g^{-1} of macroalgae. The analysis of water, plankton and macrophytes indicated the presence of palytoxin or one of its isomers (Brescanini *et al*., 2006; Barone, 2007).

In 2006 a monitoring program was initiated, aiming to prevent further harmful exposure to these algae. Bathing was forbidden in an area about 10 km in length along the coast east of Genova from 29 July to 4 August after *O. ovata* was found to be present in significant numbers. This was the only control measure that could be applied to protect human health. Despite this precaution about 20 people reported symptoms similar to those observed in 2005, but it was thought that only a few of these were caused by inhalation of aerosolised *O. ovata* fragments (Brescanini *et al*., 2006).

Clinical epidemiological studies were reported by Gallitelli *et al.* (2005) and Durando *et al.* (2007).

The report of the ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) for 2005 (see list of reports at the end of the reference section) noted this event in 2004 -

> **Spain**
>
> **Catalonia and Balearic Islands**
> High concentrations (up to 10^5cells L^{-1}) of the benthic dinoflagellate *Ostreopsis siamensis* were observed to be resuspended in the water column in touristy (sic) beaches in August. There were 40 reports of human respiratory irritation at the beach of Llavaneras.

- and the WGHABD report for 2007 noted the occurrence of these events in 2006 -

Spain

Aerosol-Borne Irritatory Syndromes
These syndromes affected sunbathers in the Mediterranean coast in summer (July–August).

In Llavaneres beach (Catalonia), 33 sun-bathers suffered nose and eye irritation during 2 days in August. The suspected cause was the benthic dinoflagellate *Ostreopsis* cf *siamensis*. Concentrations were up to 2,000 cell/L during the toxic events, but 18,000 cell/L 10 d before the irritation.

In several beaches in Almeria (Andalucia), 40-50 people (8-9 July) affected with breathing difficulties received medical attention. Water analyses revealed the presence of *Karenia* spp. (18,800 cell/l) and *Chattonella* spp. (2,080 cell/l). *Ostreopsis* spp. were detected in August.

5.3 *Karenia mikimotoi* (*Gyrodinium* cf. *aureolum*)

During blooms off New Jersey (USA) in 1984 and 1985 respiratory symptoms were recorded in lifeguards and people spending prolonged periods on the beach (Mahoney *at al*., 1990) - see sect. 3.4.2.

The WHO guidelines (Anon. 2003, chapter 7, sect. 7.3) mention in relation to aerosols of *K. brevis* that '…similar problems have now been reported in New Zealand….. which were thought to have been caused by *Karenia mikimotoi*, and cite Fernadez and Cembella (1995). However, the only reference to *K. mikimotoi* in that article appears in a section about the mouse bioassay for neurotoxic shellfish poisoning, which states:

"However, the discovery of a novel bioactive compound (*authors*'*note*: gymnodimine - not to be confused with the gymnocins mentioned in sect.3.4.1), produced by the dinoflagellate *Karenia mikimotoi*, a common species in New Zealand waters during neurotoxic events, has led the local health authorities to return to the diethyl-ether extraction procedure…."

- so the connection between that statement and the one made in the WHO guidelines is not immediately obvious.

Large and persistent blooms of *K. mikimotoi* have occurred round the British Isles and Eire in recent years (see sect. 3.4) and were extensively monitored but there were no reports of respiratory or other symptoms in humans. Analysis of samples of *K. mikimotoi* from the Irish bloom for presence of brevetoxins showed none to be present (Silke *et al*., 2005).

5.4 *Pfiesteria piscicida*

This has been reported to produce severe symptoms in humans exposed to aerosols in the laboratory, see sect. 3.5.

5.5 Cyanobacteria

Annadotter *et al*. (2005) described incidents of influenza-like symptoms in several Scandinavian towns and in Harare (Zimbabwe) that occurred shortly after people had inhaled steam whilst taking a bath, shower or sauna and also after washing dishes. The cause was shown to be due to cyanobacterial endotoxins (lipopolysaccharides) in the local water supply due to blooms in local drinking water reservoirs. It was also

found that gram-negative bacteria associated with cyanobacterial mucilage could be a source of endotoxins.

Sharma *et al*. (2006) characterized the airborne algal diversity in subtropical urban environment, the city of Varanasi in India. The city is surrounded on three sides by open agricultural land and on the other side the river Ganges. Results indicated that airborne algae are a permanent constituent of the city atmosphere. Cyanobacteria were found to dominate the aerial algal flora, which was attributed the fluctuating climates of subtropical regions. The majority of the airborne algae were of local origin, indicating short-distance transport of the algae. Soilborne algae constituted the bulk of aerial algal flora. This was attributed to their ability to withstand the dehydrating effect of the atmosphere. These workers concluded that their findings could have implications for human health as airborne algae could act as allergens (McElhenny *et al*. (1962) and Maynard (1968) cited in Sharma *et al*., 2006). They also cited a point made by Maynard that, along the shoreline of lakes and other water bodies, fragments of scums and foams with their contents of algae can be picked up by the wind and dispersed. Sharma *et al*. (2008) investigated the allergenicity of the cyanobacteria

Phormidium fragile and *Nostoc muscorum* isolated from samples of airborne particles. Of the two, the latter was more allergenic but when the two were mixed in equal amounts their allergenic effect was increased.

Cheng *et al*. (2007) carried out laboratory and field studies on sampling techniques and measurement of aerosols containing microcystin. The techniques were used in a field study to determine if aerosols containing microcystin could be found in and around a body of fresh water containing a bloom of cyanobacteria and if people engaged in recreational activities could be exposed to the aerosol. The preliminary results indicated the answer to both questions was 'yes'. Concentrations of the microcystin in the water were low (ca. 1 $\mu g.L^{-1}$) so the aerosol samples contained very low levels of microcystin, barely above the level of detection. The study demonstrated that it is possible using both high-volume and personal samplers to detect very low levels of microcystin in aerosols in areas where cyanobacteria are present and that these methods could be used in future studies to asses the exposure and dose of inhaled microcystin received by humans engaged in recreational activity.

Burns (2008) noted:

"Historically, atopic sensitivity to cyanobacteria has been reported following exposure to algae in lakes. For example, Heise (1949) found blue green algae responsible for seasonal rhinitis following exposure to algae while swimming in lakes. Human sensitivity to cyanobacteria may be related to a hereditary predisposition toward developing certain hypersensitivity reactions when exposed to specific antigens".

He goes on to comment that, as regards Florida, "….relationships between toxic cyanobacteria and their environmental consequences remain largely in the realm of incidental observation and speculation." This topic is discussed further in sect. 6.

Hudnell and Dortch (2008) presented a synopsis of research needs identified at the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB) held in 2005. Among the needs highlighted was –

"An understanding of the interactions between cyanotoxins and environmental factors is needed to assess the potential for exposure of human and other biota. Of particular interest is the transportation of cyanotoxins through aerosols, biota, and water…."

5.6 Conclusions

- The dispersal of some microalgae as aerosols and particles is well documented.
- Where dispersed via marine aerosols the toxins they contain can cause acute symptoms (known examples are the dinoflagellates *K. brevis*, *K. brevisulcata*, *O. ovata* and possibly *K. mikimotoi*). With the exception of the latter, these dinoflagellates are presently confined to warmer seas and do not, therefore, constitute a potential hazard in UK waters at present. There is no strong evidence that *K. mikimotoi* can cause serious respiratory distress in humans.
- Toxic cyanobacteria can also be dispersed as aerosols but relatively little research appears to have been carried out as to whether this presents a significant hazard. Given that the blooms reach high densities and that the free toxins are stable in water, the dispersal of cells and toxins in aerosols under suitable meterological conditions must be considered a possibility.
- When dispersed as aerial particles both eukaryotic microalgae and cyanobacteria can act as allergens. Inhalation of toxic cyanobacteria from dried scums where these become dispersed aerially in large quantities must also be considered a possibility and therefore a potential health risk.

6 Freshwater cyanobacterial blooms

Mass growths of cyanobacteria (blue–green algae), leading to the production of blooms, scums, and mats occur in nutrient-enriched water bodies throughout the world (Codd, 2000; Codd *et al*., 1999, 2005). Blooms of toxic cyanobacteria are predominantly a freshwater phenomenon, although they may also occur in estuarine and marine environments. There are over 50 species of cyanobacteria within various genera, including *Anacystis*, *Apahanizomenon*, *Cylindrospermum*, *Cylindrospermopsis*, *Microcystis*, *Lyngbia*, *Nodularia*, *Nostoc* and *Planktothrix* (=*Oscillatoria*) (Codd and Bell, 1996; Long and Carmichael, 2004).

There is a vast body of literature on cyanobacterial toxins and for details of their chemistry, biochemistry and toxicology the reader is referred to Dittmann and Wiegand (2006), van Apeldoorn *et al*. (2007) and Gago-Martinez (2007). Table 7 details the main cyanobacterial toxins and some of their properties. The microcystins, produced by *Microcystis aeruginosa*, are the most commonly encountered cyanobacterial toxins in UK freshwaters (Codd and Bell, 1996).

Table 7. Toxicological data for some of the major cyanotoxins. Data from Table 3 of Funari and Testai (2008). See also Anon. (2003; Chapter 8, Table 8.1).

Cyanotoxin produced by (*genus*)	Intraperitoneal LD_{50} [1] $\mu g.kg^{-1}$ body wt	Oral LD_{50} $\mu g.kg^{-1}$ body wt	Target organ and mechanism of action	NOEL [2] $\mu g.kg^{-1}.d^{-1}$	LOEL [3] $\mu g.kg^{-1}.d^{-1}$
Microcystin [MC] hepatotoxin (*Microcystis*)	50–1,200	5,000	Liver (PP1 and PP2A phosphatases inhibition, tumour promoter	40 (MC-LR[4]; mice; 13 weeks exposure; gavage	200
				330 (MC-LR in BGAS[5]; extracts; mice; 13 weeks; dietary	
Nodularin hepatotoxin (*Nodularia*)	50	ND	Liver (PP1 and PP2A phosphatases inhibition, tumour promoter	ND (refer to MC-LR)	ND
Cylindrospermin [CYN]	2,100 (24h)	4,400-6,900 (2-6	Kidney, liver (Parent	30 (mice; 11 weeks;	60

neurotoxin (*Cylindrospermopsis*)		days)	compound: protein synthesis inhibition; Metabolites: different but unknown mechanism; possible genotoxicity	gavage). *C. raciborski* extracts more toxic than pure CYN	
Anatoxin-a neurotoxin (*Anabena*)	375	5,000	Neuromuscular system (depolarising effect due to binding to nicotinic Ach receptor)	>510 (mice; 54 days; drinking water) Limited chronic risk	ND
Homoanatoxin-a neurotoxin (*Planktothrix*)	330	ND	Similar to anatoxin-a	ND	–
Anatoxin-a(s) neurotoxin (*Anabena flos-aquae*)	20–40		Peripheral nervous sytem (AChE inhibition; nerve hyper-excitability)	ND Limited chronic risk	–
Saxitoxin neurotoxin (*Anacystis*)	10–20	263	Neuromuscular system (Membrane Na^+ ion channel block) Human: 0.144 –0.304 mg/person: from moderate symptoms up to paralysis and death	ND Acute risk > chronic	–
Lipopolysaccharide (LPS) endotoxins (most genera)	40–190 $mg.kg^{-1}$. body wt.	ND	Possible skin and mucosa (irritation; topical effects)	ND	–

Notes: [1] *LD_{50} - the dose of toxin causing death in 50% of the experimental population* ***[2]*** *No Observed Effect Level;* ***[3]*** *Lowest Observed Effect Level****;*** ***[4]*** *the most common of the microcystin family (Gago-Martinez, 2007)* ***[5]*** *BGAS = blue-green algae nutritional (health food) supplement; ND = not determined*

Table 8. Maximum concentrations of the major cyanotoxins found in cyanobacterial blooms reported in the literature. Data from Table 2 of Funari and Testai (2008).

Cyanotoxins	Maximum concentrations	Location
Microcystins	7,300 $\mu g.g^{-1}$	China & Portugal
Microcystins	15,600-25,000 $g.L^{-1}$	Japan, Germany
Nodularins	18,000 $\mu g.g^{-1}$	Baltic Sea
Cylindrospermin	5,500 $\mu g.g^{-1}$	Australia
Anatoxin-a	2,600-4,400 $\mu g.g^{-1}$	Finland, Japan
Saxitoxins	2,040-3,400 $\mu g.g^{-1}$	Australia
Anatoxin-a(s)	3,300 $\mu g.g^{-1}$	Denmark

As regards human exposure to cyanobacteria and public health, a number of reviews are available including those by Kuiper-Goodman *et al*. (1999), Codd *et al.* (2005) and Stewart *et al*. (2006c and supplement). A more concise overview can be found in the World Health Organization's *Guidelines for Safe Recreational Water Environments* (Anon. 2003, chapters 7 and 8).

A recent comprehensive review on human health risk assessment related to exposure to cyanotoxins has been published (Funari and Testai, 2008) and exposure to cyanotoxins due to recreational activities is one of the topics discussed at some length.

They begin by saying that in locations where there are occasional and transient blooms, exposure to cyanobacteria and their toxins probably does not present a chronic risk, but in areas where there are persistent blooms of cyanobacteria and intensive recreational activities then subacute or sub-chronic exposure may constitute a risk to public health. The review considers the following:

6.1 Illness possibly caused by cyanobacteria

Evidence is cited that acute illnesses may be associated with cyanobacterial blooms: This includes a report of two soldiers who developed pneumonia after swallowing water while engaged on a canoeing exercise on a freshwater reservoir in Staffordshire. At the time there was a bloom of *Microcystis aeruginosa*. The toxin microcystin was shown to be present in the bloom material. Other soldiers involved in the exercise presented with symptoms that might have been associated with cyanobacterial poisoning, including sore throats, headaches, abdominal pains, dry coughs, diarrhoea, vomiting, and blistered mouths (Turner *et al*., 1990, and see also Codd *et al.*, 1999).

Other cases of illness reported in various parts of the world are detailed in Anon. (2003) and Stewart *et al.* (2006b; 2006c and supplement).

6.2 Allergic reactions possibly caused by cyanobacteria

The review also details evidence for cyanobacteria being responsible for cutaneous (dermal) reactions typical of allergic responses, as well as symptoms such as conjunctivitis, rhinitis, asthma and urticaria being indicative of immediate hypersensitivity responses. It has been suggested that the allergens responsible for

these reactions may be cyanobacterial lipopolysaccharide (LPS) endotoxins, but it was concluded that there is insufficient evidence to substantiate this (see Table 9).

After an extensive survey of the literature on LPS and public health, Stewart *et al*. (2006d) concluded:

"There is a danger that initial speculation about cyanobacterial LPS may evolve into orthodoxy without basis in research findings. No cyanobacterial lipid A structures have been described and published to date, so a recommendation is made that cyanobacteriologists should not continue to attribute such a diverse range of clinical symptoms to cyanobacterial LPS without research confirmation"

Funari and Testai (2008) point out that other compounds that may be present in the water (aldehydes, ketones and terpenoids) during cyanobacterial blooms could be responsible for the some of the symptoms reported.

In a comprehensive review entitled "Recreational and occupational field exposure to freshwater cyanobacteria – a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment", Stewart *et al.* (2006c) came to the conclusion:

"A range of freshwater microbial agents may cause acute conditions that present with features that resemble illnesses attributed to contact with cyanobacteria and, conversely, acute illness resulting from exposure to cyanobacteria or cyanotoxins in recreational waters could be misdiagnosed. Accurately assessing exposure to cyanobacteria in recreational waters is difficult and unreliable at present, as specific biomarkers are unavailable. However, diagnosis of cyanobacteria-related illness should be considered for individuals presenting with acute illness following freshwater contact if a description is given of a water body visibly affected by planktonic mass development."

Some of the studies on the effect of contact by whole cells and extracts from cyanobacteria on humans and animal analogues are summarized in Table 9.

Table 9. Summary of studies on allergenicity/sensitivity rections caused by cyanobacteria.

Study	Details	Reference
Allergenic (sensitization, skin and eye irritation) effects of freshwater cyanobacteria	A sensitization test on albino guinea pigs and intradermal reactivity and occular irritation test on albino rabbits were carried out with freeze-dried algal suspension in physiological salt solution. The sensitivity of guinea pigs is similar to that of humans. *Microcystis*, *Anabaena*, *Cylindrospermopsis and Aphanizomenon* bloom and strain samples were examined in sensitization and irritation tests and no correlation was found between the toxin content and the allergenic character. The most toxic sample (*Microcystis aeruginosa*) was not the most allergenic but the nontoxic *Aphanizomenon* was the most allergenic. The axenic strains were not allergenic at all. Pure microcystin-LR was only slightly allergenic even in high concentration (1.5 $mg.mL^{-1}$). Water and lipid soluble fractions were obtained from lyophilized algal suspensions. Only one of the lipid soluble fractions was skin irritative whereas the	Torokne *et al*. (2001)

	strongest irritative effect was shown by the water soluble fraction.	
Acute skin irritant effects of cyanobacteria in healthy volunteers using skin patch testing	Cell suspensions and extracts of cyanobacterial cultures of *Microcystis aeruginosa* (non-toxic strain), *Anabaena circinalis* and *Nodularia spumigena* were applied to 64 volunteers in one trial, and *Microcystis aeruginosa* (toxic strain), *Aphanocapsa incerta* and *Cylindrospermopsis raciborskii* were applied to 50 volunteers in a second trial. The conclusion was that a small proportion of healthy people (around 20%) may develop a skin reaction to cyanobacteria in the course of normal water recreation, but the reaction is mild and will resolve without treatment.	Pilotto *et al.* (2004)
Cutaneous hypersensitivity reactions to freshwater cyanobacteria – human volunteer studies	A consecutive series of adult patients presenting for diagnostic skin patch testing participated. A sample of volunteers matched for age and sex was also enrolled. Patches containing aqueous suspensions of various cyanobacteria, including *Microcystis aeruginosa*, at three concentrations were applied for 48 h; dermato- logical assessment was made 48 h and 96 h after application. 20 outpatients and 19 reference subjects were recruited into the study. A single outpatient produced unequivocal reactions to several cyanobacteria suspensions; this subject was also the only one of the outpatient group with a diagnosis of atopic dermatitis. No subjects in the reference group developed clinically detectable skin reactions to cyanobacteria. The conclusion from the study was that hypersensitivity reactions to cyanobacteria appear to be infrequent in both the general and dermatological outpatient populations. As cyanobacteria are widely distributed in aquatic environments, a better appreciation of risk factors, particularly with respect to allergic predisposition, might help to refine health advice given to people engaging in recreational activities where nuisance cyanobacteria are a problem.	Stewart *et al.* (2006a)
Cyanobacterial lipopolysachharides (LPS)	Review of evidence. LPS frequently cited in the cyanobacteria literature as toxins responsible for a variety of heath effects in humans (skin rashes to gastrointestinal, respiratory and allergic reactions). Most of the small number of formal research reports describe cyanobacterial LPS as weakly toxic compared to LPS from the Enterobacteriaceae. The literature on cyanobacterial LPS was systematically reviewed and also examined was the literature relating to heterotrophic bacterial LPS and the atypical lipid A structures of some photosynthetic bacteria. A convincing body of evidence could not be found to suggest that heterotrophic bacterial LPS, in the absence of other virulence factors, is	Stewart *et al.* (2006d)

	responsible for acute gastrointestinal, dermatological or allergic reactions via natural exposure routes in humans.	

6.3 The WHO Guidelines

The review by Funari and Testai goes on to consider the Guidelines for Safe Recreational Water Environments drawn up by the World Health Organization (Anon., 2003) and the rationale behind them, based on the epidemiological study by Pilotto *et al*. (1997) and the known toxicological properties of microcystins. They maintain that, although the guidelines represent an important tool for risk assessment, they have some limitations: (i) that the study by Pilotto *et al.* (1997) on which they are based has certain shortcomings; (ii) values related to systemic effects are based on the toxicological profile of microcystin-LR to the exclusion of other cyanobacterial toxins (see Table 7); (iii) the WHO guidelines are expressed in terms of cell densities (see Table 10), whereas in some cases, such as senescent blooms containing microcystins, high levels of dissolved cyanotoxins may occur in water. In these conditions, they say, cell density can be misleading as an indicator for the absence of toxins and/or of risk for human health. A number of cyanobacterial toxins, including microcystins and nodularins, are stable in the free state (Gago-Martinez, 2007 and see comments about stability of nodularin in sect. 3.11).

Table 10. WHO guidelines for safe practice in managing recreational waters (Anon., 2003, Chapter 8, Table 8.3).

Guidance level or situation	How guidance level derived	Health risks	Typical actions [1]
Relatively low level of adverse effects			
20,000 cyanobacterial cells.ml^{-1} or 10µg chlorophyll *a*.L^{-1} with dominance of cyanobacteria	From human bathing epidemiological study	• Short-term adverse health outcomes, e.g. skin irritations, gastrointestinal illness	• Post on-site risk advisory signs • Inform relevant authorities
Moderate probability of adverse health effects			
100,000 cyanobacterial cells.ml^{-1} or 50µg chlorophyll *a*.L^{-1} with dominance of cyanobacteria	From provisional drinking-water guideline value for microcystin-LR[2] and data concerning other cyanotoxins	• Potential for long-term illness with some cyanobacterial species • Short-term adverse health outcomes, e.g. skin irritations, gastrointestinal illness	• Watch for scums or conditions conducive to scums • Discourage swimming and further investigate hazard • Post on-site risk advisory signs • Inform relevant authorities
High probability of adverse health effects			
Cyanobacterial scum formation in areas where whole-body contact and/or risk of ingestion/ aspiration occur	Inference from oral animal lethal poisoning Actual human illness case histories	• Potential for acute poisoning • Potential for long-term illness with some cyanobacterial species • Short-term adverse health outcomes, e.g. skin irritations, gastrointestinal illness	• Immediate action to control contact with scums; possible prohibition of swimming and other water activities • Public health follow-up investigation • Inform public and relevant authorities

Notes: *[1] Action taken should be determined in light of extent of use and public health assessment of hazard.*
[2] Provisional drinking-water guideline for microcystin-LR is 1µg.L^{-1} (Anon., 1998).

A number of other epidemiological studies in relation to cyanobacteria were reviewed by Funari and Testai, including three carried out in the UK (Philipp, 1992; Philipp and Bate, 1992; Philipp *et al*., 1992). These studies were conducted on recreational users of six inland water bodies, in five of which cyanobacterial blooms were present. The results of these UK studies were not statistically significant whereas other studies, e.g. Pilotto *et al*. (1997), were statistically significant. For a detailed discussion of these references see Stewart *et al.*, 2006c, Table 2). In discussing these studies, Stewart *et al*. (2006c) commented that, despite the equivocal results, there remained the potential for serious injury or death due to exposure to the potent hepato- and neurotoxins produced by blooms of freshwater cyanobacteria, the likely exposure route for those toxins being either orally, from ingestion of recreational water and possibly by inhalation (see sect. 5.).

6.4 Possible under-reporting of symptoms

Funari and Testai comment that, because of their nonspecific and relatively mild nature, many acute symptoms caused by exposure to cyanobacteria are not being

recorded unless they require medical attention. Conversely, they say, symptoms such as dermatitis and gastro-intestinal effects may be erroneously attributed to cyano-bacterial metabolites and may be due to other causes:

"……by other chemicals dissolved in water during cyanobacterial blooms, as well as by other risk factors present in fresh waters, like avian cercariae, other bacteria and/or viruses, or physical stimuli that can induce non-allergic urticaria."

They cite Stewart *et al*. (2006d) regarding the possible role of bacterial lipopolysaccharides and see Stewart *et al*. (2006c). By 'other chemicals' they presumably mean the -

"……(aldehydes, terpenoids and ketones), some of which are endowed with irritating and sensitizing properties, are dissolved in water."

that they refer to earlier in their review (p. 108) as opposed to cyanobacterial toxins per se.

The section on recreational exposure in the review concludes:

"....on the basis of anecdotal, epidemiological and toxicological data, it appears that the risk of severe effects for bathers is posed by cyanobacteria only when they bloom or form scums."

(Table 8 illustrates the concentrations of cyanotoxins that can occur in bloom material and compare those values with the lowest observable effect limits given in Table 7). Funari and Testai go on to say:

This issue has been well addressed in an article of the new Directive of the European Union (EU Directive 2006/7/EC) concerning the management of bathing water quality. In relation to cyanobacterial risks, Article 8 states the following:

Cyanobacterial risks

1. When the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks.
2. When cyanobacterial proliferation occurs and a health risk has been identified
   or presumed, adequate management measures shall be taken immediately to prevent exposure, including information to the public.

With this article, the EU Directive on one hand recognizes the importance of cyanobacterial risk in bathing waters, and on the other, provides neither general nor specific limits, taking into account the complexity of this issue and the still scant information available on known/ unknown toxins and their toxicological profile. Local authorities are asked to assess the risk in the specific water body and to promote appropriate measures to prevent dangerous exposures, including information to the public on the possible risks. Assessing the risk, and managing it, require adequate specific skills and can be quite challenging."

6.5 Research needs

In a final section identifying research needs Funari and Testai highlight the need for the identification of repeated-dose toxicity for cyanotoxins other than the microcystin MC-LR, necessary for the derivation of guidance values and regulatory limits.

Similarly, in a synopsis of research needs presented at a large international conference, Hudnell and Dortch (2008) argued that information on the effects of cyanotoxins is largely confined to high-level exposures involving LD_{50} (see Table 7) bioassays on animals and, useful as such studies are, they do not answer questions relevant to human environmental exposures, which were identified as: (i) the relative potency of cyanotoxins through different routes of exposure (inhalation, dermal absorption, ingestion); (ii) the effects of repeated, low-level exposures; (iii) the combined effects from exposure to commonly occurring cyanotoxin mixtures (including additive and synergistic effects); (iv) factors that increase or decrease the susceptibility of individuals (and other animal species) to adverse effects from exposure.

They went on to say that human exposures to cyanotoxins are most likely to occur through contact with recreational waters and drinking water. The probability of high-level exposures through water ingestion was considered less than that for repeated, low-level exposures through recreational or drinking water contact (ingestion, dermal absorption and inhalation). However, they say, much less is known about the health risks posed by repeated, low-level exposures. Lower level exposures may cause acute illness characterized by nonspecific symptoms such as gastro-intestinal distress, skin rashes, respiratory difficulty, and influenza-like symptoms. They suggested lower level exposures also may cause chronic illness in some individuals, characterized by sustained fatigue, muscle and joint pains, and severe neurological symptoms that persist indefinitely and that clinical research is needed.

6.6 Conclusions

- Human exposure to the high concentrations of toxins present in dense cyanobacterial blooms and scums in and around recreational waters appears to constitute a real health hazard.
- There is evidence that exposure can cause a variety of symptoms, mostly minor, but few have been well characterized. The evidence is largely anecdotal. The scientific research community has clearly signaled the topics requiring further investigation, including chronic, low-level exposure in environments like recreational waters.
- In the absence of a substantial body of hard data regarding inhalation, aspiration and ectopical contact with both whole cells and dissolved toxins, probably the safest approach to assessment and risk management at present is provided in the WHO guidelines (Anon., 2003), which may be summarized by the statement in those guidelines: “For practical purposes, the present state of knowledge implies that health authorities should regard any mass development of cyanobacteria as a potential health hazard.” In addition to the WHO guidelines the risk assessment and management strategies devised by the Scottish Executive Health Department Blue-Green Algae Working Group (Anon., 2007a) and by Codd *et al.* (2005) also provide useful models.

7 Macroalgae

Bathing beaches are habitats not normally associated with dense growth of seaweed or macroalgae. Rocky outcrops or shores surrounding beaches may have varying densities of algal growth depending on degree of exposure. Bathing beaches are, however, subject to varying amounts of detached seaweed being washed up after storms and settling out on the strandline. This strandline material, with associated flotsam and jetsam, needs to be assessed on an individual beach basis in terms of its potential health risk.

As with microalgae, blooms of macroalgae appear to be becoming more frequent due to increasing nutrient loading of coastal waters. These 'nuisance' species tend to be mainly filamentous, often unattached forms, mainly green algae (e.g. *Ulva* spp., *Cladophora* spp.) in nutrient-rich, temperate waters with occasional examples of brown algae such as *Pilayella littoralis* (Valiela *et al.*, 1997; Raffaelli *et al.*, 1998). These could impact on bathing beaches but mainly for aesthetic reasons as washed-up decaying seaweed.

In a study of patients with atopic sensitivity to algae and lichens Champion (1971) included tests with extracts of several seaweeds, the thalloid green alga *Ulva*, and the brown algae *Fucus* and *Laminaria* (species not specified). Four out of five patients responded positively to skin prick tests with extracts of *Ulva* but all three subjects who were subjected to patch tests with this alga showed no reaction. One out of five patients showed a positive reaction to skin pricks of *Laminaria* and *Fucus* extracts but skin patch tests of these extracts on three patients were negative in each case.

Approximately 500–600 secondary metabolites have been isolated from marine algae and these may function either as herbivore deterrents or as allelopathic and antifouling agents (Hay and Fenical, 1988) but no adverse effects on human health, i.e. no direct chemical-based toxic effect on humans due to these compounds, could be found reported in literature.

One metabolite investigated was caulerpenyne, a bioactive terpenoid produced by *Caulerpa taxifolia.* Given the extensive growth of this species in the Mediterranean Sea where it was introduced, the cytotoxic effects of caulerpenyne were studied in different *in vitro* models using various human cell lines but the risk of cutaneous and/ or food intoxication to humans was considered to be minimal (Parent-Massin, 1996). With the trend of rising sea temperatures, it is possible that species like *Caulerpa taxifolia* and other 'exotic' seaweed species may find their way into UK waters in the future and could impact on bathing beaches.

7.1 Microalgae associated with seaweeds

Recorded health problems associated with seaweeds appear to be as a result of epiphytic micro-algae or cyanobacteria rather than caused by the macroalga *per se*. An example of this is respiratory illness associated with blooms of the normally epiphytic dinoflagellate *Ostreopsis* spp. in the Mediterranean Sea (see sect. 5.3).

Prorocentrum lima, a toxic epibenthic dinoflagellate, is common in UK waters but there are no reports of it causing symptoms in humans due to direct contact with intact cells (see sect. 4. and notes 5 and 6 in Table 6).

8.2 Seaweed dermatitis

Seaweed dermatitis (or 'swimmer's itch') is caused by direct contact with the cyanobacterium, *Lyngbya majuscula* (synonym *Microcoleus lyngbyaceus*). *L. majuscula* has been recorded in the tropical and subtropical regions in the Pacific and Indian Oceans and in the Carribbean and Mediterranean seas (Osborne *et al*., 2001).

It grows in fine strands 10–30 cm long loosely attached to seagrass, sand, coral and rocky outcrops and usually occurs in matted, filamentous clumps.

Contact seaweed dermatitis was first described from Hawaii (Grauer and Arnold, 1961). Symptoms include dermatitis involving itching, rash, burning blisters with deep desquamation and pain (Solomon and Stoughton, 1978) and respiratory irritation (Anderson *et al*., 1988). Debromoaplysiatoxin and lyngbyatoxin-a have both been isolated from *L. majuscula* and are known to be highly inflammatory and also potent protein kinase C activators and tumour promoters (Mynderse *et al*., 1977; Anon., 2003). Seaweed dermatitis caused by *L. majuscula* has also been recorded in Australia (Osborne *et al.*, 2001, 2007) and Japan (Yasumoto & Murata, 1993).

Lyngbyatoxin-a and debromoaplysiatoxin have been identified from *Lyngbya* samples collected from springs and marine embayments in Florida and severe dermatitis has also been reported following recreational activities in waters supporting *Lyngbya* blooms in Florida's springs (Burns, 2008).

Trichodesmium, another cyanobacterium that blooms frequently in sub-tropical regions can also cause swimmer's itch (Anderson *et al.*, 2001).

As *Lyngba majuscula* is presently confined to tropical and subtropical regions it is unlikely to become a problem in UK bathing waters for the foreseeable future.

8.3 Allergy due to contact with *Sargassum muticum*

'Japweed', *Sargassum muticum*, is an invasive exotic species of seaweed that has become established in Europe and in various parts of the UK. There are reports of it causing contact dermatitis in professional fishermen in the Netherlands (van der Willigen *et al*., 1988a,b) where it grows prolifically in Lake Grevelingen. The allergen appears to be iodine, which occurs in high concentrations in many seaweeds and kelps. It was concluded that allergic reactions to the alga occur 'only after prolonged and intensive skin contact'. The risk to recreational beach users in the UK, should they encounter this seaweed, therefore appears to be low.

7.4 'Dogger Bank Itch' or 'Weed Rash'

A condition referred to as 'Dogger Bank Itch' or 'weed rash',' which is an allergic contact dermatitis, is caused by an organism that may appear to be a seaweed but is, in fact, a bryozoan (see illustrations in Pathmanban *et al*., 2005). This is mentioned here as it has been mistakenly identified as a seaweed in the past. It was first described in the 1930s by fishermen in Denmark in connection with a skin complaint, which they alleged was due to contact with a seaweed known as 'sea-chervil' (Newhouse, 1966). Other names given to the bryozoan are 'curly weed'and 'amber weed', and 'ju-ju weed' is the name used by fishermen in the eastern English Channel (Pathmanban *et al*., 2005).

Dogger Bank Itch, which may become a chronic and debilitating illness, affects mainly fishermen and is caused by overexposure to the bryozoan, *Alcyonidium diaphanum*, which is often brought up as bycatch (Pathmanaban *et al.*, 2005; Sharp *et al*., 2007). The allergen causing the dermatitis was found to be a hydrophilic metabolite, (2-hydroxyethyl) dimethyl sulphoxonium ion, produced by the bryozoan (Carlé and Christophersen, 1982; Pathmanaban *et al.*, 2005).

A. diaphanum is rare intertidally, found only in locations characterised by tidal rapids, but is widely distributed sublittorally in fishing areas around the British coast (Porter *et al*., 2002; Sharp *et al.*, 2007). However, Porter *et al*. (2002) state:

"It has long been familiar to biologists and is conspicuous on the strandline in early autumn when huge colonies, in considerable quantities, are detached by storm action and washed ashore."

There is a possibility, therefore, that *Alcyonidium* could be handled by recreational beach users but the risk of sensitization seems small; Pathmanban *et al*. (2005) state that the period of exposure to *A. diaphanum* before sensitization occurs ranges from less than a year to more than 45 years.

Note: Evidence of toxins found to be present in or associated with seaweeds

The seaweeds, *Chondria armata*, *C. baileyana* and *Alsidium corallinum* were all found to contain measurable quantities of domoic acid, the amnesic shellfish poisoning (ASP) toxin, when the original incident of ASP was investigated at Prince Edward Island, Canada (Bates *et al*., 1989).

A poisoning incident resulting from the consumption of the red alga *Gracilaria coronopifolia* in Hawaii in 1994 was attributed to the presence of aplysiatoxin and debromoaplysiatoxin (Nagai *et al*., 1996). The origin of these toxins was later found to be *L. majuscula*, which was growing epiphytically on the red alga (Ito and Nagai, 1998, 2000). None of these algae occur in British waters.

8.5 Conclusions

- No evidence of direct risks to human health associated with contact with seaweeds indigenous to British shores was found in the literature. Situations where considerable amounts of weed are prone to wash up on the beach will have to be assessed on an individual basis in terms of possible health risks associated with decomposing weed. Proposals by the Scottish Government for implementing the Bathing Water Directive as regards macroalgae can be found in Anon. (2007b).
- The 'exotic', *Sargassum muticum*, has been reported to cause contact dermatitis, attributed to its high iodine content, but the risk to recreational beach users due to brief contact, e.g. picking up a frond from the strand line, is probably very small.
- Although the risk of recreational beach users developing dermatitis due to handling the bryozoan *Alcyonidium diaphanum* seems small, those responsible for inspecting beaches should perhaps be able to identify it and be aware of its possible effect.

8 Final discussion and general conclusions

In his comprehensive review of the effects of harmful algal blooms on aquatic organisms Landsberg (2002) lists some 200 species of dinoflagellates, diatoms, raphidophytes, prymnesiophytes, silicoflagellates, ciliates and cyanobacteria then known to be, suspected to be, or with the potential to be toxic or harmful to a wide range of terrestrial and aquatic organisms, including man. Fortunately, many of the effects detailed are manifestations of allelopathy - the production of compounds by an organism that inhibit the growth of competing micro-organisms (see Legrand *et al.*, 2003) and many of the deleterious effects on higher organisms are on gill-breathers. In most cases where human health is affected by algae it is due to direct consumption of phycotoxins via fish and shellfish vectors (see Leftley and Hannah, 2008), which is not part of this literature review. This leaves relatively few potential hazards to human health caused by cyanobacteria and by algae (both micro- and macro-) to be considered. Since some of these problem algae do not occur in UK waters, e.g. *Karenia brevis* (sect. 5.1) the field is narrowed even further, but such organisms as *K. brevis* have been included when reviewing potential hazards in a broader context. Attention has been focused mainly on the Environment Agency's list of 'notifiable' and nuisance algae.

The authors of this review are not toxicologists or epidemiologists and the assessment of potential hazards to human health posed by algae in and around the periphery of bathing waters is based solely on an assessment of relevant scientific and other literature and expertise in phycology. Table 11 summarises these conclusions and the details are provided in the main text.

In a UK context, the major hazard to bathers - and those on the periphery of bathing waters - is considered to be cyanobacteria, principally the freshwater cyanobacteria.

To reiterate the WHO *Guidelines for Safe Recreational Water Environments* (Vol. 1):

"For practical purposes, the present state of knowledge implies that health authorities should regard any mass development of cyanobacteria as a potential health hazard." The opinion of the international research community appears to be that research is required into the effects on humans of chronic exposure to cyanobacteria in natural waters (see sect. 6.), including those groups who frequently indulge in water sports.

The 'notifiable' planktonic and benthic algae on the EA list (see Table 1) do not appear to constitute a high risk to human health by contact, aspiration or inhalation. However, under certain circumstances very dense blooms of athecate dinoflagellates, such as *K. mikimotoi*, and flagellates such as raphidophytes, e.g. *Fibrocaps japonica*, might possibly cause respiratory distress if the bloom was subjected to strong wind and wave action, and whole and lysed cells were dispersed as an aerosol (see 3.8.2). But this is only speculation - no such incidents have been reported during large, persistent blooms of *K. mikimotoi* in the seas around the UK and Eire in recent years (see sects. 3.4 and 5.4).

There will always be sensitive individuals who display atopic (Champion, 1971) and other reactions even to inocuous algae but the literature suggests that for the general population the seaweeds and phytoplankton present in UK coastal bathing waters do not present a great hazard.

Climate change may mean that new genera or species of algae, some potentially harmful to humans health, may become established and bloom as the seas around the UK become warmer. For example, the benthic, bloom-forming, non-toxic dinoflagellate *Thecadinium yashimaense* has recently been recorded for the first time in Europe and has invaded the North Sea (Hoppenrath *et al*. 2007). Another example is the dinoflagellate *Ostreopsis ovata* that has become established in the Mediterranean and has caused respiratory problems to humans (see sect. 5.3). The same applies to seaweeds, which may be introduced via ships coming from abroad and subsequently becoming detached, or as sporelings attached to shells of imported live shellfish. Constant vigilance is required.

Table 11. Risk assessment of exposure to blooms of algae on the EA list of 'notifiable' and nuisance algae, based on reports in the literature.

	Assessment of risk to human health based on reports in the literature			
Alga or group of algae	**Contact**	**Aspiration**	**Inhalation [1]**	**Section reference**
Non-toxic marine phytoplankton causing nuisance blooms				
Noctiluca scintillans	**low**	**low**	**low**	2.1
Diatoms (see Tables 1 and 2) *Fragilaria striatula* is reported to cause contact dermatitis but only on prolonged exposure. Diatoms with setae (spines) in dense blooms might possibly cause minor irritation to sensitive tissues such as conjunctiva.	**low**	**low**	**low**	2.2
Phaeocystis spp. Two anecdotal reports that direct contact with dense blooms and associated foam may cause minor irritation.	**low**	**low**	**low**	2.3
Emiliania huxleyi	**low**	**low**	**low**	2.4
Toxic marine phytoplankton				
Prorocentrum lima	**low**	**low [2]**	**low**	3.2
Amphidinium carterae	**low**	**low**	**low**	3.3
Karenia mikimotoi (*Gyrodinium aureolum*) One anecdotal report (US) of nausea, eye irritation and lung congestion.	**low**	**low**	**low**	3.4 and 5.4
Pfiesteria spp. One report of detection in 'low numbers' at one site in UK. Considered by the authors of the present report not to be a threat in the UK. Recommend reading is the review article by Vogelbein *et al*. (2008).	**low**	**low**	**low**	3.5 and 5.5
Chrysochromulina polylepis	**low**	**low**	**low**	3.6
Prymnesium parvum	**low**	**low**	**low**	3.7
Fibrocapsa japonica In addition reports of ichthyotoxic haemolytic fatty acids there are references to possible brevetoxin-like compounds but no detailed evidence.	**low**	**low**	**low**	3.8
Heterosigma akashiwo	**low**	**low**	**low**	3.9
Nodularia spumigena (cyanobacterium) Not considered a major threat in that it does not bloom extensively in UK waters. Not mentioned in the WHO Guidelines. Nodularin toxin is regarded as equivalent to microcystin LR and an action level of $5x10^4$ cells.mL^{-1} has been recommended (see sect.3.11 and refs. therein).	**high**	**high**	**high**	3.11
Diatoms and dinoflagellates normally toxic to humans via shellfish vectors				
Pseudonitzschia (amnesic shellfish poisoning - ASP) *Dinophysis acuminata* (diahorretic shellfish poisoning - DSP) *Prorocentrum lima* (DSP) *Alexandrium* spp. (paralytic shellfish poisoning - PSP)	**low**	**low [2]**	**low**	4.

Freshwater cyanobacteria [3]				
The WHO Guidelines state that, "For practical purposes, the present state of knowledge implies that health authorities should regard any mass development of cyanobacteria as a potential health hazard." The opinion of the international research community appears to be that research is required into the effects on humans of chronic exposure to cyanobacteria in natural waters.	**high**	**high**	**high**	6.
Seaweeds				
No genera occurring in UK waters presently appear to present a high risk. There is a report from the Netherlands of dermatitis being caused by prolonged contact with *Sargassum muticum*. The allergen is iodine, which is present in many seaweeds.	**low**	**-**	**-**	7.3
Bryozoans				
The bryozoan *Alcyonidium diaphanum* ('sea chervil') is a known cause of contact dermatitis in fishermen due to the (2-hydroxyethyl) dimethyl sulphoxonium ion metabolite it produces. It could be mistaken for a seaweed on the strand line when washed ashore in large quantities after gales. The literature indicates that frequent and prolonged contact is necessary so the risk is assessed as low. Beach monitors should be able to identify it and be aware of its potential effects.	**low**	**-**	**-**	7.4
Notes: *[1] Inhalation via aerosols or wind dispersed particles of algal material, e.g. originating from dried algal or cyanobacterial scums.* [2] *See Table 6.* [3] *See Table 10.*				

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ADDENDUM

Reports of the IOC Working Group on Harmful Bloom Dynamics (IOC WGHABD)
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