

**RECREATIONAL AND OCCUPATIONAL FIELD EXPOSURE TO  
FRESHWATER CYANOBACTERIA – A REVIEW OF ANECDOTAL AND  
CASE REPORTS, EPIDEMIOLOGICAL STUDIES AND THE CHALLENGES  
FOR EPIDEMIOLOGIC ASSESSMENT**

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## **Abstract**

Cyanobacteria are common inhabitants of freshwater lakes and reservoirs throughout the world. Under favourable conditions, certain cyanobacteria can dominate the phytoplankton within a waterbody and form nuisance blooms. Case reports and anecdotal references dating from 1949 describe a range of illnesses associated with recreational exposure to cyanobacteria: hay fever-like symptoms, pruritic skin rashes and gastro-intestinal symptoms (the latter probably related to ingestion of water) are most frequently reported. Some papers give convincing descriptions of allergic responses to cyanobacteria; others describe more serious acute illnesses, with symptoms such as severe headache, pneumonia, fever, myalgia, vertigo and blistering in the mouth. A coroner in the United States recently found that a teenage boy died as a result of accidentally ingesting a neurotoxic cyanotoxin from a golf course pond; this is the first recorded human fatality attributed to recreational exposure to cyanobacteria. One of the main public health concerns with exposure to freshwater cyanobacteria relates to the understanding that some blooms produce toxins that specifically affect the liver or the central nervous system. The route of exposure for these toxins is oral, from accidental or deliberate ingestion of recreational water, and possibly by inhalation. Cyanobacterial lipopolysaccharides (LPS) are also reported to be putative cutaneous, gastrointestinal, respiratory and pyrogenic toxins.

This review introduces the topic of cyanobacteria in recreational waters with a brief discussion of the main cyanotoxins. A comprehensive review of anecdotal and case reports of human illness attributed to recreational and occupational exposure to cyanobacteria follows, with discussion of some important papers. Epidemiological

studies of recreational exposure to cyanobacteria are reviewed. A brief synopsis of the advantages and disadvantages of various common epidemiological study designs follows; their actual and potential application to the study of recreational exposure to cyanobacteria is raised. Discussion of some water-related risk factors that may be important differential diagnoses for cyanobacteria-related illness concludes the review.

## **Introduction**

Cyanobacteria are a diverse group of prokaryotes that occupy a broad range of ecological niches by virtue of their age, having first appeared some 2.5 billion years ago, and specialisation. All cyanobacteria are photoautotrophic organisms, yet many can grow heterotrophically, using light for energy and organic compounds as a carbon source [1]. The cyanobacteria are a remarkably widespread and successful group, colonising freshwater, marine and terrestrial ecosystems, including extreme habitats such as Antarctic lakes, salt works and hot springs [2 pp.273,277,299]. Cyanobacteria are common inhabitants of freshwater lakes and reservoirs throughout the world. Under favourable conditions, certain cyanobacteria can dominate the phytoplankton within a waterbody and form nuisance blooms.

Cyanobacteria have come to the attention of public health workers because many freshwater and brackish species can produce a range of potent toxins. This observation was first reported over 120 years ago, when sheep, horses, dogs and pigs were seen to die within hours of drinking from a lake affected by a bloom of the

brackish-water cyanobacterium *Nodularia spumigena* [3]. Since then, many reports of livestock and wild animal deaths have appeared in the literature. Such reports have been collated by several authors [4-7, 8 pp.40-43, 9]. Some reports are dramatic in terms of the number of animals affected or the rapid progression of illness and death, with mass deaths of thousands of animals [10], and large animals succumbing within minutes [11, 12]. Laboratory-based toxicological investigations have confirmed that freshwater and brackish cyanobacteria produce several categories of toxin that are (with one exception – the saxitoxins) unique to cyanobacteria. The topic of cyanobacterial toxins has been widely studied, and many excellent texts and reviews are available, e.g. [8, 12-25]. Details of the principal cyanotoxin groups that are significant from a public health perspective are summarised in Table 1.

Cyanobacteria poisoning of humans has occurred through known and suspected exposure to cyanotoxin-contaminated drinking water supplies [26] and reviewed in: [8 (pp.61-5), 9, 27]; confirmed and suspected exposure to contaminated dialysate by patients undergoing haemodialysis [28-32]; and through recreational and occupational contact. This review will concentrate on the latter exposures.

## **Rationale and search criteria**

All references that could be found in the medical and scientific literature, including conference proceedings, which describe specific incidents involving human illness and exposure to freshwater cyanobacteria in recreational or in-field occupational

settings are summarised in Table 2. The following citation sources were not examined for this exercise:

- Reports of cyanobacteria-associated illness from recreational exposures to marine or estuarine waters
- Publications written in languages other than English – with the exception of the Russian paper by Pashkevich [33], which we were opportunistically able to have translated.
- Newspaper reports – with three exceptions: two reports that describe the first human fatality to be attributed to recreational contact with cyanobacteria [34, 35]. At the time this review was submitted, these were apparently the only published references to this tragedy, so were included here because of their importance. The cyanobacteria research community awaits publication of a comprehensive case report in the scientific or medical literature. Another news article supplements a cursory description of cyanobacteria-associated illnesses; both the news report and the scientific publication appear to describe the same incident, with more detail provided by the journalist [36, 37]. There are undoubtedly many more publications in the news media that report suspected cyanobacteria-related human and animal morbidity and mortality: for example Duggan [38] and Ruff [39] reported on cyanobacteria blooms in Nebraska lakes that were associated with two dog deaths and more than 40 complaints of acute eye, upper respiratory, gastrointestinal and skin symptoms.

Anecdotal and case reports presented in this review were collected in a non-systematic process, by following reference lists in identified papers, and including citations sent

by other researchers in this field. Bibliographic database searches yielded few reports: a PubMed search conducted in March 2005 using the terms “(cyanobacteria\* OR blue green alga\*) AND recreation\*” generated 42 citations, only one of which [40] was suitable for inclusion in Table 2. A Web of Science search conducted at the same time yielded 69 citations, of which three [40-42] were eligible for inclusion in Table 2. Broadening the search terms to “(cyanobacteria\* OR blue green alga\*) AND health” in both databases generated only two further citations [22, 43]. Some key papers can be found on PubMed by narrowing the search terms, e.g. “(cyanobacteria\* OR blue green alga\*) AND pneumonia” [44], or “(cyanobacteria\* OR blue green alga\*) AND cutaneous” [45]. However, the tools for conducting a systematic review of morbidity associated with recreational exposure to cyanobacteria are incomplete at present. Several references in Table 2 are from book chapters [46-48] and government or quasi-government reports [49-51]. Some citations are from publications in which the reports of cyanobacteria-associated illness in humans are brief and/or incidental to the main focus of the work (usually pertaining to ecological or ecotoxicological findings), e.g. [37, 52, 53]. One recommendation that follows from this discussion is that authors include the search keyword “recreation” or “recreational” in future publications that discuss suspected cyanobacteria-related morbidity from recreational waters.

### **Recreational and in-field occupational exposure to cyanobacteria: anecdotal and case reports**

Case reports and anecdotal references presented in Table 2 date from 1949 [54], and describe a range of illnesses associated with recreational exposure to cyanobacteria:

hay fever-like symptoms, pruritic skin rashes and gastro-intestinal symptoms are most frequently reported. Some papers give convincing descriptions of allergic responses to cyanobacteria [45, 54]. Others describe more serious acute illnesses, with symptoms such as severe headache, pneumonia, fever, myalgia, vertigo and blistering in the mouth [6, 44, 55, 56]. The first and so far only description of a fatality related to recreational exposure to cyanotoxins appeared in news reports recently. A U.S. coroner concluded that a teenage boy died as a result of ingesting anatoxin-a-producing cyanobacteria from a golf course pond, although there was an unusual sequence of events preceding the death insofar as the time period between exposure and death (some 48 hours) does not square with the known mechanisms of toxicity of purified anatoxin-a, which initiates pathological signs in laboratory animals within minutes of dosing [34, 35]. The principal public health concerns regarding recreational exposures relate to the potential, presumably a now-realised potential if the aforementioned fatality is indeed attributable to cyanotoxin poisoning, for exposure to hazardous levels of cyanotoxins in untreated waters. Routes of exposure are through direct contact with skin and mucous membranes, via inhalation, and by ingestion, either accidental or deliberate.

## **Discussion – Table 2**

Some reports listed in Table 2 present scant information relevant to this topic, with little or no detail beyond location and the kind of illness reported [49, 57]. On the other end of the scale are examples of thorough, considered case reports, describing relevant medical history and diagnostic investigations [45, 54]. One reason for the

dearth of detail may be that non-specific, mild and self-limiting illnesses do not merit much discussion, however, some references to more serious illnesses leave a great deal unanswered, for example the 12 year-old boy who reportedly lapsed into unconsciousness for a six-hour period, and developed pneumonia, myalgia and arthralgia [46]. It would have been very interesting to know whether or not this boy had any predisposing medical conditions (e.g. diabetes, epilepsy) that might have explained the loss of consciousness, whether any medical attention was sought, and, if so, the details of his disease progression.

The observation that repeated water contact in a particular lake preceded a skin eruption on a six year-old girl, while other bathers appeared unaffected, helped support a diagnosis of hypersensitivity in that case [45]. One of the few reports of mass effects, with 20-30 children suffering conjunctival and upper respiratory symptoms during a school aquatic event, is tempered by the observation that that number represented about 25% of those exposed [47]. So hypersensitivity reactions affecting a sub-set of allergy-prone children may also be an explanation for the latter outbreak, although this speculation – in the absence of any other reported investigations – is solely based on that estimate of 25% of those exposed experiencing symptoms.

Those reports that have indicated symptom onset time suggest that responses can be rapid, with some urticaria and hayfever-like symptoms commencing while subjects are still in the water [47, 54]. While a disparate range of signs and symptoms are listed, many reports describe a collective group of symptoms resembling immediate or Type-I hypersensitivity reactions. Immediate hypersensitivity reactions are commonly

associated with atopy, which is the familial tendency to react to naturally occurring antigens, mostly proteins, through an IgE-mediated process. Atopy commonly manifests as a spectrum of diseases, e.g. seasonal rhinitis, conjunctivitis, asthma and urticaria. Different atopic illnesses often affect the same individual. A fundamental feature of Type-I hypersensitivity reactions is the rapid onset of symptoms – normally seconds to minutes – following exposure to antigens [58 (pp.57, 88-93, 130, 224), 59-62].

Some serious though apparently self-limiting gastro-intestinal illnesses have been reported after contact with cyanobacteria in recreational waters, presumably through ingestion of affected water. Dillenberg & Dehnel [55] describe how an adult male inadvertently swallowed lake water affected by a bloom of *Microcystis* sp and *Anabaena circinalis*. After some three hours he developed cramping abdominal pain and nausea, which progressed to painful diarrhoea followed by a fever of 38.9<sup>0</sup>C, severe headache, lassitude, myalgia and arthralgia. Such illnesses are worrying, considering the two boys that were sickened – one of whom subsequently died – after exposure to cyanobacteria in a golf course pond suffered acute and severe gastro-intestinal illnesses [34].

Occupational exposures were included in this review, although some caution should be exercised when comparing occupational and recreational exposures. Waters that are obviously discoloured or visibly affected by cyanobacteria scums may be of interest to aquatic field workers who are keen and/or obliged to collect samples. The two incidents involving UK soldiers and sea cadets conducting canoe capsizing activities, presumably under orders from their supervising officers, occurred in waters

that were reportedly subject to a “heavy bloom of *Microcystis* spp” [40] and a “scum of *Oscillatoria*...” [22]. Waters that are obviously suffering a loss of visual amenity may be shunned by many recreational users, although avoidance behaviour in such circumstances cannot be taken for granted [63 (Chapter 4)].

The other reports that are of particular interest are those grouped under “cold & flu-like symptoms”. Several publications describe individuals presenting with a flu-like illness, with signs and symptoms including fever, headache, lassitude, arthralgia, myalgia, sore throat, cough, diarrhoea and vomiting. A proposed explanation for this constellation of symptoms is that of a coordinated, cytokine-mediated, innate immune response. Fever and malaise are events that are directed by endogenous mediators; for further discussion see [63 (Chapter 6)]. This spectrum of signs and symptoms also mimics those reported in volunteer studies of intravenous Gram-negative bacterial lipopolysaccharide injection [64-67]. Mammalian responses to LPS are mediated by inflammatory cytokines (see accompanying review by Stewart *et al* [68]). Flu-like reactions to immunostimulant drugs are sometimes referred to as “acute cytokine syndromes” [69], and the flu-like syndrome of fever, rigors, tachycardia, malaise, headache, arthralgia and myalgia that accompanies interferon pharmacotherapy is thought to be due to the release of eicosanoids, IL-1 and TNF- $\alpha$  [70].

### **Epidemiology of recreational exposure to cyanobacteria**

A critique and examination of the published epidemiological studies on recreational exposure to cyanobacteria will be incorporated into a discussion for

cyanobacteriologists on the most commonly applied approaches to epidemiology. Important study types that have not been applied to the topic of recreational exposure to cyanobacteria will be presented and examples drawn from similar studies into coastal water quality and recreational exposure. The advantages and disadvantages of each kind of study will be briefly outlined. Each study type will be presented in increasing order of robustness, as there is a broadly agreed upon hierarchy of study types with respect to the ability of each to determine causation.

Six epidemiological studies of recreational exposure to cyanobacteria have been conducted to date: three analytical cross-sectional studies from the U.K. using similar survey instruments [71-73], a small case-control study from Australia [74], and two larger prospective cohort studies, also from Australia [75, 76].

### **Anecdotal and case reports**

Anecdotal and case reports represent the weakest evidence of causation, as there is no comparison with unexposed “control” subjects. However, their utility is clearly very important in alerting the medical and scientific community to unusual events and disease mechanisms, and for generating hypotheses. This community was first alerted to the toxic potential of cyanobacteria in 1878, with a report to the journal *Nature* by George Francis, who observed the rapid death of large stock animals after drinking contaminated water [3]. Anecdotal reports of unusual illness or deaths in wild and pet animals after drinking green or “scummy” water still serve as useful sentinel events to warn local authorities and communities that there is a problem in their waterbody

[77]. It is possible that the so-called flu-like illnesses reported after recreational exposures (see Table 2) are indications that some cyanobacterial products are signalling cytokine-mediated innate immune responses. The question as to whether more anecdotal reporting of these events in the medical literature is warranted remains open. Case reports are often designed to emphasise unusual findings, yet some dispute the utility of such reports, largely on the basis that if presenting illnesses are so rare, most practitioners will never encounter them [78, 79].

That cyanobacteria can produce potent and lethal toxins is not a novel observation, but it is probably not widely understood by primary healthcare providers. Exposures to aquatic cyanobacteria are common in recreational settings, and the dynamic nature of cyanobacterial bloom progression and toxin production demands vigilance by general practitioners and emergency physicians. Published case reports and case series would still be valuable educational tools, both for primary healthcare providers and the cyanobacteria research community. Reports of severe illness, mass outbreaks or post-acute morbidity, with careful attention to exposure history, predisposing conditions, duration of signs and symptoms and supplemented by near-time water quality data would be most appropriate for publication.

### **Cross-sectional studies**

In a cross-sectional study, a “cross-section” of the population is identified – i.e. without regard to either disease status or exposure – and then exposure and outcome are measured at the same time. Cross-sectional studies can be descriptive – a so-called

prevalence survey – or analytical, examining the relationship between exposure and disease. Figure 1 shows the design of a cross-sectional study.

The three studies by Philipp and co-workers are examples of analytical cross-sectional studies [71-73]. Questionnaires were distributed to recreational users of six inland waterbodies, five of which experienced cyanobacteria blooms during 1990. The questionnaires elicited information on exposure to study waters and the presence of specific symptoms in a defined period prior to receiving the form. This period ranged from 14 days [72] to four weeks [73]. One questionnaire asked about exposure to the study water on a weekend when a bloom occurred some 2½ weeks previously [71]. Recreational interest groups were used to target likely users of the waterbodies; questionnaires were mailed to members of sailing and angling clubs. Site authorities distributed questionnaires at one study lake [73]. The results of these three studies were similar: mostly minor morbidity was reported, with similar disease patterns across sites.

The theoretical advantages of this study type are that it is reasonably cost-effective, and in this context – recreational exposure to cyanobacteria – it can be conducted opportunistically to take advantage of any sudden-onset cyanobacteria blooms.

Disadvantages relate to the difficulty in establishing that exposure occurred before the outcome [80 (p.146), 81 (p.174)]. The studies conducted by Philipp and his team [71-73] were examples of analytical cross-sectional studies, in that unexposed individuals served as controls for statistical comparison of illness reporting.

## Case-control studies

A case-control study is an analysis that identifies cases of the disease of interest (case group), and suitable individuals without the disease (control group), then looks back in time to determine the exposure status of individuals in each group. Figure 2 shows a case-control design.

A case-control study of illness rates was conducted after an extensive *Anabaena circinalis*-dominant bloom along South Australia's Murray River in the summer of 1991-1992 [74]. Patients presenting with gastro-intestinal (G-I) or dermatological complaints comprised the case group; the patient presenting after each case was identified served as the control group. Exposure was determined by identifying each subject's principal source of water for drinking domestic use (bathing, dishwashing) and recreation during the week prior to consultation. Recreational exposure was categorised as no contact, direct exposure to river water, or other exposure, e.g. farm dams or treated water in swimming pools. The study found a significantly increased risk of G-I symptoms for those drinking chlorinated river water, and an increased risk of G-I and cutaneous symptoms in those using untreated river water for domestic purposes. The study found a statistically non-significant increase in the relative odds of developing G-I or skin symptoms amongst those with recreational exposure to river water, but that risk was lower than for those exposed to other sources of recreational water (tank, farm dam or another location). The number of subjects was small for the recreational exposure component of the study, with only some 50 subjects (16% of the study group) reporting any recreational exposure during the study period.

The advantages of a case-control design for investigating recreational exposure to cyanobacteria are that studies can be conducted opportunistically in response to the development of cyanobacteria blooms, and they are very useful for investigating infrequent outcomes. The study of El Saadi *et al* [74] has another advantage over other epidemiological studies into recreational exposure to cyanobacteria in that medical practitioners ascertained outcome data, as opposed to self-reporting of symptoms. General disadvantages of the case-control design principally relate to the problem of recall bias, where individuals with the disease of interest tend to overestimate relevant past exposures [80 (p.151), 81 (pp.169-70)]. Because the outcome has already occurred when exposure is measured, people with disease may systematically overestimate (or underestimate) their exposure compared to disease-free controls, leading to falsely elevated (or reduced) measures of risk associated with exposure. Another major issue with case-control studies is the difficulty of identifying an appropriate control group – i.e. people who would have been identified as cases if they had the disease of interest.

Recall bias may not be so much of a problem for investigating acute illnesses following recreational exposure to cyanobacteria, where a fairly short time lag between exposure and symptom onset can be anticipated, especially if recreational exposure is determined by a yes/no response. The main problem with a case-control study in this context will be in actually identifying cases. A case-control design would not be suitable for investigating outcomes from exposure to a cyanobacteria bloom in a lake adjacent to a city, as most recreational users who do develop symptoms would presumably seek medical attention after they return home, i.e. from one of a large number of medical practitioners. El Saadi *et al* [74] alluded to the difficulty of gaining

the cooperation of medical practitioners, as they approached practices in 11 towns along the Murray River, yet those in three towns presumably refused to participate in their study. The diffuse spread of cases from point sources of exposure (a cyanobacteria-affected waterbody) across a large town or city would make a case-control study practically unworkable. A case-control study would also be unsuitable for recruiting subjects who did not seek medical attention for symptoms occurring after exposure. However, a well-designed case-control study would be valuable if geographical location is a primary consideration. This would require enlisting the cooperation of medical practitioners in small townships near to cyanobacteria-affected recreational waters that are sufficiently remote from larger urban centres to allow recruitment of local residents and tourists who will camp nearby.

## **Cohort studies**

A cohort study compares disease outcomes in exposed and unexposed populations; exposure is estimated before determining outcomes. Figure 3 shows the design of a cohort study. The studies by Pilotto *et al* [75] and Stewart *et al* [76] are examples of prospective cohort studies.

Pilotto *et al* [75] recruited individuals at five recreational waterbodies in three Australian states. Cyanobacteria blooms were anticipated at these sites, based on occurrences in previous years. Individuals were approached and invited to participate in the study. Participants completed a face-to-face interview to determine health status and recreational water activities; two telephone follow-up interviews were conducted

at two and seven days following the day of recruitment into the study. Individuals who did not have water contact on the recruitment day served as the control group. No significant differences in symptom occurrence were reported at the 2<sup>nd</sup> day follow-up, but the authors concluded there was a significant increase in symptoms at 7 days, after excluding subjects with symptoms or previous recent recreational water exposure. The cohort size from which these significant results were drawn was rather small, with 295 exposed subjects, and 43 unexposed controls. Pilotto *et al* [75] interpreted the increased symptom reporting at 7 days but not 2 days following exposure as possibly due to delayed allergic responses, although so-called “late phase” allergic and asthmatic responses tend to occur some 4-24 hours after allergen exposure [58 (pp.59, 93), 82].

Stewart *et al* [76] also conducted a cohort study of recreational exposure to cyanobacteria. 1,331 subjects were recruited from 19 recreational waterbodies in eastern Australia and central and northeast Florida; subjects completed a self-administered questionnaire to determine recreational activity, recent illness and history of any relevant chronic diseases such as asthma, hay fever and eczema. A single follow-up telephone interview was conducted after three days post-exposure. Reference subjects were recruited at recreational waters unaffected by cyanobacteria; exposure categories (low, intermediate, high) were allocated to study subjects on the basis of cyanobacteria levels measured in study waters collected on the day they were recruited into the study. Statistically significant increased reporting of respiratory symptoms and a pooled “any symptom” category occurred amongst subjects exposed to high levels of cyanobacteria, although symptoms were predominantly rated as mild

by study subjects. A similar but non-significant relationship was also seen for reporting of skin, ear and fever symptom groups.

The studies of Pilotto *et al* [75] and Stewart *et al* [76] are both examples of a prospective cohort design, where study subjects have their exposure status determined, and are then followed forward in time to observe the development of disease. For these investigations into recreational exposure to cyanobacteria, exposure status was determined by collecting water samples on the day subjects were recruited into the study; cyanobacteria were identified and enumerated and the resultant cell counts or biomass estimates formed the basis of exposure at any given site on a particular day. One of the problems with this approach is that cyanobacterial blooms are dynamic and can change rapidly. Unless the presence of significant cyanobacterial biomass can be predicted with some degree of certainty, a prospective cohort design can result in wasted effort if the water samples reveal lower than anticipated levels of cyanobacteria. This problem undoubtedly occurred in some instances during the study conducted by Stewart *et al* [76]. One possible approach to dealing with this would be to conduct a historical cohort study, where a cohort of subjects is identified after some have experienced the outcome of interest and relevant exposure information is obtained from historical records (i.e. as in a prospective cohort study the exposure information was recorded *before* any outcomes occurred).

Ferley *et al* [83] conducted a historical cohort study to investigate the epidemiology of microbial pollution in riverine recreational waters. Study subjects were interviewed about recreational activities at eight summer camps in a French river basin on days preceding the interview. Water samples from the sites were collected and analysed

twice-weekly and these records were used to classify subjects as exposed or unexposed to microbial indicators. Applying this design to the epidemiology of recreational exposure to cyanobacteria would require a site where a sufficient number of campers stay for long enough to be questioned retrospectively about their exposure. When analysis of water samples showed that defined thresholds for cyanobacteria or cyanotoxins had been exceeded, subjects could then be enrolled into the exposed group of a cohort study. All this would still require considerable organisation and expense; probably the best way to study this topic using a cohort design would be to concentrate recruitment at chronically cyanobacteria-affected waterbodies.

Whether a cohort study is conducted prospectively or retrospectively, the basic study design is identical – exposed and unexposed groups are compared with respect to disease outcome [81 (p.152)]. General advantages of a cohort design are the ability to determine disease onset (the exposure precedes the disease), and the study of exposures in natural settings [80 (pp.148, 152)]. General disadvantages relate to confounding, which refers to differences in the distribution of risk factors other than the exposure of interest between exposed and unexposed groups. Cohort studies can be expensive and resource intensive [80 (pp.148, 152)].

### **Experimental epidemiology**

A randomised trial compares the development of disease in exposed and unexposed subjects, but unlike a cohort study, entry of study subjects to exposure groups is by random allocation. Figure 4 shows the basic design of a randomised experiment.

Randomised trials are considered the “gold standard” study in many clinical settings but are unusual tools in the field of environmental epidemiology, largely because of ethical problems, insofar as potentially harmful exposures cannot be randomly assigned [80 (p.155)]. However, there are exceptions. Fleisher *et al* [84-86] and Kay *et al* [87] describe randomised trials to investigate gastrointestinal and other illnesses in subjects exposed to coastal recreational waters with varying microbiological water quality. Study subjects were recruited from shopping centres, sporting clubs and other locations some three weeks prior to trials taking place. All subjects were given a medical examination and structured interview 2-3 days before the trial to determine any acute or predisposing illnesses. Subjects were then randomly allocated to either exposed or non-exposed groups. On the day of the trial, those in the exposed group were instructed to remain in the water for at least 10 minutes, and to immerse their heads three times. Observers were employed to monitor the activity and time each bather spent in the water. Those in the non-exposed group were restricted to a roped-off area on the beach, and observers were assigned to confirm that individuals in the non-exposed group did not enter the water. Follow-up procedures included medical examination, face-to-face interview and self-administered questionnaire. Frequent and extensive water sampling was conducted throughout the each trial; microbial water quality parameters such as faecal coliforms and faecal streptococci were the exposure measures used in statistical modelling. Ethical concerns were addressed by conducting the studies at beaches that met European mandatory directives for faecal indicator counts, although beaches used in one study failed guidance levels [84]. Only subjects 18 years or older were studied.

The most significant advantage of a randomised trial is that, provided the study is large enough, so-called confounding variables – extraneous factors that might influence the study outcome – will be evenly distributed across exposed and unexposed groups, leaving any observed differences in outcome directly attributable to the study factor [80 (p.155)]. From the perspective of water-based recreational exposures, Kay *et al* [87] correctly contrast the strengths of their design with the relative methodological weaknesses of cohort studies, pointing out that individuals who swim and those who do not will self-select into each exposure category, and may differ with respect to factors other than bathing water quality.

Randomised trials also have their own inherent weaknesses: ethical concerns, as mentioned above; they are expensive and resource intensive; subjects may not always comply with their treatment or exposure allocation; and there is a degree of artificiality introduced, so observed outcomes of the study may not be generalisable to the broader population [80 (p.155)].

The advantages and limitations of a randomised trial to investigate the epidemiology of recreational exposure to cyanobacteria will now be briefly discussed. Fleisher *et al* [84] and Kay *et al* [87] highlight another important strength of their study, which is directly applicable to the topic of planktonic cyanobacteria in recreational waters.

Their frequent water sampling regime allowed each exposed subject to be assigned an index of exposure. Significant temporal and spatial variation in indicator organism densities can be seen in marine recreational waters [86], which is exactly the same situation with planktonic freshwater cyanobacteria [88]. The advantages of such an approach for investigating recreational exposure to cyanobacteria would be that a

positive result from a well-designed randomised trial would be powerful evidence of causation. Ethical hurdles could possibly be overcome insofar as there are presently no mandatory directives for recreational exposure to cyanobacteria. Several European countries and others such as Australia and Canada have or are in the process of adopting exposure guidelines. The studies of Fleisher *et al* [84-86] and Kay *et al* [87] could be improved insofar as their work was conducted “single-blind”, i.e. the follow-up interviewers and medical examiners were unaware of the exposure status of their study subjects, but study subjects were obviously aware of their exposure status. As discussed by Stewart *et al* [76], there is a risk that the use of non-bathers as a control group for water-based recreational activity might lead to under-reporting of relevant symptoms by the control group. A randomised trial to investigate recreational exposure to cyanobacteria could conceivably be conducted as a “double-blind” enterprise, by the use of reference waterbodies unaffected by cyanobacteria. Florida would be ideally suited to such work, as oligotrophic and eutrophic inland recreational waters can be found in close proximity to one another [63 (Section 3.5.2)]. Blinding refers to the ability to make study participants and/or investigators unaware of the study intervention, so that clinical observations or responses to questions are not influenced by that knowledge [89].

On the other side of the equation are several important factors that militate against conducting a randomised trial to study the health impacts of recreational exposure to cyanobacteria, at least in the near future:

- **Cost:** compared to the limited epidemiological studies that have been conducted already in this area, the resources, organisation and planning

required to conduct a randomised study with a similar level of rigour to those conducted to examine faecal contamination of coastal waters would be formidable. Hiring observers and medical examiners, analysing large numbers of water samples for cyanobacteria and cyanotoxins, and compensating subjects for their time and travel costs would not be a trivial exercise.

- **Ethics:** while a study could conceivably be conducted because of the absence of mandatory directives, what kind of exposures should be sought in order to model realistic, real-world activities in waters affected by cyanobacteria – and more importantly, cyanotoxin-affected waters? With the presumption that the most hazardous exposure to cyanotoxins is via the oral route, should study subjects be instructed to swallow lake water? While inadvertent or deliberate consumption of cyanotoxin-affected water undoubtedly occurs in natural recreational settings, it would clearly be unethical to randomly assign such consumption.
- **Compliance:** Investigators conducting a randomised trial should anticipate a significant level of non-compliance from subjects allocated to cyanobacteria exposure. If cyanobacteria-affected study waters are visibly affected by green discoloration, surface scums or unusual odours, a number of volunteers might refuse to even dip their toes in it. Significant non-compliance could be avoided by choosing study waters that are not subject to the aesthetic impacts of cyanobacteria, but such waters would presumably be only marginally affected compared to waters affected by frank cyanobacteria blooms and simultaneously frequented by recreational users.
- **Artificiality:** Organisational demands of having volunteers swimming in a defined (and restricted) area of a lake, while being observed throughout, and

conducting an associated water sampling regime would necessitate relatively short-term exposures, say 10-60 minutes. The ability to relate cyanobacterial biomass or cyanotoxin indices to individual subjects would see significant improvements in exposure measurement over all epidemiological studies conducted to date in this field. However, this would not model the diversity of exposures seen in natural settings, where various recreational activities (e.g. swimming, skiing, sailing) occur over different spatial and temporal dimensions (one beach *vs* whole lake; minutes *vs* essentially all day). Cohort studies are ideal for observing exposures in natural settings, but, as discussed by Stewart [63 (Chapter 3)] there is a loss of precision when it comes to measuring such diverse exposures. Children make up a significant proportion of the population that participates in recreational water exposure, but ethical concerns would presumably preclude them from participating in a randomised trial.

### **Other epidemiological study designs**

The study types discussed above are the most commonly conducted approaches to answering epidemiological questions. The presentation of each design is highly simplified, as is intended only as a very basic introduction for cyanobacteriologists unfamiliar with the discipline. They are presented in ascending order of their reliability in determining causality: anecdotal and case reports < cross-sectional studies < case-control studies < cohort studies < randomised trials. The field of epidemiology is very much larger and more complex than that presented in this

introduction, with many more potential approaches to tackling this problem. Some useful methodologies may be found in ecologic studies, which use groups of people as the unit of observation, e.g. classes in schools, factories, cities or countries [90]. Pilotto *et al* [91] used an ecologic design to examine the relationship between cyanobacterial contamination of drinking water supplies and adverse perinatal outcomes, although this type of study would not be useful for investigating short-term health outcomes from recreational exposures in highly mobile population groups such as tourists. Case-crossover designs were originally used to investigate brief exposures that result in immediate, short-term effects and acute outcomes that have a rapid and unambiguous onset. Only cases are observed, i.e. subjects with outcomes of interest; relative risks are calculated by comparing the frequency of exposure before the outcome (case period) with respect to an earlier period (control period), so study subjects serve as their own controls [92, 93]. A clinical epidemiology design used to investigate various therapeutic interventions might be usefully modified for diagnostic purposes to investigate suspected cases of cyanobacteria-related illness. The n-of-1 randomised controlled trial is a double-blind crossover study conducted on individual subjects; inhalation or cutaneous exposures could conceivably be conducted with suitable volunteers to compare responses to cyanobacterial products and inert vehicles [94-100].

The above discussion highlights the choices available to epidemiologists to further investigate the topic of recreational exposure to cyanobacteria. All study designs have their own inherent strengths and weaknesses, some study designs would be inappropriate. The conclusions of Kleinbaum *et al* [101 (p.92)] apply here, in that the chosen design should be carefully tailored to the study objectives, the nature of the

disease being studied, socio-political constraints, and the availability of time, money and personnel.

### **Cyanobacteria and water-related disease: some complicating factors**

Other explanations for disease need to be considered by both clinicians and epidemiologists in their respective endeavours. Epidemiological studies usually aim to identify and adjust for confounding variables such as smoking and age of study participants. The following sections will discuss some freshwater-related risk factors, mostly microbial, that may confound epidemiological studies and complicate clinical diagnoses of cyanobacteria-related illness linked to recreational exposures. The final section of this review will discuss the possibility of misdiagnosis from the opposite direction: a water-borne disease outbreak in Finland that was subject to epidemiological scrutiny, but cyanobacterial exotoxin contamination of reticulated supplies was apparently not considered at the time.

### **Freshwater-related dermatoses**

- **Avian cercariae:** avian cercariae are schistosome larvae for which humans are an accidental host. Pruritis and macules are the initial signs and symptoms, sometimes a diffuse erythema and urticaria can develop and last for several hours [102-104, 105 (p.539)]. Fever, nausea and vomiting can also accompany severely affected cases [103, 106]. The clinical presentation of cercarial

dermatitis can be difficult to delineate from the picture of cyanobacterial dermatitis.

- **Gram-negative bacteria:** *Aeromonas hydrophila* and *Chromobacterium violaceum* are abundant in freshwater habitats. Both usually cause infection through a pre-existing skin wound, though the clinical picture in each case is not reminiscent of any of the reports listed in Table 2. *A. hydrophila* causes cellulitis and a purulent discharge; aspiration of water can cause pneumonia and septicemia. *C. violaceum* infections present with various cutaneous signs that are secondary to systemic disease, including sepsis [107]. *Vibrio vulnificus* has reportedly caused soft tissue infection after contact in brackish inland waters, though most cases are associated with estuarine contact [108 (pp.114-5)]. *Pseudomonas aeruginosa* is widely-distributed in natural and artificial aquatic environments. Cutaneous infection presents as an erythematous or urticarial rash some 18-24 hours after water contact and progresses to a follicular dermatitis. Fever and pruritis are uncommon. Most reports of pseudomonal dermatitis are related to spa pool or hot-tub exposures [108 (pp.165-171), 109]. *P. aeruginosa* in recreational waters is a common cause of otitis externa, presenting as a purulent discharge [108 (p.166)]. Diagnostic criteria include culturing the organism from skin or ear swabs; the incubation period would also help to distinguish *P. aeruginosa* infection from cyanobacteria-related dermatoses.
- **Non-allergic urticaria:** physical stimuli such as heat, cold and exercise can induce itching and hives in susceptible individuals [105 (pp.145-7), 110].

## Gastro-intestinal illness

- **Shigellosis:** Shigella outbreaks are the most commonly reported cause of disease associated with untreated inland recreational water in the USA, with 16 events affecting almost 1,300 people between 1985 and 1994 [108 (p.43)]. The incubation period is typically 2-3 days, with an upper limit of about 7 days. Illness severity is strain-dependent, with most *S. sonnei* infections being mild and self-limiting, and *S. dysenteriae* type 1 associated with severe diarrhoea which may progress to a life-threatening illness [108 (p.125)].
- ***E. coli:*** *E. coli* are markers of faecal pollution in recreational waters. Disease outbreaks traced to enterohaemorrhagic *E. coli* 0157 have been reported from recreational water exposures [108 (p.148), 111].
- **Norwalk-like viruses:** Various transmission routes, including recreational water outbreaks have been documented [111].

## Other microbial pathogens

- ***Naegleria fowleri:*** *N. fowleri* is a free-living thermotolerant amoeba found in warm or thermally polluted waters. It is the causative organism of primary amoebic meningoencephalitis, a fulminating, typically fatal illness. The entry

route is via the nasal mucosa; fit, immunocompetent children and young adults with a recent history of freshwater recreational activity are those most commonly affected. The causative organism and diagnosis are usually confirmed at autopsy. Several reviews are available, e.g. [112-119].

- **Viruses:** Pharyngo-conjunctival fever outbreaks associated with non-enteric adenoviruses in recreational waters have been reported [111].
- **Legionella:** *Legionella* infections have been associated with recreational water contact [111].

### **Possible under-diagnosis of cyanobacteria-related illness**

The examples given above highlight some of the differential diagnoses that need to be worked through when considering possible cases of cyanobacteria-related illness from recreational exposures. Competent history-taking and diagnostic microbiology support will correctly diagnose many such cases. Competent history-taking and clinical diagnostic support also operated in several of the case reports listed in Table 2, with the early dermatological testing and microscopic examination of stool and vomitus samples lending strong support to the suspicion of cyanobacteria-related morbidity.

Misdiagnosis of cyanobacteria-related disease may occur in both directions. In 1978, nearly half the population of an industrial town in Finland were affected by a flu-like illness, with symptoms of fever, fatigue, cough, dyspnoea and myalgia. Symptoms

occurred some 3-6 hours after taking a bath, shower or sauna and persisted for 8-16 hours. The outbreak lasted for some four months. This epidemic was investigated on several fronts, and provocation testing demonstrated an obvious link to the reticulated water supply. Tap water was cultured in a range of organic media for fungal and bacterial pathogens. No definitive pathogen was identified to explain the epidemic, yet in three published reports the authors describe how the shallow lake that was the town water source had taken on a distinct opaque blue-green appearance, had a musty smell, and the sand filtration system was covered by a mat of cyanobacteria. This change occurred in the same month (August, i.e. late summer) that the epidemic began. Analysis of crude lake water in the third month after the onset of the epidemic showed high coliform counts, *Aspergillus fumigatus* and unspecified blue-green algae. Investigations centred on identifying antibodies to mesophilic actinomycetes, which the authors [120] note were not pathogenic, whereas aquatic cyanobacteria were known at the time to be toxic. The health workers investigating the outbreak apparently did not consider the possibility of a cyanobacterial exotoxin breakthrough into the reticulated supply [120-124]. The epidemiological report of Aro *et al* [121] came closest to suggesting that cyanobacteria may have been involved, suggesting that “towards the end of summer...the microorganisms in the lake multiply rapidly and produce some toxic substance or allergen”, and reported that cyanobacterial endotoxin concentration in lake and tap water was high. This incident appears to have been retrospectively attributed to the presence of cyanobacterial endotoxins in the reticulated supply [125]. A similar outbreak occurred almost three years earlier in a Swedish town, though with a much smaller proportion of cases identified. Cyanobacteria were known to affect the town’s raw water supply, and the investigators did consider the possibility that cyanotoxins may have been responsible

for the outbreak [126], though the analytical technique used by investigators at the time – gas chromatography – would have failed to detect the presence of cyanobacterial exotoxins in the post-treatment water supply. While no conclusions can be made about events that occurred over 25 years ago, from the descriptions of the outbreaks and the raw water supplies, most cyanobacterial toxicologists would rate cyanotoxin exposure with a high index of suspicion.

A similar outbreak occurred more recently in Homa Bay, Kenya, in 1998. Apparently associated with a mass development of cyanobacteria in Lake Victoria, an epidemic of fever, malaise, dizziness and upper respiratory symptoms was related to hot water bathing. Symptoms lasted 12-24 hours, and returned when a shower or bath was taken again. This outbreak was reported in a conference abstract; the authors suggested cyanobacterial endotoxins were responsible, though it is not stated whether any investigation of cyanobacterial exotoxins was conducted [127].

### **Concluding remarks**

The true incidence of acute cyanobacteria-associated illness from recreational exposure is unknown, as many outcomes are likely to be mild and self-limiting, so medical attention is not sought. With a long-standing knowledge gap amongst primary healthcare providers, non-specific signs and symptoms caused by cyanobacterial products are likely to be under-diagnosed [8 (p.69)]. Codd [128] stated:

*“Evidence linking human illnesses with cyanobacterial cells and toxins is open to criticism because of shortfalls in early detailed case definitions, because diagnoses were made by exclusion, and because identification and quantification of cyanobacterial toxins in health incidents have, until recently, been lacking.”*

The collation of anecdotal and case reports of illness associated with recreational exposure to cyanobacteria in Table 2 will hopefully highlight some of the knowledge gaps. Particular attention should be given to determining the onset and duration of individual symptoms in future case reporting, as well as detailing the presence or absence of any predisposing medical conditions.

A review of the epidemiological studies conducted to date on this topic was also combined with discussion of some common study designs used to investigate similar kinds of exposures to other microbial pathogens. The benefits and shortcomings of each of these study designs if and when applied to the study of recreational exposure to cyanobacteria were discussed. This was intended to be an introductory overview of environmental epidemiology for non-epidemiologists, and interested readers should refer to standard epidemiology texts for more detail.

The most important advances in understanding the health impacts of cyanobacteria have come from the discipline of toxicology. The major toxins have been extensively studied and characterised, and while there is still much to be discovered in the field of cyanobacterial toxicology, significant advances in the future will be made at the interface of toxicology and epidemiology. Molecular epidemiology techniques using

yet-to-be discovered biomarkers of exposure, susceptibility and outcome will refine knowledge of the risks associated with various acute and chronic exposures to cyanotoxins. The collaborative skills that epidemiologists and toxicologists can bring to this endeavour were visualised with a mildly jaundiced eye by Paddle [129], whose chapter on epidemiology for toxicologists is an excellent general primer:

*“The total evidence about the risk to humans...will consist of the toxicologist’s precise, experimental data about the wrong species at the wrong exposure, and the epidemiologist’s imprecise, observational data about the right species at the right exposure.”*

In conclusion, anecdotal and case reports of variable reliability have suggested a range of symptoms are associated with exposure to cyanobacteria in recreational or occupational settings. Some reports of cutaneous reactions are strongly suggestive of allergic reactions, and symptoms such as rhinitis, conjunctivitis, asthma and urticaria also hint at immediate hypersensitivity responses. Flu-like illnesses involving a constellation of symptoms including fever, malaise, myalgia, arthralgia, severe headache, cough and sore throat are, in these authors’ opinion, explained by a cascade action of pro-inflammatory cytokines. If correct, this implies that some cyanobacterial products are ligands that signal innate immune responses, and such responses may need to be considered in terms of their potential to direct pathological changes in the liver and other organ systems.

The epidemiology of recreational exposure to cyanobacteria is incomplete at present. All common epidemiological approaches have their own inherent advantages and

disadvantages; identification of biomarkers for exposure, susceptibility and outcome in the future should lead to a significantly improved perception of the risks of bathing in cyanobacteria-affected waters.

There are significant challenges and obstacles to conducting rigorous epidemiological studies of recreational exposure to cyanobacteria. Much useful information could be gleaned from a well-conducted case series, especially if investigations into suspicious cases are supplemented with clinical investigations such as specific IgE levels. N-of-1 randomised controlled trials may be a useful approach for evaluating the risk of morbidity in relation to demonstrated prior exposure to cyanobacteria.

### **Competing interests**

The authors declare that they have no competing interests.

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## References

1. Adams DG: **Cyanobacteria**. In: *Bacteria as multicellular organisms*. Edited by Shapiro JA, Dworkin M. New York: Oxford University Press; 1997: 109-148.
2. Fogg GE, Stewart WDP, Fay P, Walsby AE: **The blue-green algae**. London: Academic Press; 1973.
3. Francis G: **Poisonous Australian lake**. *Nature* 1878, **18**:11-12.
4. Schwimmer D, Schwimmer M: **Algae and medicine**. In: *Algae and man*. Edited by Jackson DF. New York: Plenum Press; 1964: 368-412.
5. Schwimmer M, Schwimmer D: **Medical aspects of phycology**. In: *Algae, man, and the environment*. Edited by Jackson DF. Syracuse, NY: Syracuse University Press; 1968: 279-358.
6. Codd GA, Beattie KA: **Cyanobacteria (blue-green algae) and their toxins: awareness and action in the United Kingdom**. *PHLS Microbiol Dig* 1991, **8**(3):82-86.
7. Carmichael WW, Falconer IR: **Diseases related to freshwater blue-green algal toxins, and control measures**. In: *Algal toxins in seafood and drinking water*. Edited by Falconer IR. London: Academic Press; 1993: 187-209.
8. Ransom R, Soong FS, Fitzgerald J, Turczynowicz L, El Saadi O, Roder D, Maynard T, Falconer I: **Health effects of toxic cyanobacteria (blue-green algae)**. Canberra: National Health and Medical Research Council / Australian Government Publishing Service; 1994.
9. Duy TN, Lam PKS, Shaw GR, Connell DW: **Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water**. *Rev Environ Contam Toxicol* 2000, **163**:113-185.
10. Rose EF: **Toxic algae in Iowa lakes**. *Proc Iowa Acad Sci* 1953, **60**:738-745.
11. McLeod JA, Bondar GF: **A case of suspected algal poisoning in Manitoba**. *Can J Public Health* 1952, **43**(8):347-350.
12. Codd GA, Edwards C, Beattie KA, Lawton LA, Campbell DL, Bell SG: **Toxins from cyanobacteria (blue-green algae) - The Pringsheim lecture**. In: *Algae, environment and human affairs*. Edited by Wiessner W, Schnepf E, Starr RC. Bristol: Biopress; 1995: 1-17.
13. Carmichael WW, (Editor): **The water environment - Algal toxins and health**. New York: Plenum Press; 1981.
14. Falconer IR, (Editor): **Algal toxins in seafood and drinking water**. London: Academic Press; 1993.
15. Sivonen K: **Cyanobacterial toxins and toxin production**. *Phycologia* 1996, **35** (6 Suppl):12-24.
16. Chiswell RK, Smith M, Norris R, Eaglesham G, Shaw G, Seawright A, Moore M: **The cyanobacterium, *Cylindrospermopsis raciborskii*, and its related toxin, cylindrospermopsin**. *Australas J Ecotoxicol* 1997, **3**(1):7-23.
17. Codd GA, Ward CJ, Bell SG: **Cyanobacterial toxins: occurrence, modes of action, health effects and exposure routes**. *Arch Toxicol Suppl* 1997, **19**:399-410.

18. Dawson RM: **The toxicology of microcystins.** *Toxicon* 1998, **36**(7):953-962.
19. Falconer IR: **Algal toxins and human health.** In: *The handbook of environmental chemistry - vol 5 pt C.* Edited by Hrubec J, vol. 5 Part C. Berlin: Springer-Verlag; 1998: 53-82.
20. Hunter PR: **Cyanobacterial toxins and human health.** *Symp Ser Soc Appl Microbiol* 1998, **27**:35S-40S.
21. Chorus I, Bartram J, (Editors): **Toxic cyanobacteria in water - A guide to their public health consequences, monitoring and management.** London: E & FN Spon; 1999.
22. Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS: **Cyanobacterial toxins, exposure routes and human health.** *Eur J Phycol* 1999, **34**(4):405-415.
23. Botana LM, (Editor): **Seafood and freshwater toxins - pharmacology, physiology, and detection.** New York: Marcel Dekker; 2000.
24. Chorus I, (Editor): **Cyanotoxins - occurrence, causes, consequences.** Berlin: Springer-Verlag; 2001.
25. Codd GA, Morrison LF, Metcalf JS: **Cyanobacterial toxins: risk management for health protection.** *Toxicol Appl Pharmacol* 2005, **203**(3):264-272.
26. Annadotter H, Cronberg G, Lawton L, Hansson HB, Göthe U, Skulberg O: **An extensive outbreak of gastroenteritis associated with the toxic cyanobacterium *Planktothrix agardhii* (Oscillatoriales, Cyanophyceae) in Scania, south Sweden.** In: *Cyanotoxins - occurrence, causes, consequences.* Edited by Chorus I. Berlin: Springer-Verlag; 2001: 200-208.
27. Kuiper-Goodman T, Falconer I, Fitzgerald J: **Human health aspects.** In: *Toxic cyanobacteria in water - a guide to their public health consequences, monitoring and management.* Edited by Chorus I, Bartram J. London: E & FN Spon; 1999: 113-153.
28. Hindman SH, Favero MS, Carson LA, Petersen NJ, Schonberger LB, Solano JT: **Pyrogenic reactions during haemodialysis caused by extramural endotoxin.** *Lancet* 1975, **2**(7938):732-734.
29. Jochimsen EM, Carmichael WW, An JS, Cardo DM, Cookson ST, Holmes CEM, Antunes MBdC, de Melo Filho DA, Lyra TM, Barreto VST *et al*: **Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil.** *N Engl J Med* 1998, **338**(13):873-878.
30. Pouria S, de Andrade A, Barbosa J, Cavalcanti RL, Barreto VTS, Ward CJ, Preiser W, Poon GK, Neild GH, Codd GA: **Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil.** *Lancet* 1998, **352**(9121):21-26.
31. Carmichael WW, Azevedo SM, An JS, Molica RJR, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Eaglesham GK: **Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins.** *Environ Health Perspect* 2001, **109**(7):663-668.
32. Azevedo SMFO, Carmichael WW, Jochimsen EM, Rinehart KL, Lau S, Shaw GR, Eaglesham GK: **Human intoxication by microcystins**

- during renal dialysis treatment in Caruaru-Brazil. *Toxicology* 2002, **181**:441-446.
33. Pashkevich YA: **[On the etiology of skin lesions developing after contact with cyanophyceae]**. *Vestn Dermatol Venerol* 1979(May (5)):47-51.
  34. **Coroner cites algae in teen's death - Experts are uncertain about toxin's role** [<http://www.jsonline.com/news/state/sep03/167645.asp>]
  35. **Wisconsin teen's death a wake-up call about toxic algae** [<http://www.philly.com/mld/philly/news/nation/7932502.htm>]
  36. Calvin BC: **Is dog toxic lake's first victim? - officials concerned over spreading microbes**. In: *The Seattle Times*. Friday final edn. Seattle; 1997: B1.
  37. Johnston BR, Jacoby JM: **Cyanobacterial toxicity and migration in a mesotrophic lake in western Washington, USA**. *Hydrobiologia* 2003, **495**(1-3):79-91.
  38. **Algae levels at Pawnee are still high**. [<http://journalstar.com/articles/2004/07/23/local/10052795.txt>]
  39. **Drought may have triggered toxic algae in Nebraska lakes**. [<http://journalstar.com/articles/2004/07/27/nebraska/10052967.txt>]
  40. Chorus I, Falconer IR, Salas HJ, Bartram J: **Health risks caused by freshwater cyanobacteria in recreational waters**. *J Toxicol Environ Health B Crit Rev* 2000, **3**(4):323-347.
  41. Lawton LA, Codd GA: **Cyanobacterial (blue-green algal) toxins and their significance in UK and European waters**. *J Inst Water Environ Manage* 1991, **5**(4):460-465.
  42. Cronberg G: **Qualitative and quantitative investigations of phytoplankton in Lake Ringsjon, Scania, Sweden**. *Hydrobiologia* 1999, **404**:27-40.
  43. Soong FS, Maynard E, Kirke K, Luke C: **Illness associated with blue-green algae**. *Med J Aust* 1992, **156**(1):67.
  44. Turner PC, Gammie AJ, Hollinrake K, Codd GA: **Pneumonia associated with contact with cyanobacteria**. *Br Med J* 1990, **300**(6737):1440-1441.
  45. Cohen SG, Reif CB: **Cutaneous sensitization to blue-green algae**. *J Allergy* 1953, **24**(5):452-457.
  46. Dillenberg HO, cited in Schwimmer D & Schwimmer M: **Algae and medicine**. In: *Algae and man*. Edited by Jackson DF. New York: Plenum Press; 1964: 368-412.
  47. Billings WH: **Water-associated human illness in northeast Pennsylvania and its suspected association with blue-green algae blooms**. In: *The water environment - Algal toxins and health*. Edited by Carmichael WW. New York: Plenum; 1981: 243-255.
  48. Codd GA: **Blue-green algal toxins: water-borne hazards to health**. In: *Water and public health*. Edited by Golding AMB, Noah N, Stanwell-Smith R. London: Smith-Gordon; 1994: 271-278.
  49. National Rivers Authority (NRA): **Toxic blue-green algae. Water Quality Series No.2**. London: National Rivers Authority; 1990.
  50. NSW Blue-Green Algae Task Force: **Final report of the NSW Blue-Green Algae Task Force**. Parramatta: NSW Department of Water Resources; 1992.

51. Carmichael WW: **Assessment of blue-green algal toxins in raw and finished drinking water**. Denver: AWWA Research Foundation and American Water Works Association; 2001a.
52. Cronberg G, Annadotter H, Lawton LA: **The occurrence of toxic blue-green algae in Lake Ringsjon, southern Sweden, despite nutrient reduction and fish biomanipulation**. *Hydrobiologia* 1999, **404**:123-129.
53. Pizzolon L, Tracanna B, Prósperi C, Guerrero JM: **Cyanobacterial blooms in Argentinean inland waters**. *Lakes Reserv Res Manage* 1999, **4**(3-4):101-105.
54. Heise HA: **Symptoms of hay fever caused by algae**. *J Allergy* 1949, **20**(5):383-385.
55. Dillenberg HO, Dehnel MK: **Toxic waterbloom in Saskatchewan, 1959**. *Can Med Assoc J* 1960, **83**:1151-1154.
56. Carmichael WW, Jones CLA, Mahmood NA, Theiss WC: **Algal toxins and water-based diseases**. *CRC Crit Revs Environ Contr* 1985, **15**(3):275-313.
57. Christopher P, Davis P, Falconer I, Bowling L, Dyson J: **Blue-green algae hit Lake Cargelligo**. *NSW Public Health Bull* 1991, **2**(11):110, 113.
58. International Programme on Chemical Safety (IPCS): **Environmental Health Criteria 212: Principles and methods for assessing allergic hypersensitization associated with exposure to chemicals**. Geneva: World Health Organization; 1999.
59. Behrendt H, Becker WM: **Localization, release and bioavailability of pollen allergens: the influence of environmental factors**. *Curr Opin Immunol* 2001, **13**(6):709-715.
60. Borish LC, Steinke JW: **2. Cytokines and chemokines**. *J Allergy Clin Immunol* 2003, **111**(2 Suppl):S460-475.
61. Lemanske RF, Jr., Busse WW: **6. Asthma**. *J Allergy Clin Immunol* 2003, **111**(2 Suppl):S502-519.
62. Prussin C, Metcalfe DD: **4. IgE, mast cells, basophils, and eosinophils**. *J Allergy Clin Immunol* 2003, **111**(2 Suppl):S486-494.
63. Stewart I: **Recreational exposure to freshwater cyanobacteria: epidemiology, dermal toxicity and biological activity of cyanobacterial lipopolysaccharides**. *PhD thesis*. Brisbane: University of Queensland; 2004.
64. Martich GD, Boujoukos AJ, Suffredini AF: **Response of man to endotoxin**. *Immunobiology* 1993, **187**(3-5):403-416.
65. Burrell R: **Human responses to bacterial endotoxin**. *Circ Shock* 1994, **43**(3):137-153.
66. Brandtzaeg P: **Significance and pathogenesis of septic shock**. *Curr Top Microbiol Immunol* 1996, **216**:15-37.
67. Wright SD: **Innate recognition of microbial lipids**. In: *Inflammation: Basic principles and clinical correlates*. Edited by Gallin JI, Snyderman R, 3rd edn. Philadelphia: Lippincott Williams & Wilkins; 1999: 525-535.
68. Stewart I, Schluter PJ, Shaw GR: **Cyanobacterial lipopolysaccharides and human health - a review**. *Submitted for publication* 2005.

69. Descotes J: **Importance of immunotoxicity in safety assessment: a medical toxicologist's perspective.** *Toxicol Lett* 2004, **149**(1-3):103-108.
70. Vial T, Choquet-Kastylevsky G, Descotes J: **Adverse effects of immunotherapeutics involving the immune system.** *Toxicology* 2002, **174**(1):3-11.
71. Philipp R: **Health risks associated with recreational exposure to blue-green algae (cyanobacteria) when dinghy sailing.** *Health Hyg* 1992, **13**:110-114.
72. Philipp R, Bates AJ: **Health-risks assessment of dinghy sailing in Avon and exposure to cyanobacteria (blue-green algae).** *J Inst Water Environ Manage* 1992, **6**(5):613-620.
73. Philipp R, Brown M, Bell R, Francis F: **Health risks associated with recreational exposure to blue-green algae (cyanobacteria) when windsurfing and fishing.** *Health Hyg* 1992, **13**:115-119.
74. el Saadi OE, Esterman AJ, Cameron S, Roder DM: **Murray River water, raised cyanobacterial cell counts, and gastrointestinal and dermatological symptoms.** *Med J Aust* 1995, **162**(3):122-125.
75. Pilotto LS, Douglas RM, Burch MD, Cameron S, Beers M, Rouch GJ, Robinson P, Kirk M, Cowie CT, Hardiman S *et al*: **Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities.** *Aust N Z J Public Health* 1997, **21**(6):562-566.
76. Stewart I, Webb PM, Schluter PJ, Fleming LE, Burns JW, Jr., Gantar M, Backer LC, Shaw GR: **Epidemiology of recreational exposure to freshwater cyanobacteria - an international prospective cohort study.** *Submitted for publication* 2005.
77. Backer LC: **Cyanobacterial harmful algal blooms (CyanoHABs): developing a public health response.** *Lake Reserv Manage* 2002, **18**(1):20-31.
78. Hoffman JR: **Rethinking case reports.** *West J Med* 1999, **170**(5):253-254.
79. Thornton J: **Case reports; original, educational or sexy--yes please, just rare--no thanks.** *Eur J Obstet Gynecol Reprod Biol* 2001, **96**(1):7.
80. Gerstman BB: **Epidemiology kept simple - an introduction to classic and modern epidemiology.** New York: Wiley-Liss; 1998.
81. Gordis L: **Epidemiology**, 3rd edn. Philadelphia: Elsevier Saunders; 2004.
82. McConnell WD, Holgate ST: **The definition of asthma: its relationship to other chronic obstructive lung diseases.** In: *Asthma*. Edited by Clark TJH, Godfrey S, Lee TH, Thomson NC, 4th edn. London: Arnold; 2000: 1-31.
83. Ferley JP, Zmirou D, Balducci F, Baleux B, Fera P, Larbaigt G, Jacq E, Moissonnier B, Blineau A, Boudot J: **Epidemiological significance of microbiological pollution criteria for river recreational waters.** *Int J Epidemiol* 1989, **18**(1):198-205.
84. Fleisher JM, Jones F, Kay D, Stanwell-Smith R, Wyer M, Morano R: **Water and non-water-related risk factors for gastroenteritis among bathers exposed to sewage-contaminated marine waters.** *Int J Epidemiol* 1993, **22**(4):698-708.

85. Fleisher JM, Kay D, Salmon RL, Jones F, Wyer MD, Godfree AF: **Marine waters contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom.** *Am J Public Health* 1996, **86**(9):1228-1234.
86. Fleisher JM, Kay D, Wyer MD, Godfree AF: **Estimates of the severity of illnesses associated with bathing in marine recreational waters contaminated with domestic sewage.** *Int J Epidemiol* 1998, **27**(4):722-726.
87. Kay D, Fleisher JM, Salmon RL, Jones F, Wyer MD, Godfree AF, Zelenauch-Jacquotte Z, Shore R: **Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure.** *Lancet* 1994, **344**(8927):905-909.
88. Carmichael WW, Gorham PR: **The mosaic nature of toxic blooms of cyanobacteria.** In: *The water environment - Algal toxins and health.* Edited by Carmichael WW. New York: Plenum; 1981: 161-172.
89. Schulz KF, Grimes DA: **Blinding in randomised trials: hiding who got what.** *Lancet* 2002, **359**(9307):696-700.
90. Rothman KJ, Greenland S: **Types of epidemiologic studies.** In: *Modern epidemiology.* Edited by Rothman KJ, Greenland S, 2nd edn. Philadelphia: Lippincott-Raven; 1998: 67-78.
91. Pilotto LS, Kliwer EV, Davies RD, Burch MD, Attewell RG: **Cyanobacterial (blue-green algae) contamination in drinking water and perinatal outcomes.** *Aust N Z J Public Health* 1999, **23**(2):154-158.
92. Maclure M: **The case-crossover design: a method for studying transient effects on the risk of acute events.** *Am J Epidemiol* 1991, **133**(2):144-153.
93. Wang PS, Schneeweiss S, Glynn RJ, Mogun H, Avorn J: **Use of the case-crossover design to study prolonged drug exposures and insidious outcomes.** *Ann Epidemiol* 2004, **14**(4):296-303.
94. Guyatt G, Sackett D, Adachi J, Roberts R, Chong J, Rosenbloom D, Keller J: **A clinician's guide for conducting randomized trials in individual patients.** *CMAJ* 1988, **139**(6):497-503.
95. Guyatt GH, Keller JL, Jaeschke R, Rosenbloom D, Adachi JD, Newhouse MT: **The n-of-1 randomized controlled trial: clinical usefulness. Our three-year experience.** *Ann Intern Med* 1990, **112**(4):293-299.
96. Rochon J: **A statistical model for the "N-of-1" study.** *J Clin Epidemiol* 1990, **43**(5):499-508.
97. Johannessen T, Petersen H, Kristensen P, Fosstvedt D: **The controlled single subject trial.** *Scand J Prim Health Care* 1991, **9**(1):17-21.
98. Patel A, Jaeschke R, Guyatt GH, Keller JL, Newhouse MT: **Clinical usefulness of n-of-1 randomized controlled trials in patients with nonreversible chronic airflow limitation.** *Am Rev Respir Dis* 1991, **144**(4):962-964.
99. Cook DJ: **Randomized trials in single subjects: the N of 1 study.** *Psychopharmacol Bull* 1996, **32**(3):363-367.
100. Schluter PJ, Ware RS: **Single patient (n-of-1) trials with binary treatment preference.** *Stat Med* 2005, **24**:IN PRESS.

101. Kleinbaum DG, Kupper LL, Morgenstern H: **Epidemiologic research. Principles and quantitative methods.** New York: Van Nostrand Reinhold; 1982.
102. Hoeffler DF: "**Swimmers' itch**" (cercarial dermatitis). *Cutis* 1977, **19**(4):461-465, 467.
103. Gonzalez E: **Schistosomiasis, cercarial dermatitis, and marine dermatitis.** *Dermatol Clin* 1989, **7**(2):291-300.
104. Marquardt WC, Demaree RS, Grieve RB: **Parasitology and vector biology,** Second edn. San Diego, CA: Academic Press; 2000.
105. Habif TP: **Clinical dermatology - a color guide to diagnosis and therapy,** 4th edn. St Louis, Missouri: Mosby; 2004.
106. Anon.: **Swimmers' itch, a surfacing problem? An outbreak at a Suffolk watersports park.** *Commun Dis Intell Bull* 1988, **88**(9):3-6.
107. Auerbach PS: **Natural microbiologic hazards of the aquatic environment.** *Clin Dermatol* 1987, **5**(3):52-61.
108. Hunter PR: **Waterborne disease. Epidemiology and ecology.** Chichester: John Wiley & Sons; 1997.
109. Sausker WF: **Pseudomonas aeruginosa folliculitis ("splash rash").** *Clin Dermatol* 1987, **5**(3):62-67.
110. Brooks C, Kujawska A, Patel D: **Cutaneous allergic reactions induced by sporting activities.** *Sports Med* 2003, **33**(9):699-708.
111. Moe CL: **Waterborne transmission of infectious agents.** In: *Manual of environmental microbiology.* Edited by Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV. Washington, DC: American Society for Microbiology; 1997: 136-152.
112. Marciano-Cabral F: **Biology of Naegleria spp.** *Microbiol Rev* 1988, **52**(1):114-133.
113. Bottone EJ: **Free-living amebas of the genera Acanthamoeba and Naegleria: an overview and basic microbiological correlates.** *Mt Sinai J Med* 1993, **60**(4):260-270.
114. Barnett NDP, Kaplan AM, Hopkin RJ, Saubolle MA, Rudinsky MF: **Primary amoebic meningoencephalitis with Naegleria fowleri: clinical review.** *Pediatr Neurol* 1996, **15**(3):230-234.
115. Hannisch W, Hallagan LF: **Primary amoebic meningoencephalitis: a review of the clinical literature.** *Wilderness Environ Med* 1997, **8**(4):211-213.
116. Marshall MM, Naumovitz D, Ortega Y, Sterling CR: **Waterborne protozoan pathogens.** *Clin Microbiol Rev* 1997, **10**(1):67-85.
117. Martinez AJ, Visvesvara GS: **Free-living, amphizoic and opportunistic amebas.** *Brain Pathol* 1997, **7**(1):583-598.
118. Schuster FL, Visvesvara GS: **Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals.** *Int J Parasitol* 2004, **34**(9):1001-1027.
119. Schuster FL, Visvesvara GS: **Amebae and ciliated protozoa as causal agents of waterborne zoonotic disease.** *Vet Parasitol* 2004, **126**(1-2):91-120.
120. Ojanen TH, Katila ML, Mantyjärvi R, Seppänen H, Muittari A, Kuusisto P, Virtanen P: **Exposure of water consumers to mesophilic actinomycetes.** *J Hyg (Lond)* 1983, **91**(3):535-541.

121. Aro S, Muittari A, Virtanen P: **Bathing fever epidemic of unknown aetiology in Finland.** *Int J Epidemiol* 1980, **9**(3):215-218.
122. Muittari A, Kuusisto P, Virtanen P, Sovijarvi A, Gronroos P, Harmoinen A, Antila P, Kellomaki L: **An epidemic of extrinsic allergic alveolitis caused by tap water.** *Clin Allergy* 1980a, **10**(1):77-90.
123. Muittari A, Rylander R, Salkinoja-Salonen M: **Endotoxin and bath-water fever.** *Lancet* 1980b, **2**(8185):89.
124. Muittari A, Kuusisto P, Sovijarvi A: **An epidemic of bath water fever--endotoxin alveolitis?** *Eur J Respir Dis Suppl* 1982, **123**:108-116.
125. Rapala J, Lahti K, Rasanen LA, Esala AL, Niemela SI, Sivonen K: **Endotoxins associated with cyanobacteria and their removal during drinking water treatment.** *Water Res* 2002, **36**(10):2627-2635.
126. Atterholm I, Ganrot-Norlin K, Hallberg T, Ringertz O: **Unexplained acute fever after a hot bath.** *Lancet* 1977, **2**(8040):684-686.
127. **A malaria-like syndrome after baths and showers in cyanobacteria-contaminated water: the importance of lipopolysaccharide endotoxins**  
[\[http://www.inweh.unu.edu/lvfo/lv2000%20abstracts.htm\]](http://www.inweh.unu.edu/lvfo/lv2000%20abstracts.htm)
128. Codd GA: **Cyanobacterial toxins, the perception of water quality, and the prioritisation of eutrophication control.** *Ecol Eng* 2000, **16**(1):51-60.
129. Paddle GM: **Epidemiology.** In: *Experimental toxicology The basic principles.* Edited by Anderson D, Conning DM. Cambridge: The Royal Society of Chemistry; 1990: 436-456.
130. Domingos P, Rubim TK, Molica RJR, Azevedo SMFO, Carmichael WW: **First report of microcystin production by picoplanktonic cyanobacteria isolated from a northeast Brazilian drinking water supply.** *Environ Toxicol* 1999, **14**(1):31-35.
131. World Health Organization: **Guidelines for safe recreational water environments - Volume 1: coastal and fresh waters.** Geneva: World Health Organization; 2003.
132. Takai A, Harada KI: **Freshwater hepatotoxins: ecobiology and classification.** In: *Seafood and freshwater toxins - pharmacology, physiology, and detection.* Edited by Botana LM. New York: Marcel Dekker; 2000: 603-612.
133. Skulberg OM, Carmichael WW, Andersen RA, Matsunaga S, Moore RE, Skulberg R: **Investigations of a neurotoxic Oscillatorialean strain (cyanophyceae) and its toxin. Isolation and characterization of homoanatoxin-a.** *Environ Toxicol Chem* 1992, **11**(3):321-329.
134. Fitzgeorge RB, Clark SA, Keevil CW: **Routes of intoxication.** In: *Detection methods for cyanobacterial toxins.* Edited by Codd GA, Jefferies TM, Keevil CW, Potter E. Cambridge: The Royal Society of Chemistry; 1994: 69-74.
135. Sivonen K, Jones G: **Cyanobacterial toxins.** In: *Toxic cyanobacteria in water - a guide to their public health consequences, monitoring and management.* Edited by Chorus I, Bartram J. London: E & FN Spon; 1999: 41-111.
136. Carmichael WW, Evans WR, Yin QQ, Bell P, Moczydlowski E: **Evidence for paralytic shellfish poisons in the freshwater**

- cyanobacterium Lyngbya wollei (Farlow ex Gomont) comb. nov.**  
*Appl Environ Microbiol* 1997, **63**(8):3104-3110.
137. Lagos N, Onodera H, Zagatto PA, Andrinolo D, Azevedo SM, Oshima Y: **The first evidence of paralytic shellfish toxins in the fresh water cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil.** *Toxicon* 1999, **37**(10):1359-1373.
138. Pomati F, Sacchi S, Rossetti C, Giovannardi S, Onodera H, Oshima Y, Neilan BA: **The freshwater cyanobacterium *Planktothrix* sp FP1: molecular identification and detection of paralytic shellfish poisoning toxins.** *J Phycol* 2000, **36**(3):553-562.
139. Sivonen K: **Freshwater cyanobacterial neurotoxins: ecobiology, chemistry, and detection.** In: *Seafood and freshwater toxins - pharmacology, physiology, and detection.* Edited by Botana LM. New York: Marcel Dekker; 2000: 567-581.
140. Pereira P, Li R, Carmichael WW, Dias E, Franca S: **Taxonomy and production of paralytic shellfish toxins by the freshwater cyanobacterium *Aphanizomenon gracile* LMECYA40.** *Eur J Phycol* 2004, **39**(4):361-368.
141. Ohtani I, Moore RE, Runnegar MTC: ***Cylindrospermopsis raciborskii*: a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*.** *J Am Chem Soc* 1992, **114**(20):7941-7942.
142. Harada KI, Ohtani I, Iwamoto K, Suzuki M, Watanabe MF, Watanabe M, Terao K: **Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method.** *Toxicon* 1994, **32**(1):73-84.
143. Terao K, Ohmori S, Igarashi K, Ohtani I, Watanabe MF, Harada KI, Ito E, Watanabe M: **Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*.** *Toxicon* 1994, **32**(7):833-843.
144. Seawright AA, Nolan CC, Shaw GR, Chiswell RK, Norris RL, Moore MR, Smith MJ: **The oral toxicity for mice of the tropical cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska).** *Environ Toxicol* 1999, **14**(1):135-142.
145. Stewart I, Seawright AA, Schluter PJ, Shaw GR: **Primary irritant and delayed-contact hypersensitivity reactions to the freshwater cyanobacterium *Cylindrospermopsis raciborskii* and its associated toxin cylindrospermopsin.** *Submitted for publication* 2005.
146. Moore RE: **Public health and toxins from marine blue-green algae.** In: *Seafood toxins ACS Symposium Series No 262.* Edited by Ragelis EP. Washington, DC: American Chemical Society; 1984a: 369-376.
147. Moore RE: **Structure-activity studies of aplysiatoxin-type tumor promoters.** In: *Cellular interactions by environmental tumor promoters.* Edited by Fujiki H, Hecker E, Moore RE, Sugimura T, Weinstein IB. Tokyo: Japan Scientific Societies Press; 1984b: 49-57.
148. Moore RE, Blackman AJ, Cheuk CE, Mynderse JS, Matsumoto GK, Clardy J, Woodard RW, Craig JC: **Absolute stereochemistries of the aplysiatoxins and oscillatoxin A.** *J Org Chem* 1984, **49**(13):2484-2489.

149. Fujiki H, Ikegami K, Hakii H, Suganuma M, Yamaizumi Z, Yamazato K, Moore RE, Sugimura T: **A blue-green alga from Okinawa contains aplysiatoxins, the third class of tumor promoters.** *Jpn J Cancer Res* 1985, **76**(4):257-259.
150. Reif C, cited in Billings WH: **Water-associated human illness in northeast Pennsylvania and its suspected association with blue-green algae blooms.** In: *The water environment - Algal toxins and health.* Edited by Carmichael WW. New York: Plenum; 1981: 243-255.
151. van Hoof F: **The occurrence of toxic cyanobacteria in Europe (excluding the UK and Scandinavia).** In: *Toxic cyanobacteria: current status of research and management: 1994;* Adelaide: Australian Centre for Water Quality Research; 1994: 29-33.
152. Williamson M, Corbett S: **Investigating health risks from riverine blooms of blue green algae.** *NSW Public Health Bull* 1993, **4**(3):27-29.
153. Probert CS, Robinson RJ, Jayanthi V, Mayberry JF: **Microcystin hepatitis.** *Arq Gastroenterol* 1995, **32**(4):199.
154. el Saadi O, Cameron AS: **Illness associated with blue-green algae.** *Med J Aust* 1993, **158**(11):792-793.

## Figure legends

**Figure 1. Cross-sectional study design.** Adapted from Gordis [81 p.173].

**Figure 2. Case-control study design.** Adapted from Gordis [81 (p.160)].

**Figure 3. Cohort design.** The timeline on the left describes a prospective cohort study; the timeline on the right describes a retrospective cohort study. Adapted from Gordis [81 (p.152)].

**Figure 4. Randomised intervention design.** Adapted from Gordis [81 (p.116)].

**Table 1. Cyanotoxins with public health significance**

<b>Toxin or toxin group</b>	<b>Classification by principal target organ systems</b>	<b>Toxin-producing genera</b>	<b>LD<sub>50</sub> (i.p. mouse)</b>	<b>References</b>
Microcystins	Hepatotoxins	<i>Microcystis</i> , <i>Anabaena</i> , <i>Nostoc</i> , <i>Planktothrix</i> , <i>Aphanocapsa</i> , <i>Anabaenopsis</i> , <i>Hapalosiphon</i>	25->1000 µg/kg	[18, 25, 130, 131 (pp.140- 1)]
Nodularins	Hepatotoxins	<i>Nodularia</i>	30-60 µg/kg	[8 (pp.31-2), 25, 132]
Anatoxin-a, homoanatoxin-a	Neurotoxins	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Oscillatoria</i> , <i>Planktothrix</i> , <i>Microcystis</i> , <i>Phormidium</i>	200- 375µg/kg	[8 (pp.27-9), 17, 25, 133- 135]
Anatoxin-a(S)	Neurotoxin	<i>Anabaena</i>	20-40µg/kg	[8 (pp.28-9), 25, 135]
Saxitoxins	Neurotoxins	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Lyngbya</i> , <i>Cylindrospermopsis</i> , <i>Planktothrix</i>	10-30µg/kg	[25, 131 (p.140), 135- 140]
Cylindrospermopsin	General cytotoxin (multiple organ systems affected, incl. liver, kidney, gastrointestinal tract, heart, spleen, thymus, skin)	<i>Cylindrospermopsis</i> , <i>Aphanizomenon</i> , <i>Umezakia</i> , <i>Raphidiopsis</i>	2.1mg/kg (24 hours) 200µg/kg (5-6 days)	[8 (p.32), 16, 135, 141- 145]
Aplysiatoxin, debromoaplysiatoxin	Dermal toxins	<i>Lyngbya</i>		[146-149]

**TABLE 2. Reports of human morbidity and mortality attributed to recreational exposure to freshwater cyanobacteria**

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
Hayfever-like symptoms (conjunctivitis, rhinitis, sneezing)														
1934	Late summer	Muskego Lake, Waukesha County, WI, USA	Report of single subject, male	N/S	57	Swimming	Itching of eyes, nasal congestion	"weedy lake"	3 hours	<48 hours		History of frequent sinus infections		[54]
1935	Late summer	Muskego Lake, WI, USA	Same subject	N/S		Swimming	Same symptoms, + mild asthma		As above	As above				[54]
1936-1946	Late summer	North Lake, Waukesha County, WI, USA	Same subject	N/S		Swimming	Nasal discharge and congestion, conjunctivitis, mild asthma	Oscillatoreaceae (sample taken in late summer, 1944)	N/S	N/S	Surface scum extracts gave immediate skin reactions. Cutaneous injection of 0.03mL of 1:1,000 dilution resulted in mild asthma within 20 mins. Control subjects did not react to scum extracts	Subject swam in North Lake during summer months over the ten-year period, without incident until mid-August each year, when swimming was followed by symptom onset. Desensitisation injections over 4 years were successful		[54]
1979	Late summer	Lake Wallenpaupack, PA, USA	5 (family group)	N/S	N/S (parents + son + daughter + friend)	Swimming	"Severe" hayfever-like symptoms (sneezing, nasal discharge, eye irritation): 3/5 earache: 2/5	N/S. Lake developed a distinct green colour several days prior to incident. Three weeks later: heavy bloom of <i>Anabaena</i>	Hayfever symptoms: during exposure; earache: several hours after exposure	N/S		N/S		[47]
1979	Late summer	Lake Wallenpaupack, PA, USA	20-30	60-90 (those affected comprised approx 25% of aquatic event participants)	N/S (high school students)	Participating in school aquatic event	Eye irritation, sore throat, earache, sneezing, nasal discharge, swollen lips	N/S. see above	During exposure or within 2-3 hours	Within 2-3 days  *Subsequent exposure reportedly caused re-occurrence of symptoms		N/S	Affected individuals were members of a high school summer aquatic program. The event was cancelled because of these illnesses	[47]
N/S	N/S	N/S	Single male	N/S	N/S (adult)	Swimming	Hayfever-like symptoms	Bloom of <i>Microcystis</i>	N/S	N/S		N/S		[150]

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
Cutaneous symptoms														
1949-1952	Summer	Lake Carey, PA, USA	Report of single subject, female	Reported skin rash "never appeared in other bathers swimming in the same water"	6 (condition first seen at age 3 years)	Swimming	Seasonal erythematous papulo-vesicular rash, occasionally progressing to oozing and crusting. Rash limited to face, neck, shoulders, upper chest and extremities; rash never seen on areas covered by swimsuit	<i>Anabaena</i>	1-2 hours	< 2 weeks (if no further contact with lake water)	Skin patch testing: strong positives to <i>Anabaena</i> filtered from lake water, chloroform extract of same, and phycocyanin extract	None (apart from reported condition)	Rash never seen after swimming in artificial pools or ocean. Only seen after water exposure at Lake Carey, and once after bathing in a Canadian lake	[45]
Late 1940s & mid 1950s	Late summer	Lake Ringsjön, Scania, Sweden	"several" members of a family	N/S	N/S ("young girl" + siblings)	Swimming	Pruritic skin rashes	<i>Gloeotrichia echinulata</i>	N/S	N/S				[42, 52]
1977	Summer	Mingechaur Reservoir, Azerbaijan	7	13	N/S	Swimming	Slightly raised, erythematous spots, 2-6mm diameter; seen on skin bordering and outside swimsuit	Benthic <i>Lyngbya kützingii</i> ; also "planktonic blue-green algae colouring the water"	2 <sup>nd</sup> day after swimming	10-12 days				[33]
1985	N/S	UK	N/S	N/S	N/S	Sail-boarding	Skin rashes	N/S ("toxic blooms")	N/S	N/S		N/S		[49 p.43]
1989	N/S	Japan (lake)	1	N/S	N/S (adult)	Collecting algal scum *	Rashes	<i>M. aeruginosa</i> containing microcystins	N/S	N/S		N/S		[49 p.59]
1989	Late summer – early autumn	Bewl Water, Kent, UK	1	N/S	N/S	Fishing	'blotchy' skin on face and hands after handling water on three separate occasions	<i>Microcystis</i> sp	Within two hours	Several days		N/S		[49 p.100]
1989	N/S	NRA South West Region, UK	N/S ("some NRA staff")	N/S	N/S (adults)	Sampling water *	Tingling sensations on hands	<b>"Potentially toxic cyanobacteria species"</b>	N/S	N/S		N/S		[49 p.102]
1989	Early-mid autumn	Welton Water, Yorks, UK	N/S	N/S	N/S	N/S ("water users")	Rashes	<i>Anabaena</i> sp	N/S	N/S		N/S		[49 p.105]
1991	Late spring – early summer	Darling-Barwon river system, NSW, Australia	N/S	N/S	N/S (adults)	Sampling water*	Skin irritation	<i>Anabaena</i> spp	N/S	N/S		N/S		[50 p.32]

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
Gastro-intestinal symptoms (nausea, vomiting, diarrhoea, abdominal pain)														
1959	Summer	One of Katepwa Lakes, SK, Canada	Report of single subject, male	N/S	N/S (adult)	Swimming	Headache, nausea, G-I upset	N/S	During night after swimming	<48 hours.	Stool sample showed "many tiny greenish spheres which resembled in size and morphology the cells of <i>Microcystis</i> ". Stool specimen negative for <i>Salmonella</i> and <i>Entamoeba</i>	N/S	Subject sought medical advice for gastroenteritis, admitted to hospital, given oral chloramphenicol. Recovery within 24 hours of admission	[55]
1959	Summer	Long Lake, SK, Canada	10	N/S	N/S (children)	Swimming	Diarrhoea, vomiting	N/S. Subjects swam in "algae-covered lake water". Dried <i>Microcystis</i> and <i>Anabaena</i> scum found later on shore	N/S. Reported to local medical officer with illness on the day after exposure	N/S	Stool specimen from one child contained cells resembling <i>Anabaena</i> "in great numbers"		Local farmer reported that two cows died after drinking from the lake 12-16 hours previously, during an algal bloom. A third sick cow recovered after receiving penicillin injections	[55]
N/S	N/S	N/S (lake)	Single subject, male	N/S	4	Fell into lake, swallowed water #	Abdominal pain, nausea, vomiting, diarrhoea, wooziness, headache, thirst	N/S	Day of exposure: G-I symptoms; next day: wooziness, headache, thirst	N/S	<i>Aphanizomenon</i> found in stool and vomitus specimens	N/S		[46]
1961	N/S	N/S	4	N/S	N/S (students)	Swimming	Headache, malaise, diarrhoea	Heavy growth of <i>Microcystis</i> and <i>Anabaena</i>	N/S	N/S		N/S		[46]
1989	Early autumn	Rutland Water, Leics, UK	N/S	N/S	2 & 3 year-olds	Playing at edge of scum	Vomiting & diarrhoea	<i>M. aeruginosa</i>	N/S	N/S	Microcystin-LR found to be principal cyanobacterial toxin present	N/S	20 sheep and 15 dogs died over approx 3 weeks in late summer – early spring after contact with bloom scum	[6]
1990	N/S	Unnamed lake, Tiel, The Netherlands	N/S	N/S	N/S	Swimming	"G-I complaints"	<i>Anabaena flos-aquae</i>	N/S	N/S	Water quality parameters and food-borne pathogen testing negative	N/S	Mouse bioassay and HPLC-UV confirmed presence of anatoxin-a	[151]

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
Progression to fatal illness														
2002	Mid-summer	Golf course pond, Milwaukee, WI, USA	5	0	17 yo male and 4 friends	"splashing and diving". Two most severely affected boys had their heads under water for varying periods of time #	17-year-old developed nausea, vomiting, progressed to "shock" and "seizure", acute heart failure; death ensued approx. 48 hours after exposure. One teenager who also apparently ingested water developed severe diarrhoea and abdominal pain. The other three youths developed "minor symptoms"	Presumably <i>A. flos-aquae</i> containing anatoxin-a, determined from blood and stool samples of the boy that died and the other severely affected youth	N/S	N/S	Analysis of "tissue, blood and other fluid samples" from the two severely affected teenagers. Anatoxin-a found in unspecified sample/s. Autopsy showed "acute heart damage" but no evidence of meningitis or encephalitis. Analyses for pesticides, parasites and other pathogens negative	N/S.	The unusual feature of this case is the length of time – 48 hours – that ensued between exposure and ingestion of contaminated water and subsequent death	[34, 35]
Cold & flu-like symptoms (incl. fever, headache, myalgia, arthralgia, sore throat, respiratory and gastro-intestinal)														
1959	Summer	Echo Lake, SK, Canada	Report of single subject, male	N/S	N/S (adult)	Intending to swim – fell into lake, swallowed an estimated half-pint of water #	Abdominal cramps and pain, nausea, vomiting, painful diarrhoea, fever, severe headache, weakness, pain in limb muscles and joints. Stools and vomitus were slimy and green	N/S. Lake had a visible bloom on day of exposure. <i>Microcystis</i> and <i>Anabaena</i> bloom 35 days prior to exposure	3 hours: abdominal pain, nausea, vomiting 5 hours: diarrhoea; next morning: fever, headache, myalgia, weakness	Recovering when questioned on 2 <sup>nd</sup> day after exposure	Stool specimen showed "innumerable spheres of <i>Microcystis</i> and 2-3 well-preserved curved chains of <i>A. circinalis</i> per high-power field"	N/S	During bloom 35 days prior to exposure, an unknown number of dogs and geese died after swimming in the lake. Other dogs sickened after drinking lake water. Also fish kills	[55]
N/S	N/S	N/S (swimming hole)	Single subject, male	N/S	12	Swimming	Fever, loss of consciousness for six hours, dyspnoea, pneumonia, myalgia, arthralgia	Abundant <i>M. aeruginosa</i>	N/S (onset reported as sudden, with subsequent myalgia and arthralgia)	N/S	Stool sample: <i>Aeromonas</i> (Gram -ve bacteria), <i>Spirogyra</i> and <i>Mougeotia</i> (both green algae)	N/S		[46]

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
1979	Late summer	Lake Wallenpaupack, PA, USA	15	N/S	N/S	Swimming	Vomiting, nausea, diarrhoea, eye irritation, sore throat, fever, earache	N/S. See above.	Symptoms occurred "a short time after contact with lake water"	G-I symptoms: 24-48 hours		N/S	Affected individuals all holidaying in rental cottages. Well water to cottages free of bacterial contamination	[47]
1979	Late summer	Arrowhead Lake, PA, USA	Single female	N/S	N/S ("young girl")	Swimming #	Chills, sore throat, fever, nausea, diarrhoea	N/S	"within several hours"	Approx. 3 days		N/S		[47]
1989	Early autumn	Rudyard Lake, Staffs, UK	2	N/S	16	Swimming and canoeing exercises, the latter involving 360° rolls * #	Malaise, sore throat, circum-oral blistering, left-sided pleuritic pain, dry cough, vomiting, central abdominal pain, diarrhoea, fever	<i>M. aeruginosa</i>	Day after exposure	<2 weeks	Microcystin-LR found to be principal cyanobacterial toxin present	N/S	Both were hospitalised, having developed left lower-lobe pneumonia accompanied by thrombocytopenia	[22, 40, 41, 44, 48, 51 p.19]
1989	Early autumn	Rudyard Lake, Staffs, UK	8	N/S	N/S (soldiers)	As above * #	Sore throat, headache, abdominal pain, dry cough, diarrhoea, vomiting, blistered mouth	<i>M. aeruginosa</i>	N/S	N/S	See above	N/S	All subjects were soldiers who had partaken in canoeing exercises, subsequently admitted to barracks medical centre	[22, 40, 41, 44, 51 p.19]
1992	Mid-spring	Darling River, Wilcannia, NSW, Australia	2	N/S	N/S - teenagers	Swimming	Gastroenteritis and myalgia	<i>Anabaena</i> sp	N/S	Symptoms resolved after 48 hrs	One subject required admission to hospital.	N/S	Some Wilcannia residents reported itchy skin rashes after showering, even after carbon filtration of the town water supply	[152]
N/S	N/S	Unnamed reservoir, UK	Single subject, male	N/S	39	Windsurfing	Fever, nausea, vomiting	N/S, but microcystin isolated from reservoir; several sheep and dogs died	N/S "shortly after windsurfing trip"	N/S	Liver function tests, liver biopsy	No significant past history, no medications	Mild hepatic dysfunction investigated six weeks after exposure	[153]

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
Mixed symptoms														
1945	Late summer	Lake Keesus, Waukesha County, WI, USA	Report of single subject, female	N/S	39	Swimming	Gross eyelid oedema, nasal congestion, generalised urticarial rash	N/S	"while swimming"	N/S	Skin test with Oscillatoreaceae extract (0.03mL of 1:10,000 dilution) resulted in immediate and severe local reaction treated by local injection of adrenaline	Long-standing history of autumn hay-fever and seasonal asthma	Oscillatoreaceae extracts elicited positive (but unspecified) reactions in "many individuals who knew that swimming caused hay fever" at dilutions up to 1:100,000	[54]
1946	Late summer	Lake Keesus, WI, USA	Same subject	N/S		Swimming	As above	N/S	N/S	N/S				[54]
1973-4	Summer	Belgrano Park pond, Santa Fe City, Argentina	N/S	N/S	N/S	Swimming and bathing	G-I symptoms, dermatitis, otitis, conjunctivitis	Mainly <i>M. aeruginosa</i> ; bloom contained "up to 60,000 colonies/mL"	N/S	N/S	N/S	N/S		[53]
1979	Late summer	Arrowhead Lake, PA, USA	Initial reports: 20-30 children, several adults. 12 children + 1 adult investigated	N/S	4-12, + adult	Swimming	Headache: 8/13 stomach cramps: 9/13 nausea: 5/13 vomiting: 7/13 diarrhoea: 11/13 fever: 5/13 rash: 1/13 sore or inflamed throat: 3/13	N/S	During exposure, to maximum 12 hours after exposure. Rash on arms & legs of adult ♀ developed "shortly after wading along lake edge"	Most symptoms: <72 hours All symptoms: ≤5 days	Stool samples of four children negative for <i>Salmonella</i> and <i>Shigella</i> . Throat swab of one child with sore throat negative for viral involvement	N/S	Routine weekly monitoring for faecal coliforms showed counts were ≤40/100mL prior to incident	[47]
1980	Mid-summer	Pocono Highlands Lake, PA, USA	N/S "swimmers"	N/S	N/S	Swimming	Eye irritation, earache, sore throat	<i>Anabaena</i> sp.	N/S	N/S	Water samples produced signs of poisoning in mice suggestive of hepatotoxin. LD <sub>50</sub> = 90 mg/kg (i.p. mouse)	N/S		[56]
1980	Late summer	Lake Lahonton, NV, USA	N/S "several... affected"	N/S	N/S	Water skiing, swimming	Erythema, eye irritation, dizziness, nausea, stomach cramps, diarrhoea	<i>Aphanizomenon flos-aquae</i>	N/S	N/S	LD <sub>50</sub> = 500 mg/kg body weight (i.p. mouse)	N/S		[56]

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
1980	Mid-summer	Camp William Penn, PA, USA	75-100	N/S	N/S	N/S ("campers affected from contact with water")	Conjunctivitis, sore-red throat, headaches, diarrhoea, nausea	<i>Anabaena</i> sp.	N/S	N/S		N/S		[56]
1981	Mid-summer	Harveys Lake, PA, USA	N/S ("many reports...")	N/S	N/S	N/S	Skin irritation, nausea, dizziness, diarrhoea	<i>Anabaena flos-aquae</i>	N/S	N/S	Mouse toxicity testing of water showed both neurotoxins and hepatotoxins present. LD <sub>50</sub> = 125 mg/kg	N/S	Lake closed to public access until early spring	[56]
1989	Early autumn	Rutland Water, Leics, UK, and other UK lakes	N/S ("several")	N/S	N/S	Sail-boarding #	Skin rashes, nausea, vomiting, blistering inside mouth, severe thirst	<i>M. aeruginosa</i>	N/S	N/S	Microcystin-LR found to be principal cyanobacterial toxin present	N/S	20 sheep and 15 dogs died over approx 3 weeks in late summer – early spring after contact with bloom scum	[6, 41, 49 p.92]
1990	Spring	Lake Cargelligo, NSW, Australia	2 or 3	N/S	N/S	Swimming	Two cases of conjunctivitis, one case of rash	<i>Anabaena circinalis</i>	N/S	N/S		N/S	Lake closed as water supply for one month while bloom evident	[57]
1991	Summer-Autumn	Lakes Alexandrina and Albert, SA, Australia	1	N/S	N/S (adult)	Crayfishing	Skin and/or eye symptoms (pruritis, skin rash, sore red eyes)	<i>Nodularia</i> sp	N/S	N/S	Case identified retrospectively by either interview with local residents, local health workers or surveillance through local GPs	N/S	7 other cases identified, all with skin and/or eye symptoms. Two of them also had asthma symptoms, one had hay fever symptoms, another a sore throat. Water contact was by showering or bathing	[43]
1991	Late spring – early summer	Darling River, Wilcannia, NSW, Australia	1	Sole report after surveillance requests to report confirmed or suspected cyanobacteria-related illness during an extensive riverine bloom	N/S (adult)	Water skiing	Skin rash, conjunctivitis, diarrhoea, respiratory difficulty	<i>Anabaena</i> spp	N/S	N/S		N/S	All schools along the river system were asked to report increases in illness-related absenteeism, especially for G-I symptoms. No such increase was reported	[50 p.32]

Year	Season	Location	Number affected	Estimates of number unaffected	Age	Water activity	Signs & Symptoms	Dominant plankton	Time of onset after exposure	Symptom duration	Diagnostic criteria	Predisposing conditions	Notes	References
1991-2	Summer	River Murray, SA, Australia	11	N/S	1-64	"water sport... particularly skiing". One of these cases had skin contact through both water sport and residential water use	Contact exposure (n=2): rash, itching, mouth blistering, eye irritation; oral ingestion (n=3): diarrhoea, vomiting, nausea, muscle weakness, sore throat, respiratory difficulty, headache; contact + oral exposure (n=6): mixture of above symptoms	Predominantly <i>A. circinalis</i>	N/S	N/S	Cases investigated following telephone or personal complaints to health authorities and water supply management	N/S	15 further cases with exposure to River Murray water from reticulated water supply	[154]
1996	N/S	Hollingworth Lake, UK	11	N/S	N/S (sea cadets)	Canoe capsizing trials *	Facial rashes, asthmatic signs, dry sporadic cough, vomiting	<i>Planktothrix agardhii</i> , containing three microcystins	Day of, and after exposure	N/S		N/S		[22]
1997	Autumn	Lake Sammamish, WA, USA	N/S ("several")	N/S	"young children"	Swimming	G-I complaints, rashes	<i>M. aeruginosa</i> , microcystins measured at approx. 500 µg/g dry weight	N/S	N/S	N/S	N/S	A dog began "heaving and coughing", died four hours after exposure to bloom	[36, 37]

# – cyanobacteria-affected water reportedly ingested (table column: "Water activity")

\* – occupational exposure (table column: "Water activity")

N/S – not stated



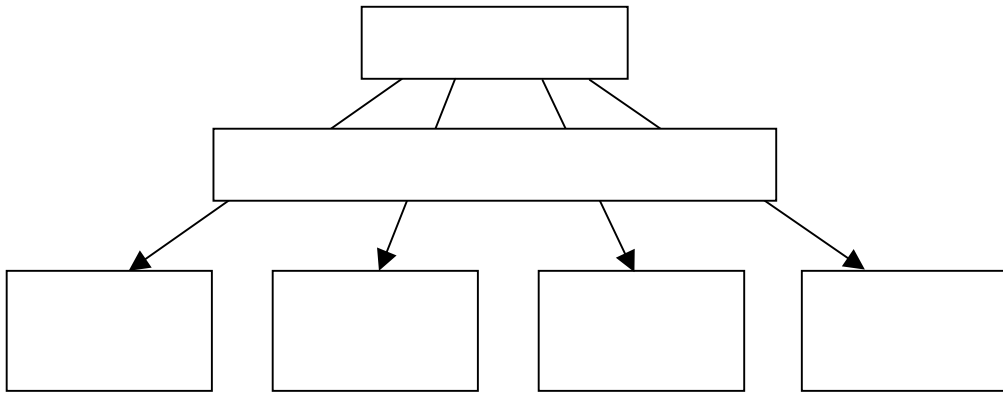


Figure 1

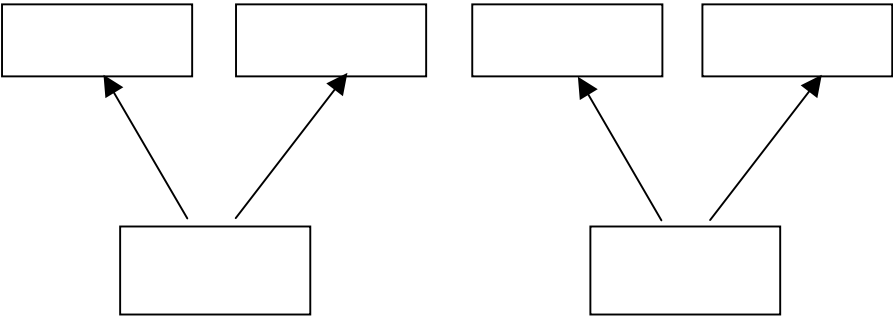


Figure 2

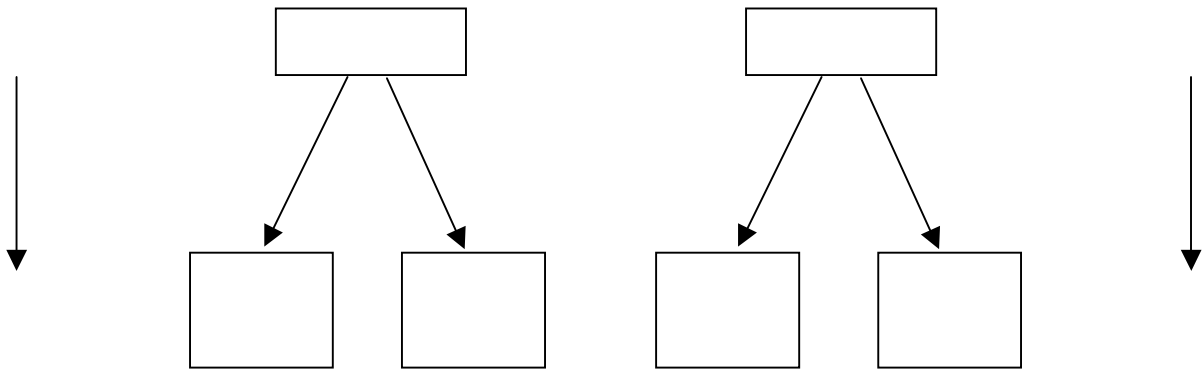


Figure 3

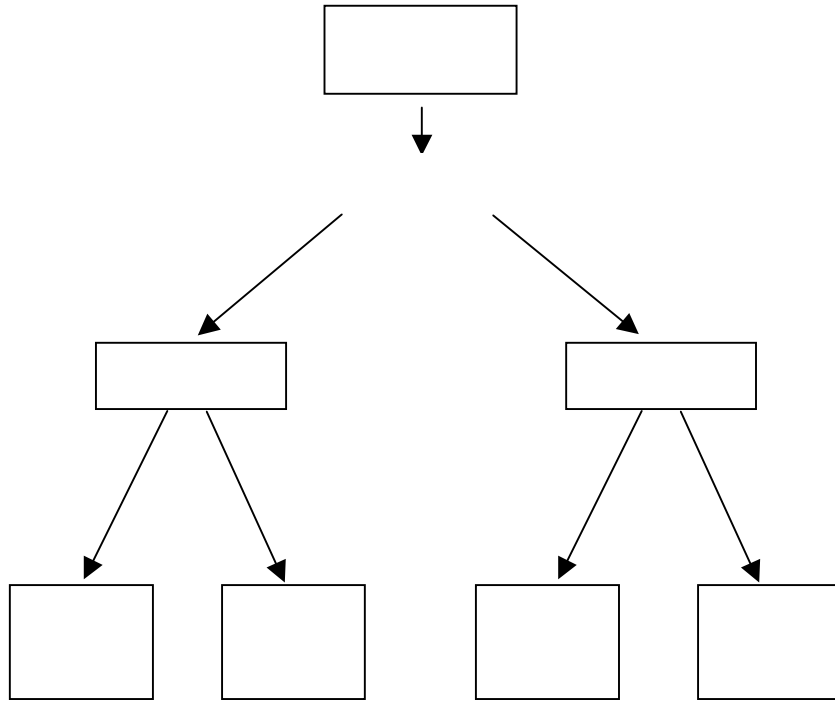


Figure 4

**Additional files provided with this submission:**

Additional file 1: iantest.enl : 924KB

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