Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities

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Abstract: The aim of this study was to investigate effects on health of exposure to cyanobacteria as a result of recreational water activities. Participants, who were aged six years and over, were interviewed at water recreation sites in South Australia, New South Wales and Victoria on selected Sundays during January and February 1995. Telephone follow-up was conducted two and seven days later to record any subsequent diarrhoea, vomiting, flu-like symptoms, skin rashes, mouth ulcers, fevers and eye or ear irritations. On the Sundays of interview, water samples from the sites were collected for cyanobacterial cell counts and toxin analysis. There were 852 participants, of whom 75 did not have water contact on the day of interview and were considered unexposed. The 777 who had water contact were considered exposed. No significant differences in overall symptoms were found between the unexposed and exposed after two days. At seven days, there was a significant trend to increasing symptom occurrence with duration of exposure ($P = 0.03$). There was a significant trend to increasing symptom occurrence with increase in cell count ($P = 0.04$). Participants exposed to more than 5000 cells per mL for more than one hour had a significantly higher symptom occurrence rate than the unexposed. Symptoms were not correlated with the presence of hepatotoxins. These results suggest symptom occurrence was associated with duration of contact with water containing cyanobacteria, and with cyanobacterial cell density. The findings suggest that the current safety threshold for exposure of 20 000 cells per mL may be too high. (*Aust N Z J Public Health* 1997; 21: 562–6)

CYANOBACTERIA (blue-green algae) are persistent prokaryotic organisms. Certain species produce biotoxins potentially hazardous to animal and human health.[1–3] These organisms are photosynthetic autotrophs whose growth tends to be favoured by warm water temperatures,[4] and calm, stable weather conditions. Under optimum conditions, which usually occur in the summer months in Australia, these organisms can form massive populations or blooms in rivers and lakes. These blooms have recently become recognised as a significant issue for water supply management in Australia.[5,6] Most important is the link between increasing eutrophication, by which excessive plant growth is

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stimulated by nutrients entering water sources, leading to increasing bloom formation.[7] Cullen believes increasing eutrophication is one of the major water quality problems facing Australia and leads to excessive algal growth that is seriously degrading use of our waterways.[8] Most reported instances of blooms have occurred in southeastern Australia, with some occurrences in the far southwest and northwest, and a scheme for the systematic recording of bloom formation in all Australian states has been under development under the direction of the Agriculture and Resource Management Council of Australia and New Zealand. However, while the problem is widespread, its extent may not yet be appreciated fully. This, then, poses a public health concern for Australia, especially because affected waterways are a source of drinking water for some communities, and are common sites for recreational activities.

The first reported episode of algal poisoning occurred at Lake Alexandrina in South Australia in 1878.[9] Since then, a number of outbreaks and case reports of adverse health effects in animals and humans, probably attributable to cyanobacterial toxicity, have been reported worldwide.[10–14] A recent Australian study found elevated risks of gastrointestinal and dermatological symptoms associated with drinking and domestic use of water from the Murray River during a period of raised cyanobacterial cell counts.[15] In Australia, members of the genera *Microcystis*, *Anabaena*, *Nodularia* and *Cylindrospermopsis* produce hepatotoxins, some *A. circinalis* produce paralytic shellfish poisons, and members of most species may produce lipopolysaccharide endotoxins.[16] However, there is very little information from epidemiological studies to assist with assessing dangers to health from algal exposure.[10] Risk to humans, especially from the recreational use of waters infested with cyanobacteria, is an area that is poorly understood. The responsibility for closure of lakes and rivers to recreational use causes major problems for councils, regulatory authorities and health agencies, especially in the absence of definitive data on risk. This study investigates the risk to health of recreational exposure to quantified levels of cyanobacteria in public waterways in three Australian states.

Method

The study was conducted during January and February 1995 at Goolwa on Lake Alexandrina in South Australia, at Carcoar Dam and the Hawkesbury River (Cliftonville and Dargle) in New South Wales, and at Lakes Narracan and Boga in Victoria. Based on previous experience, algal blooms were expected to occur at these sites during summer. At the same time, these sites were expected to be used for recreational water activities, such as jet-skiing, water-skiing, swimming and windsurfing.

Interviewers attended these sites on various Sundays and invited all people in attendance who were aged six and over to participate. Carcoar Dam was used on three separate occasions, Cliftonville once, and the other four sites on two separate occasions, giving a total of 12 site attendances. A structured questionnaire was used by the interviewers to determine the health status and recreational water-related activities (including the duration of water contact) of each participant for the day of interview and for the previous five days. At the time of interview, appointments were made for telephone follow-up two and seven days later. At follow-up, any diarrhoea (two or more loose motions on one day), vomiting, flu-like symptoms (cough, cold or flu symptoms), skin rashes, mouth ulcers, fevers or eye or ear infections lasting more than 24 hours since the initial contact were recorded. These symptoms were those most commonly referred to in other literature. Any recreational water activities after the day of initial interview were also recorded.

At each site on the Sunday of the initial interview, water samples were collected at 10 a.m. and 2 p.m. On each occasion, 10 replicate integrated column samples, taken with a 25 mm diameter rigid plastic tube 2 m in length, were collected at evenly spaced distances and in a regular pattern across the exposure site and then pooled to form a composite sample. A 200 mL subsample was then taken from the composite sample and preserved immediately with Lugol's iodine (1 per cent) and stored in an amber glass bottle. The samples for cell counts were then sent to one of four water laboratories aligned with investigators in the study. Samples were concentrated by sedimentation and cyanobacteria identified and enumerated with either a compound microscope and Sedgwick–Rafter chambers or an inverted microscope and modified Utermohl settling chambers. Identification of cyanobacteria followed the work of Baker.[17,18] Cell counts for the dominant cyanobacteria were counted to a specified precision of plus or minus 20 per cent.

Toxicity was determined as part of a separate investigation into cyanobacterial toxicity by the Australian Water Quality Centre, and results were made available to this study. For the determination of toxicity, a concentrated cell sample was collected in addition to the pooled composite samples for cell counts. This was done by towing a plankton net (25 μm mesh) horizontally across the entire exposure site to obtain a representative concentrated cell sample. This sample was further concentrated by standing for two to four hours. The scum was siphoned off, frozen and subsequently freeze-dried. Toxicity was determined by mouse bioassay, by intraperitoneal injection of a water extract of these freeze-dried cells at a single dosage of 500 mg dried cells per kg. This dose threshold of 500 mg/kg is effectively regarded as the lower limit for mouse toxicity testing of crude cell extracts in this laboratory. Mice were observed for 24 hours and sacrificed for post-mortem if they had not died beforehand. Symptoms of death and gross organ damage at autopsy were used as indicators of the mechanism of acute toxicity (that is, hepatotoxicity or neurotoxicity).

We used SPSS for tabulations and bivariate comparisons of the follow-up rates, demographic data and the incidence of the different types of symptoms observed during follow-up.[19] Five separate exposure variables were examined: water contact; duration of

water contact; cyanobacterial cell density; a composite measure of time in the water and cyanobacterial cell density; and toxin presence. We used GENSTAT for logistic regression modelling, using the marginal method of Breslow and Clayton, applying the generalised linear mixed-model procedure to take account of clustering of participants in household groups.[20,21] Adjustment was made for age, sex and swimming in the follow-up period. Analyses were conducted initially including all participants who had been followed up, and then on those who remained after the exclusion of people who experienced symptoms or had engaged in recreational water activities in the five days prior to initial interview.

Results

Of 1029 people approached by interviewers, 921 (90 per cent) participated in the study. About two-thirds of the participants were male, a proportion that was similar for nonparticipants. This male-to-female ratio was fairly consistent across all age groups, and the greatest number was aged between 20 and 29. The higher male-to-female ratio was also consistent across study sites. Of the 921 participants, 845 (92 per cent) took part in the second-day follow-up, and 852 (93 per cent) in the seventh-day follow-up interviews. Of the 852 participants who took part in follow-up, 75 did not have water contact on the day of interview and were considered not exposed. The remaining 777 with water contact were considered exposed, since cyanobacteria were present at all sites on the Sundays of interview.

Of the 852 participants who took part, 469 had water contact and 124 had symptoms (79 had both) in the five days prior to the study. This provided 43 unexposed and 295 exposed participants who had not experienced symptoms or engaged in recreational water activities in the five days prior to the initial interview.

The dominant cyanobacteria recorded on the survey days across all sites included *Microcystis aeruginosa, Microcystis* sp., *Anabaena* sp., *Aphanizomenon* sp. and *Nodularia spumigena.* Hepatotoxins were identified in concentrated samples at one site on two separate interview days, and were present at three other sites on one day only. There was no evidence of neurotoxins at any site.

Less than a quarter of the participants experienced one or more symptoms, the most common being cold and flu-like symptoms (Table 1). For each symptom, apart from eye irritation, there tended to be a higher rate of occurrence in the exposed participants. However, since the occurrence of each individual symptom was low, the presence of one or more symptoms was chosen as the outcome variable for comparative analyses.

In the two days after exposure, no significant differences in the occurrence of symptoms were found between the exposed and unexposed subjects, before and after exclusion of those who experienced symptoms or engaged in recreational water activities in the five days prior to the initial interview. As well, no significant trends were found for increasing symptom rates with increasing duration of water contact or cyanobacterial cell counts.

At seven days, after adjustment for age, sex, and swimming in the follow-up period, there was no significant difference in the occurrence of symptoms between the exposed and unexposed persons either before or after exclusion. However, there was a higher odds ratio (OR) for exposed participants after exclusion (OR = 1.87) than before exclusion (OR = 1.12) (Table 2, Model 1).

Prior to exclusion of previously exposed or ill subjects, a significant difference was not demonstrated between unexposed participants, those with up to one hour of water contact, and those with more than one hour of water contact. After exclusion, however, there was a significant trend ($P = 0.03$) of increasing symptom rates with increasing duration of water contact. (Table 2, Model 2).

To further investigate the dose–response relationship, participants were classified as unexposed, or exposed to water with cyanobacterial cell counts of less than 5000, 5000 to 20 000, 20 000 to 80 000, and

Table 1: Study of exposure to cyanobacteria: proportions of participants who experienced symptoms during the seven-day follow-up period

	Group							
	All participants (before exclusion)				After exclusion[a]			
	Unexposed[b] n = 75		Exposed n = 777		Unexposed n = 43		Exposed n = 295	
Symptom	n	%	n	%	n	%	n	%
Vomiting or diarrhoea	4	5	41	5	1	2	10	3
Cold and flu symptoms	8	11	92	12	3	7	28	9
Mouth ulcers	1	1	32	4	0	0	9	3
Eye irritation	2	3	14	2	1	2	3	1
Ear irritation	2	3	22	3	0	0	5	2
Skin rash	0	0	22	3	0	0	6	2
Fever	0	0	13	2	0	0	4	1
At least one symptom	14	19	178	23	4	9	56	19

Notes:
(a) After exclusion of those who had had recreational water contact or symptoms in the five days prior to the initial interview.
(b) Participants who did not have contact with water on the day of the interview, and were considered not exposed to cyanobacteria.

Table 2: Study of exposure to cyanobacteria: adjusted odds ratios[a] and 95% confidence intervals (CI) for four logistic regression models, before and after exclusion of participants who experienced symptoms or engaged in recreational water activities in the five days prior to the initial interview, for the reported incidence of at least one symptom during seven days' follow-up associated with exposure to water containing cyanobacteria, the duration of contact and cell density

Model	Group							
	All participants (before exclusion)				After exclusion[b]			
	n	Odds ratio	CI	Trend *P*	*n*	Odds ratio	CI	Trend *P*
Model 1: exposure								
Unexposed	75	1.00			43	1.00		
Exposed	776	1.12	0.60 to 2.07		295	1.87	0.68 to 1.54	
Model 2: duration of water contact								
Unexposed	75	1.00			43	1.00		
≤60 minutes	320	0.98	0.51 to 1.89		129	1.45	0.44 to 4.84	
>60 minutes	411	1.26	0.66 to 2.40	0.2	151	2.70	0.83 to 8.80	0.03
Model 3: cell density (cells/mL)								
Unexposed	75	1.00			43	1.00		
<5 000	267	1.05	0.54 to 2.06		89	0.92	0.30 to 2.81	
5 000–20 000	183	1.49	0.75 to 2.96		79	2.71	0.92 to 8.03	
20 000–80 000	260	0.80	0.41 to 1.59		80	1.43	0.47 to 4.30	
>80 000	66	1.49	0.65 to 3.42	1.0	47	2.90	0.95 to 8.88	0.04
Model 4: duration and cell density (cells/mL)								
Unexposed	75	1.00			43	1.00		
≤60 minutes, ≤5 000	102	1.00	0.46 to 2.18		31	0.55	0.10 to 2.95	
>60 minutes, ≤5 000	157	1.14	0.55 to 2.35		58	1.47	0.41 to 5.24	
≤60 minutes, >5 000	218	0.98	0.50 to 1.94		98	1.89	0.61 to 5.86	
>60 minutes, >5 000	251	1.34	0.68 to 2.63	0.3	93	3.44	1.09 to 10.82	0.004

Notes:
(a) Adjusted for age, sex, and swimming in the follow-up period, and accounting for clustering within families.
(b) After exclusion of those who had had recreational water contact or symptoms in the five days prior to the initial interview.

more than 80 000 cells per mL. These categories are somewhat arbitrary, but are considered broad enough to represent real differences in cyanobacterial cell density, taking into account the errors and low precision of cell density estimates by conventional counting techniques. These categories also allowed a comparable distribution of participants across categories, especially after exclusion. Prior to exclusion, there was no obvious trend to increasing symptom rates with increasing cell density. A significant trend ($P = 0.04$), however, was observed after exclusion (Table 2, Model 3).

To account for the combined effect of duration of water contact and cell density, unexposed participants were compared with those exposed for up to 60 minutes and for more than 60 minutes to water with up to 5000 cells per mL and to water with more than 5000 cells per mL. The cut-off of 5000 cells per mL was chosen because of the high level of symptom occurrence (OR = 2.71) with exposure to 5000 to 20 000 cells per mL. A significant trend of increasing symptom occurrence with increasing levels of exposure before exclusion was not found. After exclusion, however, a significant trend ($P = 0.004$) was observed, and participants exposed to more than 5000 cells per mL for more than one hour experienced a significantly higher symptom occurrence rate than the unexposed (Table 2, Model 4).

No significant association was found between the presence of hepatotoxins and symptom occurrence at two and seven days after exposure.

Discussion

We concluded that exposure to cyanobacteria during recreational water-related activities was associated with symptom occurrence. This association involved a significant trend of increasing symptom occurrence with increasing duration of water contact and with increasing cyanobacterial cell density. There was also a significant trend of a higher level of symptoms in association with longer duration of water contact combined with higher cyanobacterial cell counts. These trends emerged after exclusion of participants who had been ill or engaged in recreational water contact in the five days prior to initial interview. This suggests there were substantial background symptoms, in both exposed and unexposed participants, that were associated with prior water contact and illness. While exclusion of these subjects resulted in loss of power to discriminate categorically between different levels of exposure, it allowed a greater level of discrimination to demonstrate trends.

The durations of exposure experienced by participants in this study were not unusual among recreational users, and the cell densities in the water were not unlike those often present in waterways in Australia in the summer months. Under these conditions, the positive findings in this study pose a substantial concern for public health regulators in relation to user safety. Ressom et al. have suggested 20 000 cells per mL as an acceptable threshold for cyanobacterial cell contact,[22] and this level is recog-

nised by several regulatory authorities in Australia. However, we have shown a significant trend of increasing symptom occurrence, using a cut-off of 5000 cells per mL. When, then, should warning signs of potential hazards be posted, and even more importantly, when should signs be replaced by lake closure? These are important considerations, and our findings suggest that a threshold for exposure of 20 000 cells per mL may be too high.

The absence of a significant association between the presence of hepatotoxins and symptom occurrence is not unexpected, since the symptoms under study were not specific to liver injury. These symptoms are more likely to be related to the allergenicity of cyanobacterial cells, rather than the toxins they contain. However, on the basis of this study, we cannot exclude the hepatotoxins from being responsible for symptom development in some participants. The fact that participants were exposed to hepatotoxins is troubling, since it is possible these would have been ingested by some people in the course of their water-related activities.

This is the first study to examine prospectively the association between the presence of cyanobacteria and health under normal recreational conditions. The relatively low overall symptom rates in the exposed group may relate to a healthy-user effect. Eighty per cent of the subjects were aged under 40, and they were obviously active on the day of interview. Also, repeated exposure may have resulted in some level of tolerance acquired in this cohort over time. However, if symptoms were related to allergy, the low symptom occurrence rate might also be explained by a low number of sensitive individuals in the cohort under study.

The trends observed, together with the timing of symptoms and their biological plausibility, suggest the occurrence of symptoms was likely to be caused by exposure to cyanobacteria. The ability to detect trends at seven days rather than two days after exposure may indicate a delayed rather than an immediate allergic response. However, we cannot exclude the possibility that these symptoms may have been caused by other causative factors, for example, other microorganisms, that may have correlated with the presence of cyanobacteria.

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