Chapter 29: Epidemiology of cyanobacteria and their toxins

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Introduction

Epidemiology is defined as the study of the distribution and determinants of health–related states or events in specified populations, and the application of this study to the control of health problems (Last 2001). In this context, "study" includes observation, hypothesis testing, analytic research, and experiments. In turn, each of these methods has an increasing level of sophistication that provides results with differing strength of evidence linking human exposure and health outcome. The World Health Organisation and other agencies, including the National Health and Medical Research Council (NHMRC) in Australia, have developed a classification system for these levels of evidence based on rigor, quality and the minimisation of bias. The NHMRC's new pilot classification allows for studies about aetiology. The classification is tiered and extends from the strongest Level I, that obtained from a systematic review of all studies using a prospective cohort design (Level II evidence) to Level IV, that obtained from case series and cross–sectional studies (Table 1 right hand column). Such aetiological studies are different than intervention studies where the investigators have the opportunity to determine exposure. Intervention studies vary from case–series where the outcomes prior to and after some prescribed intervention are measured and compared, to randomised controlled trials and their systematic reviews (Table 1 left hand column). Of the intervention studies, randomised controlled trials are the gold standard, which by their very nature, are designed to minimise or eliminate bias and allow inferences about exposure and health related effects. However, little of the current evidence about the health effects of cyanobacterial exposure has been

obtained from intervention studies. This pilot classification is useful as it acknowledges that evidence in some instances, by the potentially hazard effects of some agents, cannot be derived from intervention studies, and provides a classification for non–experimental studies that supply evidence about aetiology. In this context, this paper examines the epidemiological evidence for cyanobacteria in the aetiology of adverse human health effects (essentially the right hand column in the table). The literature cited is not comprehensive, but has been selected to highlight the well–documented events and to sort the evidence they provide into their relative strengths.

Table 1 Designations of levels of evidence* according to type of research question

Level	Intervention	Aetiology †††
I *	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A prospective cohort study
III–1	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)	All or none §§§
III–2	A comparative study with concurrent controls: • Non–randomised, experimental trial • Cohort study • Case–control study • Interrupted time series with a control group	A retrospective cohort study
III–3	A comparative study without concurrent controls: • Historical control study • Two or more single arm study • Interrupted time series without a parallel control group	A case–control study
IV	Case series with either post–test or pre–test/post–test outcomes	A cross–sectional study

††† If it is possible and/or ethical to determine a causal relationship using experimental evidence, then the 'Intervention' hierarchy of evidence should be utilised. If it is only possible and/or ethical to determine a causal relationship using observational evidence (i.e., cannot allocate groups to a potential harmful exposure, such as nuclear radiation), then the 'Aetiology' hierarchy of evidence should be utilised.

* A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence.

§§§ All or none of the people with the risk factor(s) experience the outcome. For example, no smallpox develops in the absence of the specific virus; and clear proof of the causal link has come from the disappearance of small pox after large–scale vaccination.
(Adapted from NHMRC additional levels of evidence and grades for recommendations for developers of guidelines. PILOT PROGRAM. 2005. http://www.nhmrc.gov.au/consult/index.htm)

Case series (Level IV evidence)

Case series provide descriptive information about illness events that raise hypotheses about causation. However, they are not designed to test such hypotheses, and at best provide clues about causation to be further investigated using analytic study designs. There are a number of case series reports in the literature linking cyanobacterial exposure and adverse effects in humans.

Outbreaks of gastroenteritis were reported in Charleston, West Virginia, and in towns along the Ohio River after a period of low rainfall and increased bloom formation (Veldee 1931; Tisdale 1931). Approximately 15% of the 60000 residents of Charlestown were affected. It is likely that people were exposed to the bloom through contaminated drinking water.

In 1959, thirteen people became ill after swimming in a Canadian lake containing cyanobacteria that had recently been linked to a number of livestock deaths. *Microcystis* spp and *Anabaena circinalis* were identified in the stools of a doctor, who had accidentally ingested some of the water (Dillenberg et al. 1960).

On Palm Island just off north–east Australia, an outbreak of a hepatitis–like illness including gastroenteritis affected 138 children and 10 adults. The cause was initially considered a mystery (Byth 1980). It was later revealed that copper sulphate was used to remove an algal bloom from the drinking water reservoir, and the illnesses occurred a week later. It is likely that the copper sulphate lysed the cyanobacterial cells, releasing dissolved cyanotoxins that were not removed from the water by the usual treatment processes, leading to illness over the ensuing week.

While the above reports implicate ingestion as the principal mode of exposure, a British Medical Journal report described two severe pneumonia deaths and 16 cases of gastrointestinal symptoms in health military recruits after canoe training on water containing a bloom of *Microcystis aeruginosa* (Turner et al. 1990). Training involved carrying full packs and taking

part in canoe rolls. This report implicates inhalation and/or aspiration as an effective exposure route for adverse effects in humans.

Anabaena and *Microcystis* blooms were found in the newly constructed Itaparica Dam's reservoir that supplied drinking water to a community in Brazil in 1988. Two thousand people developed gastroenteritis leading to 88 deaths over 42 days (Teixeira et al.1993). Investigations implicated the water impounded by the dam as the source of the outbreak with toxins from cyanobacteria considered to be responsible.

While it is highly probable and we are all likely to agree that cyanobacteria and their toxins were responsible for these events, in the absence of proper control groups, quantification of ingestion and inhalation, demonstrated dose–response effects and the like, as epidemiological evidence, this is not considered strong proof of aetiology. Certainly this evidence does not meet epidemiological criteria for causality, does not lend itself to dose–response investigation and is not helpful in assisting in the development of safety guidelines for exposure.

Cross–sectional/ecological studies (Level IV evidence)

A cross–sectional study measures disease occurrence and exposure status at the same time in a given population. Sometimes the exposure status of individuals prior to or even at the time of the onset of the disease is not necessarily known (ecological study). In these studies, exposure to an agent is assumed, based for example, on known contamination of a water supply, but the individuals are not individually asked if they actually consumed the water. Lack of specific individual exposure information prior to the onset of illness limits causality inferences. However, such studies are certainly suggestive and raise issues and hypotheses for further analytic investigations.

An important such study implicating cyanobacteria in the development of liver damage in humans was carried out in Armidale, Australia. Significantly increased levels of the liver enzyme gamma glutamyl transferase (GGT) were found in blood samples from a population of people supplied with a drinking water source from the Malpas dam containing a bloom of *Microcystis* and treated with copper sulphate. These GGT concentrations were significantly greater than in blood samples from people supplied with drinking water from a different source. (Falconer et al.1983). The raised GGT coincided with the bloom which makes it highly suggestive that it was responsible (Fig. 1). The other liver enzymes were not reported to be

significantly different between samples from people with access to the different drinking water sources.

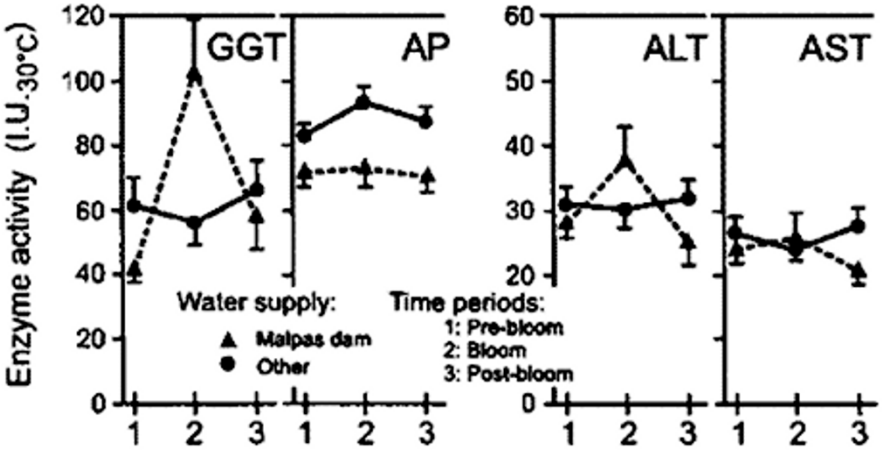

Fig. 1 Liver enzyme levels in blood samples from a pathology laboratory in Armidale, Australia, during a bloom of Microcystis aeruginosa. (GGT – gamma glutamyl transferase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; AP – alkaline phosphatase)

One hundred and fifty–six communities in South–Eastern Australia, providing 32,700 singleton live newborns during 1992–94 were studied to examine the link between potential cyanobacterial exposure through drinking water and birth outcomes. This study linked birth outcomes with reported bloom occurrences in water bodies providing drinking water to the communities involved. Cyanobacterial occurrence and cell density (alert level) in drinking water sources during the first and last trimesters and for the whole gestational period for each birth were used as estimates of exposure. There were statistically significant differences between the proportion of time during the first trimester with cyanobacterial occurrence and the percentage of births that were low birth weight (LBW) and very low birth weight. Significant differences were also found among various categories of first trimester exposure based on average cell density and LBW, prematurity and congenital defects. However, the pattern of these results does not suggest a causal link to cyanobacteria. There were no clear dose–response relationships even with such a large cohort. Analyses based on exposure during the last trimester and total gestation also showed no significant dose–response effects. The results of this study provide no clear evidence for an association between cyanobacterial contamination of

drinking water sources and adverse pregnancy outcomes (Pilotto et al.1999).

Yu (1995) reported that six large epidemiological studies have confirmed that populations receiving drinking water from pond–ditch water experience higher hepatocellular carcinoma mortality rates than populations receiving deep–well water. The findings from a study of water sources supported the hypothesis that microcystin in the drinking water of ponds–ditches and rivers, as opposed to deep–well water, was one of the risk factors for the high incidence of primary liver cancer in China (Ueno et al. 1996).

As before, although these studies suggest a link (or not) between cyanobacterial exposure and adverse health effects, they do not take individual exposure into account. The studies work on the assumption that the individuals involved did consume water from the affected water sources but we cannot be sure of this at an individual level. They were not individually asked, or if they were asked, contamination of the water by cyanobacteria (toxins) at the time of the study might not have been examined. Hence, while these studies are highly suggestive, they do not provide epidemiological proof of causality. They certainly raise the hypotheses for further investigation in humans.

Retrospective case–control study (Level III–3 evidence)

A case–control study is an analytic study that compares a group of people with disease to a similar group of people without that disease. Participants are selected into the study on the basis of having the disease. Controls are then selected based on certain inclusion criteria such as age matching. The levels of exposure to some agent each group had before appearance of the disease is then compared, and provides information about that agent being the likely cause for the outcome. Importantly, the exposure of the individuals involved in the study is known, and this information is usually obtained by questionnaire or other recorded information. Often, however, a question is raised about the accuracy of exposure information that relies on subjective recall. This may lead to recall bias, which has been identified as a major consideration to be taken into account when considering the results of case–control studies.

Along 8 Murray River towns in Australia, the risk of gastrointestinal symptoms was significantly associated with drinking chlorinated river water (RR 2.37; 95% CI 1.25, 4.49) during a period of raised cyanobacterial cell counts compared to rain water, and the risk of gastrointestinal (RR

5.20; 95% CI 1.13, 23.95) and skin symptoms (RR 5.21; 95% CI 1.01, 26.80) was associated with using untreated river water rather than rain water for domestic purposes (el Saadi et al. 1995). The width of the confidence intervals does raise doubt about the robustness of these findings. Also, the weekly mean log cyanobacterial count, although weakly correlated (r = 0.52) with the weekly proportions of patients presenting to medical practitioners with skin symptoms, this correlation was not statistically significant. This study was based on reports from general practitioners who supplied the researchers with the type of occurrence of illness, and the principal source of drinking and domestic use water, as well as recreational exposure to water. *Anaebaena* (some toxic), *Aphanizomenon* and *Oscillatoria* were the most common species present.

Prospective cohort study (Level II evidence)

A cohort study is an analytic study that involves identification of a cohort of people in which at least two groups can be identified, one that did receive the exposure of interest, and one that did not, and following these groups forward and comparing them for the outcome of interest. It is important in these studies that participants do not have the outcome of interest at the start of the study. This ensures that the risk factor occurs before illness, adding temporality in support of causality.

A prospective cohort study was conducted to investigate health effects of exposure to cyanobacteria as a result of recreational water activities. Participants aged 6 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 2 and 7 days later to record any subsequent diarrhoea, vomiting, flu–like symptoms, skin rashes, mouth ulcers, fevers, 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 between the unexposed, those exposed for up to 60 minutes and those exposed for more than 60 minutes (p = 0.03). A significant trend to increasing symptom occurrence was also found between unexposed subjects, and those exposed to water with cell counts of less than 5,000; 5,000 to 20,000; 20,000 to

80,000; and greater than 80,000 cells per mL ($p = 0.04$). Participants exposed to more than 5,000 cells per mL for more than one hour experienced a significantly higher symptom occurrence rate than the unexposed. *Microcystis* spp, *Anaebaena* spp, *Aphanizomenon* spp and *Nodularia* spp, some toxic, were identified. However, symptoms were not correlated with the presence of hepatotoxins, but might be due to direct contact with the lipopolysaccharide endotoxins on the surface of cyanobacteria. These results suggest increasing symptom occurrence was associated with increasing duration of contact with water containing cyanobacteria, and with increasing cyanobacterial cell density. The findings suggested that the currently accepted threshold for exposure of 20,000 cells per mL might be too high (Pilotto et al. 1997).

Another cohort study was conducted following an outbreak of liver failure at a dialysis centre in Caruaru, Brazil in the first half of 1996. One hundred and sixteen (89%) patients became ill (50 died from acute liver failure). Symptoms included visual disturbances, nausea and vomiting, headache, muscle weakness, epigastric pain, confusion, bleeding, fever and seizures. To examine risk factors for acute liver failure and death a case definition was established to allow comparison of risk factors for patients receiving dialysis at the city's two dialysis centres. A case was defined as any patient who had dialysis in either centre during February 1996 and who had acute liver failure. Results confirmed that all 101 case patients came from the same dialysis centre, and that centre had its water ("unfinished") supplied by truck from the municipal water–treatment plant as it was not linked to the water distribution system at that time. This water was not filtered or chlorinated. The nearby dialysis centre that recorded no cases received water from the same plant, but the water was sand filtered and chlorinated prior to being distributed to the water distribution system to which it was attached. Microcystins were found in the water reservoir, the delivery truck, the water holding tank and carbon and ion resin water treatment devices at the affected centre, and in the serum and liver tissue of case patients. No microcystins were found at the other dialysis centre. Unfortunately water samples from the time of likely exposure were not available, so it was not possible to quantify individual exposure of case patients (Jochimsen et al. 1998). So while this study provides good evidence for the acute systemic effects of microcystin exposure, it does not allow for an examination of dose response relationships that would contribute to safety guidelines.

Randomised controlled trial (RCT) (Level II evidence)

A RCT is an experimental study in which investigators randomly (usually using computer generated numbers) assign an intervention to a cohort of participants to form two groups, a treatment group and a control group, among which health outcomes can subsequently be compared. In some situations, such as with skin patch testing, it is possible to conduct a modified experimental study where patients become their own controls by randomly allocating a toxic agent, along with positive and negative control agents, to the skin of the same person. While not a true RCT where different individuals are randomised to receive different doses of toxin, both the participant and the person recording skin reactions remains blind to the position of the toxic agent.

A study using this approach on human volunteers was used to assess the skin irritant potential of a range of laboratory grown cyanobacterial species. Cell suspensions and extracts of cyanobacterial cultures of *Microcystis aeruginosa* (non–toxic strain), *Anabaena circinalis* and *Nodularia spumigena* were applied to 64 volunteers in one trial, and *Microcystis aeruginosa* (toxic strain), *Apanocapsa incerta* and *Cylindrospermopsis raciborskii* were applied to 50 volunteers in a second trial. Six cell concentrations of each organism in the range from less than 5000 to greater than 200,000 cells/mL were applied in random order using adhesive skin patches (Finn Chambers®). In addition, the applications included two treatments of each cyanobacterial species, involving whole and lysed cells, and positive (sodium lauryl sulphate) and negative (culture media) controls. Patches were removed after 24 hours and assessment of erythema was made by a dermatologist blinded to the species, cell type and concentration. On average, between 20% and 24% of individuals with 95% confidence interval ±8% reacted across the concentration range tested for these cyanobacterial species. The reaction rates were lower (11% to 15%) among the subset of subjects not reacting to negative controls. The reaction was mostly mild, and in all cases was resolved without treatment. This was the case for both whole and lysed cells with little difference in reaction rates between these two treatments. There was also no dose–response across the concentration range for any of the cyanobacterial species tested. Similar patterns of reaction were observed for atopic and non–atopic individuals. This study provides evidence that a small proportion of healthy people (around 20%) may develop a skin reaction to cyanobacteria in the course of normal water recreation, but the reaction is likely to be mild and resolve without treatment (Pilotto et al. 2004). From these results, skin irri-

tation is not readily translated into a quantitative guideline for recreational water activities.

Conclusion

The evidence clearly links cyanobacteria (toxins) to adverse heath effects, particularly gastrointestinal illness, liver disease, neurological effects, skin reactions and cancer in humans. Exposure is through ingestion, inhalation and/or aspiration and dermal contact. Unfortunately most of the research related to these health effects provides lower levels of evidence, which do not take into account individual exposure and/or exposure levels and lack associated control groups. Individual exposure information, coupled with other potential confounding information, allows an examination of the risks for such exposures to cause adverse effects. Case–control, cohort or randomised controlled trails are required to meet these criteria. However, the very toxic nature of cyanobacteria means they are not readily available for investigation at such an analytic level in humans. This then makes the current body of evidence very limited in its capacity to assist in the development of safety guidelines, especially for ingestion and inhalation. The adverse effects of such toxins essentially restrict their use in experimental studies (RCTs) of ingestion and inhalation in humans.

Opportunities do exist to further refine our knowledge in relation to dermal contact exposure. It would be possible to develop a protocol based on recreational exposure using a RCT design. Appropriate recreational sites could be identified and, with their consent, people attending these sites could be randomised into an intervention group that engages in a prescribed water–related activity and a control group that is provided with an alternate form of recreation. Ideally the control group activity should be water related but not in the water containing cyanobacteria. Thought would need to be given to the range of microorganisms that would need to be measured, other confounding factors that would need to be accounted for, the outcome variables to be recorded, and the sample size required to have confidence in the findings. Ethical considerations require that study sites would be restricted to those believed to be low risk for the occurrence of adverse human health effects. Such a study would overcome many of the biases inherent in cohort and case–control studies would allow an accurate estimation of individual personal exposure and would provide a stronger level of evidence of the effects of recreational exposure to cyanobacteria than currently exists.

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