

Reviewer's report

Title: Occupational and environmental hazard assessments for the isolation, purification and toxicity testing of cyanobacterial toxins

Version: 1 **Date:** 15 September 2009

Reviewer: Ingrid Chorus

Reviewer's report:

1. Does it address an important or timely issue? yes
2. Is it well reasoned? Yes:
3. Is it relatively balanced, or does it make plain where the author's opinions might not represent the field as a whole? Yes, it is well balanced.
4. Is the standard of writing acceptable? Yes, though the MS can be tightened by 10-20%: If condensed much more strongly, the MS would then lose its current charm of giving a nicely comprehensive information base and background for the assessment given for each potential exposure pathway and critically discussing the messages gleaned from the available studies. However, I suggest to move away from the current "essay" style (which gradually develops the scientific knowledge for each sub-title and then deduces protective controls) to a tighter structure within each chapter. It may help to start with a summary of the facts and uncertainties, including a statement on whether human exposure data are available or whether all we can currently do is to base conclusions on information gleaned from the animal experiment work, and then to move on (as the text now does) to underpin these conclusions by going through the available information on exposure pathways, and (iii) to derive the protective controls which are adequate both in face of our knowledge and in face of the uncertainties.

- Major Compulsory Revisions

The author must respond to these before a decision on publication can be reached.

none

- Minor Essential Revisions

The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

This is a very important and timely paper which comprehensively covers the range of conceivable exposure pathways and relates them to our current knowledge about cyanotoxins. It should definitely be published: not only may it have a major impact on the way cyanotoxins are handled in some labs, but - equally valuable - it will help create confidence in hazard analyses and risk assessments already performed in many labs.

The only criticism is that it is a bit wordy and repetitive and could be tightened by

10-20 %. If condensed much more strongly, the MS would then lose its current charm of giving a nicely comprehensive information base and background for the assessment given for each potential exposure pathway and critically discussing the messages gleaned from the available studies. However, I suggest to move away from the current "essay" style which gradually develops the scientific knowledge for each sub-title and then deduces protective controls to a more tightly structured style within each chapter. It may help to start with a summary of the facts and uncertainties, including a statement on whether human exposure data are available or whether we base conclusions mostly on information we glean from the animal experiment work, and then to move on (as the text now does) to underpin these by going through the available information on exposure pathways, and (iii) to conclude the protective controls which are adequate both in face of our knowledge and in face of the uncertainties.

Comments in detail are:

Introduction: should Microcystins be listed as potent tumour promoters, along with the others? Or is their potency less well established?

The sentence: "Figure 1 shows field collection of bloom biomass for the purpose of extracting cyanotoxins" leaves readers to guess what this means in relation to the previous sentences highlighting the greater risk from culture material.

Actually, comparing the 2 photos, it seems the field situation is likely to present a much higher exposure risk. How about embedding Fig. 1 along the following lines:

"Although heavy blooms as the one shown in Figure 1 may attain very high concentrations in the field, such situations are not frequently encountered by sampling staff, and lab cultures grown for toxin harvesting are likely to produce higher concentrations of specific toxins than are usually encountered in the field. Regular occupational handling of these may therefore present a greater human health risk should accidental exposures occur."

"These hazard assessment guidelines... ": which ones? "The hazard assessment guidelines presented in the following ..." Also, why the limitation "for research purposes"? what about staff in monitoring or in commercial toxin production (which may well be in rather small enterprises or semi-commercial settings, i.e. in university labs).

Separate the following into two sentences; the statements have little direct connection: "Toxic concentrations and doses will vary significantly depending on the particular cyanotoxin being processed and the exposure route, as will the expected health effects; in the absence of specific risk assessment guidelines for handling individual cyanotoxins, these recommendations should be regarded as preliminary and intentionally non-specific."

Oral exposure: this part is off balance: though the safety measures are fine, they are primarily important because of the risk of drowning and hypothermia, not because of acute intoxication to the point where I wouldn't be able to use my cell

phone! Seriously, this is overplaying the issue, particularly the discussion about the swallowing reflex in colder water. Certainly nobody should be out there sampling alone and without life jacket, but because of the risk of falling into the water in full gear and drowning ! This discussion needs to be (i) put in perspective and (ii) substantially tightened. The overall flavour might be that for sampling, safety measures to avoid falling into the water are primarily important to avoid drowning and near-drowning accidents. As experience shows a potential for discrepancies between theory and practice, staff needs to understand that workers surviving near-drowning involving substantial scum ingestion may additionally be at risk of suffering rapid neuro-intoxication or slower liver failure from cyanotoxins. Colleagues in such situations need to be aware of this risk in order to watch for breathing problems and make sure a sample for cyanobacterial and cyanotoxin identification is taken in order to assess whether liver functions may need to be monitored for several days following the accident.

Cutaneous exposure: This part is fine 1

First line p. 11: Why use "water column" and not just "water" ?

Bottom p. 12: "The toxicity of pelagic marine cyanobacteria is a poorly-understood and under-researched topic". Agreed, but will the readers know the meaning of "pelagic", and will they know that up to then, most of the discussion about marine cyanobacteria was about benthic taxa? The latter needs to be explicitly stated earlier, and here "pelagic" may need to be explained.

Bottom p. 13: "Harvesting littoral zone benthic cyanobacteria such as *L. majuscula* from beaches or rock ledges in strong surf conditions is fundamentally unsafe in any case, due to the risk of imbalance and potentially catastrophic immersion." I don't agree - not "in any case". Here, you do touch upon the issue of cyanotoxin risk in relation to other - higher - risks, and that is good. But I am confident that the risk of "imbalance and catastrophic immersion" (:)) I like the wording ! :)) can indeed be well controlled, thus these harvesting exercises need not be fundamentally unsafe in any case

Inhalational exposure risks (up to bottom p. 13): overall, I appreciate the attempt at a balanced assessment of the current state of knowledge. To have such a discussion is good and overdue, particularly as the scant evidence tends to be overplayed. Yet, particularly in face of the poor indication of risk, the chapter is too long. It could be tightened by starting out with stating that a few studies have suggested such a risk may exist, but none of them are very convincing. Then go through them, but a bit condensed, and conclude, as you do, that the little bit of evidence we have does not suggest a high risk, but due to lack of understanding of this hazard, avoiding sampling in spray situations is an adequate precautionary approach.

Harvesting and handling dry cyanobacterial material: how about increasing visibility of this important point by starting p. 14 with this separate sub-title?

Cultures: bottom p. 15: talking about vented, decontaminated air is fine, but a

simple measure is very important: make sure staff waits 5 minutes after switching off the aeration before opening a culture for cleaning or sampling, so that whatever aerosol the bubbling has generated has time to settle.

Why would inhalation be the highest risk when recovering a spilt culture? Does staff splash around so vigorously when cleaning it up?

Top of p.16: delete: "and use it for its intended purpose" (that may not at all be adequate for the intended purpose because a culture that has been on the floor may be contaminated with other stuff.)

Bottom p. 17: "will make an explosive spray inevitable" is overplaying it: stuff squirting out is not "spray" and not "explosive" - rephrase !

Middle p. 18: safe storage to prevent mis-use should be briefly mentioned here as well, in spite of the detailed discussion on p. 27 ff.

Handling and producing lyophilised material to me has always seemed the by far most relevant pathway for occupational exposure, as the dust may be very fine and fluffy - ready to fly through the lab at a breeze. I would like to see more emphasis on safe handling of this stuff, similar to the emphasis given earlier to the hazard collecting dry cyano-material. I always tell folks in my lab that if they handle this carelessly, the cleaning staff will be the ones to suffer exposure as well when they sweep it up without having a clue that the dust contains toxins. (For glassware, you state this, but to me it seems most important with respect to the "dust".)

Table 1: for "Concentration, lyophilisation, powdering" add "inhalation exposure" ! and for "laboratory culture" also add "inhalation exposure if cultures are bubbled".

Concluding remarks: How about something along the following lines: "Exposure scenarios in cyanotoxin monitoring and research strongly depend on the type of work done. As with all occupational safety exercises, the efficacy of protection measures not only depends on their adequate choice, but in particular on their acceptance by the staff that needs to use them. This is best achieved by activating staff interest and engagement in workshops identifying hazards from potential exposure pathways in the respective lab, discussing the risk of these pathways to occur and the potential for controlling the risk by safety measures as well as documenting the outcome of such workshops in lab-specific safety memos. It is important that the risk of exposure for third parties such as lab cleaning staff or the aquatic environment be included in the analysis."

- Discretionary Revisions

These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

Please note that both the comments entered here and answers to the questions below constitute the report, bearing your name, that will be passed on to the authors and published on the website if the article is accepted.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.