Filename: Public Comment on Two Pilotto et al References as they relate to Cyano cell counts Critique Revised 2020 Version 1.0.

Critique of the relevance of two references used by several management agencies, including UDWQ to justify cyanobacteria cells counts as it relates to human health.

Public Comment

To:
Utah Division of Water Quality, Salt Lake City, UT

From:
David C. Richards, Ph. D.
OreoHelix Ecological
Vineyard, UT
Phone: 406.580.7816
Email: oreohelix@icloud.com

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Introduction

Utah Division of Water Quality (UDWQ) has requested public comment on its "Utah Updated 2020 HAB Guidance document (https://deq.utah.gov/water-quality/utah-updated-2020-hab-guidance). The document was based on several agencies' guidance and guidelines for recreational use during cyanobacterial blooms including CWQMC (2016), EPA (2019), OHA (2019), WHO (1999), and WHO (2003). These groups cited dozens of references to help determine cyanobacterial guidelines and several, including UDWQ, relied on papers by Pilotto et al 2017a and Pilotto et al. 2017b. Although UDWQ may have decided not to use these references in their current guidelines, UDWQ's 2016 draft IR cites these papers and I assume UDWQ still relies on these papers. In a previous public comment, I reviewed the Pilotto et al 1997a paper and had some major misgivings concerning their conclusions that needed to be addressed. In this public comment I will review that paper once again and also review and critique Pilotto et al 1997b.

In short, neither the Pilotto et al. 1997a or 1997b papers provide support that cyanoHABs have detrimental effects on human health. These reports were either poorly analyzed or their observed effects were no more severe than simple allergies common to 30% or more of the population and that minor effects are very short lived. I recommend that UDWQ not base its cyanobacteria guidelines on the findings of these two papers and that UDWQ critically evaluate other papers that they use in their proposed guidelines.

My critique on two Pilotto et al. 1997 papers is as follows:

1. Pilotto et al. 1997a. "Health effects of exposure to cyanobacteria (blue–green algae) during recreational water related activities."

As is stated, I have already reviewed this paper in a previous public comment to UDWQ. Here, I will add additional review and critique.

Symptoms vs. exposure (Table 1)

No analyses were conducted by Pilotto et al. 1997a to determine statistical significance between the different symptoms in their Table 1 (Table 1). The authors only reported percentages; hence no conclusions can be made from this table regarding exposure effects. The authors could have easily conducted statistical analyses that included randomized resampling of the data that provided error rates (e.g. confidence intervals) and allowed for more rigorous statistical examination of effects.

One major problem was the very small sample sizes used with unexposed compared to exposed in both groups (Table 1). There were more than 10 times the number of exposed participants compared with unexposed participants in the "All participants' group and almost 7 times as many exposed participants compared to unexposed participants in the "After exclusion" group (Table 1). Statistical conclusions based on such uneven sample sizes need to account for this bias, especially when concluding significant effects of only one or two percentage points

(Table1). Without a statistical test, given the likely random error in any of the eight symptoms and the very uneven sample sizes of the unexposed group compared with the exposed groups before and after exclusion (Table1), no valid conclusions could have been made by the authors. However, if they would have performed a simple Kruskal-Wallis rank test (with ties) on the percent unexposed after exclusion vs the percent exposed after exclusion the test would have resulted in a $\chi^2 = 2.68$ with 1 d.f. and a $p = 0.10$ (with ties). These results would suggest, statistically, that there was likely no strong exposure effect and much less confidence in concluding that there was an effect.

Also, management agencies or the public could unwisely conclude that exposure to cyanobacteria may actually reduce eye irritation based on a superficial examination of their Table 1 (Table 1). In their Table 1 (Table 1), eye irritation decreased by 1% after exposure in both the 'All participants' group and the 'After exclusion' group. Such small changes in percentages should always be evaluated with caution.

Table 1. Table 1 from Pilotto et al. 1997.

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

| Symptom | All participants (before exclusion) | | | | After exclusion[a] | | | |
| | Unexposed[b] n = 75 | | Exposed n = 777 | | Unexposed n = 43 | | Exposed n = 295 | |
	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.

As another example, ear irritation, as with all of the types of symptoms listed in Table 1 (Pilotto et al 1997), can also be caused by other factors. Swimming in the ocean or a swimming pool often causes ear infections regardless of whether cyanobacteria are present or not. Obviously, someone who didn't swim would likely have not gotten ear irritation as opposed to someone who went swimming anywhere prior. A more useful study would have included participants who had recreational water contact in non-cyanobacteria waters (e.g. swimming pools, ocean, etc.) In all likelihood a significant proportion of the population that went swimming in a public pool free of cyanobacteria or in the ocean would have exhibited some of these symptoms (in the Pilotto et al case > 2%, Table 1).

Here is a quote from WebMD (www.webmd.com) titled, "Beware of Recreational Water Illnesses":

> "Recreational water illnesses refer to any illness or infection caused by organisms that contaminate water in pools, lakes, hot tubs, and oceans, resulting in diarrhea, skin rashes, swimmer's ear, and other conditions. And they are on the rise. The rate has more than doubled in the past 10 years, according to data from the CDC. Infection-producing germs that can lurk in water include Pseudomonas aeruginosa, which causes swimmer's ear (an infection of the outer ear canal, known medically as otitis externa) and skin rash (dermatitis). Others include cryptosporidium, Giardia lamblia, shigella, and E. coli, which can cause diarrhea. Each year, 10,000 RWI cases of diarrhea and 6.2 million cases of swimmer's ear occur, according to the CDC. "You can catch respiratory illnesses and colds but by far, skin rashes, swimmer's ear, and gastrointestinal bugs are the most common," he says. Diarrhea may occur when contaminated water is swallowed and driven into the mouth or nose, Greene explains. It may not begin immediately after a swim; sometimes it comes on one to two weeks later".

Symptoms listed in Pilotto et al 1997a, Table 1 need to be carefully reevaluated by UDWQ for usefulness and relevance to cyanobacteria blooms.

Odds Ratios[1]

There was an apparent error in the odds ratios reported for Model 1: exposure, after exclusion (Table 2 Pilotto et al 1997) (Table 2 this comment letter). The upper 95% CI value was reported as 1.54 which was less than the mean value reported of 1.87 (Figure 1 in this critique). Given that there were twelve authors in Pilotto et al. 1997a and likely several reviewers before publication, this obvious error should have been corrected. Unfortunately, this error in Model 1: exposure for the "after exclusion" group invalidates its use.

Table 2. Table 2 from Pilotto et al. 1997.

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[1] Odds ratio = (exposed with symptoms/not exposed with symptoms) ÷ (exposed without symptoms/not exposed without symptoms)

	Group							
	All participants (before exclusion)				After exclusion[b]			
Model	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.

Model 1
from Picotto et al. 1997

Figure 1. Mean and 95% CIs of odds rations for Model 1: unexposed vs. exposed. There were no statistically significant differences between unexposed and expose (i.e. 95% CIs of exposed overlapped the unexposed odds ratio of 1.00. Also, there was an editorial error of the mean and upper 95% CI for exposed group that was not adjusted for in this figure. The odds ratio scale was modified to scale for values < 1.00.

I created figures of the odds ratio data reported by Pilotto et al 1997a for Models 1, 3 and 4 to illustrate the shortcomings of their analyses (Figure 1, Figure 2, and Figure 3). Predictor and

response variability were so great that none of the 'treatment' odd ratios in any of the 4 models was significantly different than the control, 'unexposed' group or each other, except for one. All of the odds ratio's 95% CIs include values < 1.00, except for the Model 4; '> 60 minutes, >5000', 'after exclusion' treatment. Thus, there is no evidence that any of the categories in any of the models except one, differed in exposure effects including the control (Figures 1 -3 in this critique) (Table 2, Pilotto et al. 1997a).
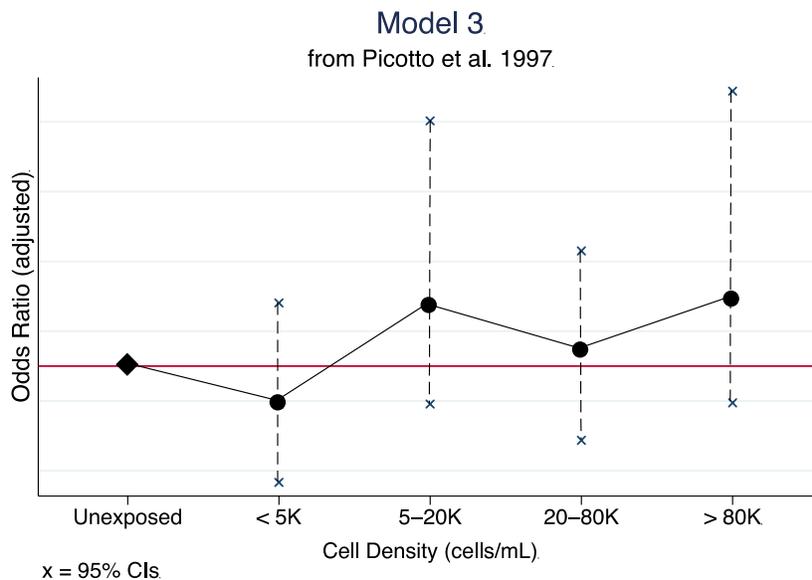
Figure 2. Mean and 95% CIs of odds rations for Model 3: unexposed vs. exposed to four different cyanobacteria cell count densities. There were no statistically significant differences between the unexposed group and any of the exposed treatments or between any of the exposed treatments (i.e. 95% CIs of all the exposed treatments overlapped the unexposed odds ratio of 1.00). Obviously, treatments were categorical with varying ranges of values within and between treatments which demands categorical response type statistical analyses. Note: Odds ratio scale was modified to scale for values < 1.00.
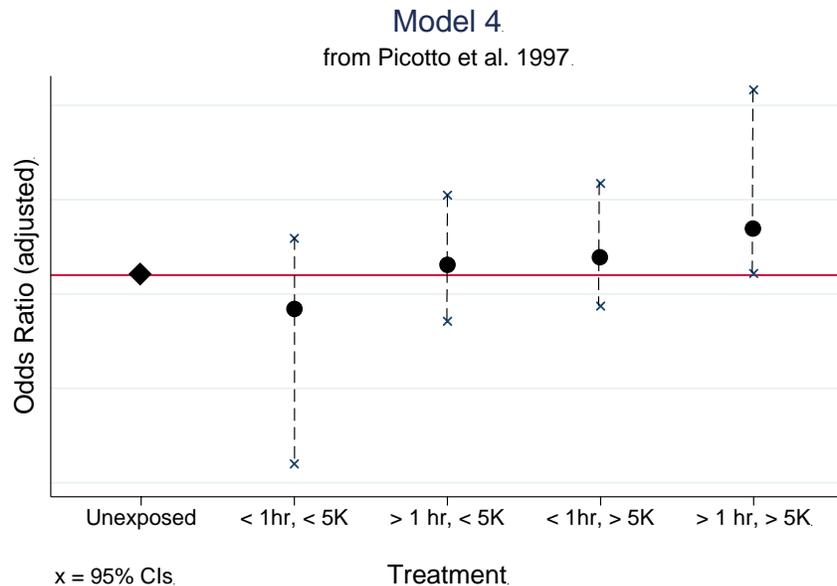
Figure 3. Mean and 95% CIs of odds rations for Model 4: unexposed vs. exposed to four different cyanobacteria cell count densities and durations. There were no statistically significant differences between the unexposed group and any of the exposed treatments or between any of the exposed treatments, (i.e. 95% CIs overlapped the unexposed odds ratio of 1.00), except for the treatment: > 1 hr., > 5K cells. No trend line was added to this figure because all treatments were categorical and not ordinal. In. particular, there was no basis for grouping the > 1 hr, < 5K before the < 1 hr, > 5K exposure other than post hoc superficial results. Note: The odds ratio scale was modified to scale for values < 1.00.

Improper trend analysis

Even though the 'after exclusion' analysis for Models 2, 3, and 4 (Table 2, Pilotto et al 1997a) showed statistically significant trends (P < 0.05), their medical exposure relevance is questionable in Models 2 and 3 and possibly irrelevant in Model 4. For example, in linear regression models, the well-known and easily interpretable R^2 value indicates how well the model fits that data and how well the predictor affects the response variable. However, R^2 values can be very small and still be statistically significant. As an example, an R^2 value of 0.1 or even less can be statistically significant but have very poor predictability or of no use determining exposure/medical relevance. P- values associated with R^2 values are a measure of whether the linear prediction line (slope) differs than zero slope. Even a slight deviation from zero slope can result in a significant p-value. Error rates of odds ratio trend p-values can also be calculated via Monte Carlo simulation but were not done in the Pilotto et al. 1997a report. It is safe to assume that some percentage of Monte Carlo type simulations of their data would result in statistically significant decreasing symptom trends with increased exposure in contrast to their results that showed increased risk. Additional analyses such as ANOVA type treatment effects models might also be appropriate and help determine the magnitude of the effects.

Model 4 (Table 2, Pilotto et al 1997a) reported trend is mostly irrelevant because the combination of duration and cell density response categories, as listed, have not been determined to be increasing exposure risk, particularly the two categories; '> 60 minutes, <

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5000 cells' and '< 60 minutes, > 5000 cells'. There was no *a priori* knowledge of whether duration (< 60 minutes, > 60 minutes) was a greater risk than cell density (< 5000, > 5000 cells). Trend analyses of odds ratios are dependent on increasing risk categories (ordinal). The four categories used in Model 4 were not entirely ordinal categories; that is there was no clear ordering of two of the five variables. If the two categories in question are kept in the analysis, then the odds ratio analysis for Model 4 technically should have been based on categorical response values not ordinal. Odds ratio trend analysis is not a viable method for categorical values. This is somewhat of a minor criticism but the over reliance on trend p-values to make conclusions and ignoring the magnitudes or lack of significance within and between the treatments themselves is important and discussed in the paragraph above.

Also, no interaction effects between exposure duration and cell density were reported by Pilotto et al. 1997a. It is unclear from Table 2 if there were interaction effects.

If we follow Pilotto et al 1997a's lead based on their odds ratio analysis presented in Table 2 (illustrated in Figures 2 and 3); we can conclude that cyanobacteria exposure for > 0 but < 1 hour and concentrations > 0 but < 5000 cells/mL are beneficial and can reduce illness; almost two times better than without exposure. Pilotto et al. 1997a results would be an additional multiple line of evidence that low exposures of cyanobacteria are a health benefit consistent with the health food industry's stance that cyanobacteria (i.e. blue green algae) supplements are beneficial and along with many other types of vitamin and nutrient supplements, low dosages are beneficial while higher dosages are not. If we are to assume that Pilotto's conclusion that high exposures have a negative effect on health, then we also have to assume that low exposures are beneficial.

In addition, if we ignore confidence intervals, the greatest difference in adjacent odds ratios in Model 3 occurs between < 5K and 5-20K cells/mL (Figure 2 this critique). At < 5K cells/mL cyanobacteria seems to be beneficial and reduces symptoms but at 5-20K there is a > 2X negative affect of cyanobacteria. This phenomenon occurs in Model 4 also. As stated in the preceding paragraph, < 5K cell/mL for < 60 minutes improves health by almost 2X but at the same concentration has negative health effects after 60 minutes of exposure.

Also, the proper method for evaluating trends using ordinal (ordered categorical) variables would be ordinal regression. Because Pilotto et al 1997a based much of their conclusions on odds ratios; ordinal logit or ordinal probit would have been the proper statistical method employed. Ratios typically are not statistically modeled using standard linear regression because they do not follow normal distributions.

2. Pilotto et al. 1997b. "Health effects of exposure to cyanobacteria (blue-green algae) due to recreational water-related activities."

Even though several agencies cited by UDWQ include this paper in their literature cited and as additional lines of evidence of the negative effects of cyanobacteria on health, there is little support for this conclusion. I include the abstract from Pilotto et al. 1997b for reference:

Abstract

To assess the skin irritant potential of a range of laboratory grown cyanobacterial species using skin-patch testing on human volunteers. 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. A small proportion of healthy people (around 20%) may develop a skin reaction to cyanobacteria in the course of normal water recreation, but the reaction is mild and resolved without treatment.

As stated in their abstract, even after 24 hours of direct skin exposure; reactions were mild, resolved themselves without treatment, and no dose response across concentrations or cyanobacterial species tested. Obviously, no recreationalist would ever expose themselves to direct contact with high concentrations of cyanobacteria for 24 hours. Pilotto et al. 2017b concluded that a small proportion, around 20% may develop an easily resolved and mild skin reaction. I interpret the meaning of their conclusion that exposure is not a problem. In addition, a potential 20% of the population having a mild skin reaction to cyanobacteria exposure is in line with percentages of individuals with allergies in general. The percentage of adults who have allergies in the U.S. is around 30%, while the percentage of children with allergies in the U.S. is around 40% (https://www.webmd.com/allergies/allergy-statistics). Or in other words, it should be expected that a low proportion of the population that have allergies could also have a slight allergic reaction to cyanobacteria just like they would to hay fever from other plants and allergens.

Conclusion

In Pilotto et al. 1997a, the experimental design and categorization of predictor and response variables resulted in such high variability compounded by the improper use of statistics to make any conclusions as to the negative health effect of cyanobacteria exposure highly suspect and mostly just speculation, other than that Pilotto et al. 1997a showed no significant differences between non exposure and exposure treatments (categories). Improved experimental design and the most appropriate statistical evaluation may or may not have resulted in detection of

significant cyanobacteria exposure effects but based on results presented in their paper, this would be highly unlikely.

The Pilotto et al. 1997b paper showed nothing more than that a proportion of the public that is likely already susceptible to allergies may also have very slight allergies to cyanobacteria if exposed for an unreasonable amount of time.

There is real cause for concern on my part that when management agencies rely on a single study or several studies to develop important guidelines, such as cyanobacteria exposure values, *without a thorough evaluation of methods and results of those papers* that erroneous values will be applied that in this case, will likely negatively affect recreational use and other parts of Utah's economy and encourage more scrutiny of water quality agency's decisions purportedly using the best available science.

I suggest that UDWQ eliminate both Pilotto et al. references as part of their multiple lines of evidence for cyanobacteria guidelines. In addition, those additional papers that had the most influence on UDWQ's guidelines need to be more thoroughly, rigorously, and critically evaluated. One approach would be to conduct a formal meta-analyses based on data obtained from a more exhaustive literature review and by critically evaluating and weighting those studies prior for use in meta-analysis or in criteria development.

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