PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Lung function in volunteers before and after exposure to trichloramine in indoor pool environments and asthma in a cohort of pool workers
AUTHORS	Nordberg, Gunnar ; Lundstrom, Nils-Goran; Forsberg, Bertil; Hagenbjork Gustafsson, Annika; Lagerkvist, Birgitta; Nilsson, Johan; Svensson, Mona; Blomberg, Anders; Nilsson, Leif; Bernard, Alfred; Dumont, Xavier; Bertilsson, Helen; Eriksson, Kare

VERSION 1 - REVIEW

REVIEWER	Lars Barregard, Dept Occupational and Environmental Medicine, Sahlgrenska University Hospital and University of Gothenburg. Competing interests: Some ongoing collaboration with Nordberg and Forsberg in other areas, but no co-authorship.
REVIEW RETURNED	22-May-2012

RESULTS & CONCLUSIONS	Do the results answer...? The objectives are stated as: " To perform...". I assume that the research questions are: 1) Are there changes in lung function or pulmonary epithelial integrity after exposure..." and 2) Does swimming pool work cause respiratory symptoms and/or asthma? Then I would say that Q1 has been answered but not Q2. Are the interpretation and conclusions ...?" I would answer yes to the experimental study results biut no the conclusion about asthma (in the Conclusions on p. 20 and in the abstract)
GENERAL COMMENTS	This ms reports the findings of two studies: 1. The first one is an experimental study of airway effects of 2 h exposure to swimming pool air (37 healthy persons and 14 pool workers). Spirometry and serum pneumoproteins were assessed before and after exposure in a swimming pool setting, as well as before and after a 2 h visit in a control setting (chamber with filtered air). Moderate exercise was performed during both rounds. Measurements of trichloramine (NH3Cl; the compound suspected to cause airway effects) were performed during exposure and (for comparison) also in a number of other swimming pools. The authors found a slight but significant decrease of FEV1 after exposure in the swimming pool setting compared to filtered air, but no difference between swimming pool air and filtered air for pneumoproteins (CC16 and SP-D). 2. The second study is a case control study of self-reported asthma

among persons who had been employed at swimming pools. About 1100 (out of 1740 who according to a census had such employment) answered a questionnaire. Exposure (self-reported hours in pool areas) was classified (0, 1, or 2) in 44 self-reported asthma cases and 128 controls. The authors found an odds ratio of 2.3 (not statistically significant) for those with highest exposure. The prevalence (probably) of self-reported asthma among the responding swimming pool employees was also compared with that of a general population sample.

Assessment

The issue is interesting, important, and subject to an ongoing debate.

The design of the experimental study is good and straight-forward with established outcome measures, and probably a reasonable power. The epi part is weaker, but it is nice to combine two techniques in the same ms, and I know of no previous effort to study this occupational group.

I have a couple of concerns, suggestions, and questions:

<u>Spirometry</u>

1. In Table 1, the mean changes (∆-values, post minus pre) are shown , and p-values in the footnote show whether these ∆-values are significantly different from zero. But the most important metric is the mean (paired) difference between changes after pool air vs filter air (∆ pool air minus ∆ filtered air). The p-values for these difference are given in the footnote (p=0.01 and 0.004). I suggest that the authors add a final column showing this "difference in changes" in Table 1. I suggest the same for Table 2. In this Table p-values for the differences in changes should be given, as in Table 1.

<u>Pneumoproteins</u>

2. There is no information about the time of the day the experiments were performed. If the exposed and unexposed sessions were in the same time of the day for each subject, then there is no need for adjustment for diurnal variation of the pneumoprotein levels, and the authors should use the original unadjusted levels. If sessions were not at the same time of the day within individuals, this is a limitation, and should be commented upon. The reason is that the equation used for correction for diurnal variation (Helleday 2006) of CC16 includes a considerable uncertainty. Although the Helleday paper has the best empirical dataset published (18 individuals and 6 time points per day x 2) the confidence intervals for changes over the day range about 1.5 µg/L, which might be more than the expected mean changes of exposure. Our own studies on CC16 also show the clear diurnal variation with decreasing levels over the day, but not fitting the Helleday equation very well. As the authors point out also SP-D

	has a clear diurnal variation, which is not taken into account in the ms. Therefore, if exposure and control sessions were matched in time – pls use the unadjusted pneumoprotein levels. If not, discuss this limitation and possible consequences for results. 3. The authors analyze the CC16corr levels and note that a) for group A levels are significantly higher before exposure to pool air than before filtered air, b) that baseline and post-exposure levels are higher for group A than for group B and c) that group B increase their levels over time but group A decrease them over time. But (as commented in point 1 above) the most important metric is the mean (paired) difference between changes after pool air vs filter air (∆ pool air minus ∆ filtered air). The authors tell us that there was no significant difference in changes between pool air and filtered air (p.13), but it is not clear whether this applies to both groups (A and B). Pls clarify. Also for SPD, there was no difference in changes after pool air vs filtered air (p. 13). Pls indicate whether this was the case for group A as well as for B. 4. The authors discuss the finding of decreased CC16 over time in spite of the correction for time, and state that the correction may be inadequate. What about the effect of exercise? Could it be different in group A (mean age 25) compared to group B (mean age 40)? 5. The authors state that data on diurnal variation of SPD are not available. But diurnal variation with lower levels in the middle of the day than in the morning has been reported previously (Hoegh 2010, Stockfelt 2012). IgE 6. The meaning of the lines on IgE on p. 14 is unclear. It is OK to include IgE levels in the ms to describe the groups. But why compare with IgE levels in asthmatic in another study? It is well known that asthmatics (on average) have higher IgE than non-asthmatics. This comparison could be deleted. One line with the levels is enough. The epidemiological study 7. This is a matched case-control study. Why were the 44 asthma case matched to 128 controls? For example 3 controls per case would give 132 controls. 8. Pls state how asthma cases were defined, and whether also as childhood asthma was included. 9. During which period was the number of hours estimated? Last

	year? Or was it a cumulative measure? 10. In the first para on p. 14 (and on p. 18 in the Discussion section) incidence of symptoms are reported. The term incidence is normally used for a rate (cases per person-time), but the figures given are percentages. Are the figures cumulative incidence? If so, during a restricted time period (sometimes called “period prevalence”)? Pls clarify. And was this analysis performed in the total group (0,1, and 2)? 11. In the second para prevalence data are reported, e.g. 12.3 % self-reported asthma. Is this figure similar to what is called incidence in the first para? If prevalence – when? Last year? Or is it cumulative incidence? Does the question permit differentiation between adult-onset asthma and asthma in childhood? 12. The prevalence is compared with that of the RHINE study. This is less relevant than the internal comparison. The authors state that RHINE data are from 1990 (p. 10), but the questionnaire to pool employees was probably administered much later (which year?). In addition, the questionnaire sent to pool employees also included questions on exposure, and thus the comparison with the population study might be subject to recall bias. The comparison with RHINE data could be deleted. 13. In the third para the OR for asthma in highly exposed <u>after starting their pool work</u> is given. How many of the 44 asthma cases? 14. The forth para describes that the risk of asthma was <u>negatively</u> associated with number of hours worked – if they had worked in such an environment for >1 year. This is strange, given the fact that the OR was higher for those who had high exposure. Pls clarify. Was this analysis only performed in subjects from group 2? Or – if it included all groups, does it mean that the increased OR was due to asthma cases occurring after less than 1 year of pool work? 15. Asking for exposure and symptoms in the same questionnaire opens for recall bias, which could be the only or part of the explanation to some of the associations reported here. This is a classic issue in epidemiology and must be discussed. 16. Another classic issue which should be discussed is the likelihood of “healthy workers selection”. <u>All outcomes</u> 17. Please discuss the power of the studies (for spirometry, pneumoproteins, and prevalence/incidence of asthma). <u>Minor issues</u>

	18. References 11-14 seem to be incorrectly numbered. On p. 10 “less that” should be “less than”.

REVIEWER	Dr Joanna Szram Clinical Senior Lecturer Department of Occupational and Environmental Medicine National Heart and Lung Institute, Imperial COllege London
REVIEW RETURNED	06-Jun-2012

THE STUDY	I believe there are other limitations to address and the overall tone of the message should be rebalanced.
RESULTS & CONCLUSIONS	More information is required to aid presentation and i believe the interpretation and conclusions do need to be redrafted.
GENERAL COMMENTS	This paper examines the relationship between exposure to swimming pool environments on the respiratory systems in two ways: 1. following brief exposure in a small number of healthy volunteers (37 referents and 14 pool workers). Lung function and biomarkers were measured before and after a 2 hour exercise period within an indoor pool environment. a) Statistically significant - but small - changes in lung function were found FEV1 change in healthy volunteers - 50ml in pool air v 10 ml in filtered air; in workers the changes were larger in filtered air (50ml) than pool air (20ml). b) Chloramine levels were measured and compared to lung function: in healthy referents an exposure of 0.23mg/m3 was associated with statistically significant fall in FEV1 % predicted. In swimming pool workers similar results were found with exposure to 0.15mg/m3 – the median concentration across 10 pool environments was 18mg/m3. 2. comparison of swimming pool workers and referents (healthy unexposed adults) in a cohort study. 1102 of 1741 pool workers identified using census data returned a completed postal questionnaire. Asthma data were compared to a 1990 study carried out by one of the authors of this study; this allowed age and sex stratified data to be generated (the same questionnaire items were used in both studies). In a nested case-control study, 44 cases of self-reported asthma were compared to 128 age and sex matched controls without asthma were selected. Exposure was defined using work titles into 0-none (e.g. cashier at a swimming pool/leisure centre), 1-low (occasional poolside work e.g. manager) and 2-high (constant pool side work). a) A raised but not statistically significant risk of asthma within pool workers (adjusted for family history) compared to the referent population (12.3% v 8.1%; in logistic regression analysis with adjustment for age and sex this difference was not statistically significant. b) A higher prevalence was found in those with highest exposure levels (2) compared to the other two exposure categories; this result was statistically significant (OR 2.31 95% CI 0.79-6.74). The authors conclude that exposure to NCl3 may contribute to asthma development. This is an interesting field of research and the two studies are complementary.

	A few comments: 1. Whilst the lung function changes seen in the first study were statistically significant, they do not reflect clinically significant airway obstruction and are within the normal variability of repetitive FEV1 measurement (or “reproducibility”). 2. The changes in pool workers were greater when exposed to filtered air than in pool air – what is the explanation for that? 3. changes in lung function in healthy volunteers - 50ml in pool air was reported as statistically significant; in workers the changes were larger in filtered air (50ml) than pool air (20ml) and the former was reported as “small”. The difference is the same in absolute terms although the statistical analysis compares the mean difference so the second finding is not significant. Confidence intervals should be included to allow the reader to make sense of the statistical findings. 4. The statistical power of findings in group B appears to be due to the larger numbers in group A than B(n=37 in group A v n=14 in group B) as well as the ability to use paired t tests due to normal distribution of data in group A. 5. Exposure was unblinded – this is likely to affect the results, potentially more so in the workers than the controls. This is worth mentioning in the discussion. 6. In addition, exposure of the two groups were significantly different (SDs did not overlap at 95% CIs); this needs to be mentioned and the implications of this discussed. Epidemiological study: 7. Please state the response rate of the postal questionnaire study in the text and any measure of response bias that could (or could not) be made. 8. Please add the standard deviations for the ages of the two groups in the epidemiological study. 9. Appropriate tables should be attached for the epidemiological study, to help the reader make sense of the results, e.g. First sentence of the Epidemiological study section quotes a statistically significant reduction in time spent in the pool environment and the incidence of acute symptoms $p<0.01$ “logistic regression” – this gives no idea of the magnitude of the effect or the confidence, or the effect of adjustments. 10. Page 15 lines 3-10 (last paragraph of results). The first sentence does not make sense – consider revision or addition of numerical values to provide clarity for the reader (e.g. table as in point 8). 11. Confounders related to working as a swimming pool teacher/attendant should be discussed; individuals who are fit enough for these types of jobs tend to exercise more regularly and are more likely to notice and report respiratory symptoms and acquire a diagnostic label of asthma. The same is true of swimming in general and also of other sports. 12. What evidence is there (in the literature if not from the authors’ study) that NCl_3 is the only relevant exposure? 13. I would suggest the impact of the findings of the study as reported in the first sentence of the conclusion be toned down: the magnitude of change does not suggest a clinically significant effect. In particular the examination of causality of association is not robust (see Bradford Hill criteria). In general the findings have been rather overinterpreted – the differences seen are small in absolute terms and may be due to a number of other factors. The discussion should be redrafted with this in mind. Nevertheless this is an interesting paper with two different but complementary studies that merit consideration of publication.

VERSION 1 – AUTHOR RESPONSE

Reviewer Lars Barregard:
1) Tables 1and 2: a new column has been added showing “difference in changes”
2) Information is included that exposures started at somewhat different time of day (I e around 8 AM in some cases and around 10AM in other cases. This was necessary in order to use facilities efficiently and include an acceptable number of observations in the study. This information and the fact that the correction introduces an additional element of uncertainty is included on page 17 in the revised manuscript
3) Information that the lack of statistically significant differences apply for both Groups A and B is included on page 14
4) Concerning the influence of exercise, the same schedule of moderate exercise was used both in the studies forming the basis for the correction and in our present studies. The age factor between group A and B is discussed on page 17.
5. The reference showing diurnal variation is included and the text modified on page 18
6. The comparison with mild asthmatics has been removed.
7. We defined the age interval and other criteria for the controls and intended to find 3 controls per case. A few of the randomly selected controls did not fulfill the criteria and were therefore excluded. The resulting 128 controls were still considered to constitute a sufficient number and were used in this study.
8 and 9) ”Self reported asthma” was derived from a positive answer to the following question: “Do you suffer from asthma or have you suffered from asthma?” Whether the asthma started before or after he/she was hired as a pool worker was derived from the combination of questions about year hired as pool worker and time when the first symptoms of asthma occurred. Because the nested case-referent study was limited to workers with asthma occurring after they were hired as pool workers, childhood asthma was not included.Under the general heading “Acute symptoms when working in a swimming-pool environment” there was a question “How large a part of a working day did you usually spend in the swimming pool environment Hours [Swedish: Hur stor del av arbetsdagen brukar/brukade du befinna dig i simhallen? Timmar]” There was thus no specification of the time period when the assessment of “number of hours” should be done. It can be assumed that the workers meant number of hours during such periods when they regularly worked in the swimming pool environment, but it cannot be said to represent a time period like “during the last year” or cumulative ¨. This information is included on pages 9 and 10.
10) The term incidence is replaced by percentage, because this is the proportion of the total number of workers (regardless of belonging to exposure groups 1, 2 or 3) that reported such symptoms.
11 and 12) The comparison with RHINE is excluded according to the recommendation of the reviewer.
13) The exact number of cases and controls have been included in the revised paper (page 15)
14) The nested case-control study included all workers regardless of exposure time. It considered the type of exposure (based on job titles) and not the length of exposure. Because there was a tendency (not statistically significant) of lower risks of asthma with the number of years worked (above 1 year), it can be assumed that the increased OR in the total data set is probably most related to the shorter work histories.
15) Discussion included on page 19.
16) Healthy worker selection is discussed on page 19, 2nd para.
18) References 11-14 have been renumbered, thank you for pointing this out.

Reviewer Johanna Szram
1) Discussion of the findings in relation to clinical “obstruction” is found on page 16
2) The lack of differences in Δ values among workers are pointed out in the revised manuscript and new column with such values are included in Tables 1 and 2. A comment has been included in the abstract “(but no significant change of other measurements of lung function)” meaning that the statistically significant decrease in FEV% may not be so important

3) Standarad deviations showing the dispersion of values are found in Tables 1 and 2.
4) Yes, the findings in Group A are more robust than in Group B. This is now more evident in the revised manuscript.
5) D
6) Comment included on p 13
7) Included on page 9
8) Sd of ages included on page9.
9) The magnitude of findings of various acute effects given as percentages of responders reporting such symptoms.
10) Text modified, page 15
11) Comments about exercise, noting contribution to confounding included on page 20
12) Comment included on page 4
13) This sentence changed to:" For the first time in adults, statistically significant but small decreases in lung function were found in previously unexposed subjects after exposure to pool air containing 0.23 mg/m3 of NCl3 compared to filtered air."
14) The discussion, conclusions and abstract has been modified
On behalf of all authors,
Gunnar Nordberg

VERSION 2 – REVIEW

REVIEWER	Lars Barregard, consultant, professor, Dept Occupational and Environmental Health, Sahlgrenska University Hospital and University of Gothenburg
REVIEW RETURNED	17-Aug-2012

THE STUDY	In my previous review I recommended (point #17) that the authors discuss the power of the studies (spirometry, pneumoproteins, and prevalence of asthma). This has not been done, but the ms can be accepted anyway.

REVIEWER	Dr Joanna Szram Honorary Senior Lecturer National Heart and Lung Institute Imperial College London UK
REVIEW RETURNED	06-Aug-2012

- The reviewer completed the checklist but made no further comments.