



Lung function in volunteers before and after exposure to trichloramine in indoor pool environments and asthma in a cohort of pool workers

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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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Lung function in volunteers before and after exposure to trichloramine in indoor pool environments and asthma in a cohort of pool workers

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List of abbreviations:

- CC16: Clara Cell protein 16
- FEV₁: Forced Expiratory Volume in 1 second, liters
- FEV_%: FEV₁x100/FVC
- FVC: Forced Vital Capacity, liter
- NCl₃: Nitrogen trichloride or trichloramine
- OR: Odds Ratio
- RHINE: Respiratory Health in Northern Europe
- SPD: Surfactant protein D

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Summary:**1) Article focus:**

Exposure to trichloramine (NCl₃) in swimming-pool air is known to cause mucous membrane and pulmonary effects, but statistically significant changes in lung function among adults have not been reported.

Epidemiological studies of asthma among pool workers are not available.

2) Key messages:

In this study we found for the first time, statistically significant decreases in lung function in volunteers after exposure to pool air with commonly occurring levels of NCl₃.

We found a tendency towards a higher odds ratio (OR) for asthma in a nested case reference study within a cohort of 1102 pool workers.

Our findings support the notion that current workroom exposures of NCl₃ may contribute to asthma development.

3) Strengths and limitations: This is the first study showing small but statistically significant decreases in lung function after exposure to pool air. This is the first nested Case/Control study in pool workers. It reports an OR for asthma of 2.31 (95% CI 0.79-6.74) among pool workers with the highest exposure (after correction for heredity), but this finding did not reach statistical significance.

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ABSTRACT

Objectives: Exposure to trichloramine (NCl_3) in indoor swimming pool environments is known to cause mucous membrane irritation, but if it gives rise to changes in lung function or asthma in adults is not known. 1: We determined lung function in volunteers before and after exposure to indoor pool environments 2: We studied the occurrence of respiratory symptoms and asthma in a cohort of pool workers.

Design/Methods/Participants: 1. We studied two groups of volunteers, 37 previously non-exposed healthy persons and 14 pool workers, who performed exercise for two hours in an indoor pool environment. NCl_3 in air was measured during pool exposures and in 10 other pool environments. Filtered air exposures were used as controls. Lung function and biomarkers of pulmonary epithelial integrity were measured before and after exposure. 2. We mailed a questionnaire to 1741 persons who indicated in the Swedish census 1990 that they worked at indoor swimming-pools.

Results: 1. In previously non-exposed volunteers, statistically significant decreases in FEV_1 and $\text{FEV}_\%$ ($p=0.01$ and $p=0.05$ respectively) were found after exposure to pool air (0.23 mg/m^3 of NCl_3). In pool workers, a statistically significant decrease in $\text{FEV}_\%$ ($p=0.003$) was seen after exposure to 0.15 mg/m^3 of NCl_3 . In the 10 other pool environments the median NCl_3 concentration was 0.18 mg/m^3 . 2. Our nested Case/Control study in pool workers found an OR for asthma of 2.31 (95% CI 0.79-6.74) among those with the highest exposure. Exposure-related acute mucous membrane and respiratory symptoms were also found..

Conclusions: This is the first study in adults showing statistically significant decreases in lung function after exposure to NCl_3 . An increased OR for asthma among highly exposed pool workers did not reach statistical significance, but the combined evidence supports the notion that current workroom exposures may contribute to asthma development. Further research on sensitive groups is warranted.

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INTRODUCTION and OBJECTIVES

Mono-, di- and trichloramines are formed following a reaction between ammonia (NH₃) or other nitrogen containing substances present in swimming pool water when hypochlorite is used as a disinfectant. Trichloramine (NCl₃) is the most volatile chloramine and is emitted into the air of indoor swimming pools. Exposure to this substance was the suspected cause of outbreaks of short-incubation ocular and respiratory illness [1,2], but concentrations of NCl₃ in pool environments were not known in these outbreaks. It is known, however, that acute respiratory and eye symptoms may occur among recreational swimmers in relation to measured levels of NCl₃ in pool environments [3].

Only few and inconclusive studies have been performed on lung function among adults after exposures to measured levels of NCl₃ in pool environments [4,5] and additional studies are required.

Clara cell protein 16 (CC16) is an epithelial protective protein in peripheral lung tissue and changes in its serum levels are used as a biomarker of epithelial integrity [6]. It has been shown to be decreased in relation to frequency of pool attendance [7]. However, changes in serum levels of CC16 have not been studied after short term exposure to NCl₃.

Thickett et al 2002 [8] reported three cases of occupational asthma among British pool workers exposed to NCl₃. There is a lack of epidemiological studies on asthma among those working in swimming pool environments.

The objectives of the present study were 1. To perform a controlled human exposure study of lung function and biomarkers of pulmonary epithelial integrity in volunteers before and after

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1
2
3 exposure to indoor swimming pool environments. 2. To perform an epidemiological study of
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5 self-reported asthma and subjective symptoms in a cohort of indoor swimming pool workers
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DESIGN, MATERIALS AND METHODS**Air sampling and determination of NCl_3 :*****Exposure measurements in human exposure study***

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21 In the two pool environments where our study of volunteers and pool-workers took place
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23 hypochlorite was used as disinfectant. Air samples were collected in the breathing zone: one
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25 sample for each 2-hour exposure, in total 51 samples.
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Determination of NCl_3 at other indoor swimming pools:

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32 Additional determinations of NCl_3 were performed 2004-2008 at 10 different pool
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34 establishments (7 conventional ones and 3 “adventure water lands”) in northern Sweden with
35
36 totally 30 indoor pools. Hypochlorite was used as disinfectant. At each swimming-pool, air
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38 was sampled during 3 hours at 3 to 4 different locations in close vicinity of the pool. The
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40 equipment was mounted on a stand with the filter at a height of approximately 1.5 meter.
41
42 Sampling was performed on three different days during winter and three different days during
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44 summer.
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51 ***Air collection and Analysis:*** 1L/min of air was pumped through a filter (quartz filter QM-A
52
53 37 mm Whatman International Ltd., Maidstone, England). The filter was soaked in a solution
54
55 of sodium carbonate and arsenic trioxide (AsO_3) and dried as presented earlier [9]. When
56
57 NCl_3 is collected on the filter it is reduced to chloride ion (Cl^-) [9]. After sampling, the filters
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3 were extracted with 10 ml of ultra-pure water, shaken for 30 minutes and filtered through a 13
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5 mm syringe filter (IC Acrodisc®, PALL). The chlorides were analyzed in a suppressed ion
6
7 chromatography system (Triatlon 900 autosampler, Spark, The Netherlands); ICSep AN1,
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9 Anion column (CETAC, Omaha, USA); SCX membrane suppressor column (Sequant, Umeå,
10
11 Sweden); JD-21 conductivity detector (Costech Microanalytical Ltd, Tallin, Estonia)). The
12
13 eluent was 7.5 mM NaOH and the suppressor 5 mM H₂SO₄. Control samples of two known
14
15 chloride concentrations (0.5, 3.0 mg l⁻¹) and at least two blanks were run together with the
16
17 samples in each run. The chloride concentrations in the blanks were subtracted from the
18
19 concentration in the samples. The detection limits of NCl₃ (1.78 and 1.18 µg m⁻³ for 2 h and 3
20
21 h samplings, respectively) were determined as three times the mean standard deviation of the
22
23 amount collected on filters of 10 blanks. The limits of quantification (5.9 µg m⁻³ and 3.9 µg m⁻³
24
25 for 2 h and 3 h samplings respectively) were determined as ten times the mean standard
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27 deviation for the same blanks.
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Human exposure study

Study groups:

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41 Group A: 37 healthy subjects (20 men and 17 women, mean age 24.5 years). They were not
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43 regular swimming pool visitors and they had not visited a swimming pool within four weeks
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45 before study start.
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48 Group B: 14 workers at swimming pools (5 men, 9 women, mean age 39.9 years).
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52 All participants were non-smokers with normal lung function and had no history of allergy or
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54 pre-existing lung disease. Subjects were free of airway infection for ≥4 weeks prior to the first
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56 exposure and throughout the remainder of the study.
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Study design

The study was conducted in a crossover control fashion. Each volunteer was exposed to filtered air in an exposure chamber and on another occasion to an indoor pool environment. In the exposure chamber, located in a separate building away from swimming-pools, incoming air was adjusted to room temperature and filtered through a particle filter. The exposures were performed in random order. Successive exposures were separated by ≥ 2 weeks. The exposures (pool environment or filtered air) lasted for 2 hours. The study subject was exercising on a bicycle ergometer with moderate exercise (minute ventilation $20 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$), during 15-minutes followed by 15 minutes of rest, i.e. four periods of exercise and four periods of rest.

Lung function:

FVC and forced expiratory volume in 1 sec (FEV_1) was determined using a portable spirometer connected to a computer (KoKo Spirometer and KoKo DigiDoser; Pulmonary Data Service Instrumentation, Inc, Louisville, KY, USA), calibrated in the morning and after every 10th measurement. $\text{FEV}_\%$ was calculated as a percentage of FVC ($\text{FEV}_\% = \text{FEV}_1 \times 100 / \text{FVC}$). Lung function was measured immediately before and after exposure in a room with non-detectable levels of NCl_3 ($< 0.002 \text{ mg NCl}_3/\text{m}^3$) or in a room adjacent to the exposure chamber.

Blood sampling and determination of biomarkers.

We obtained blood samples from the antecubital vein at 0 h and 2 h, i.e. before and after exposure, and at 4, 6 and 8 hours. Peripheral blood was collected into BD Vacutainer tubes (BD, Plymouth, UK). Each sample was allowed to clot for 1-2 h at room temperature, centrifuged at $3,000 \times g$ and serum was transferred to cryotubes and frozen at -80°C . These samples were sent to the Industrial Toxicology Unit at the Catholic University of Louvain in Brussels (IUTUCL), Belgium for determination of Clara Cell protein 16 (CC16) and

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3 Surfactant Protein D (SPD). CC16 was determined by latex immunoassay using a rabbit anti-
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5 CC16 antibody (Dakopatts, Glostrup, Denmark) and CC16 purified at (IUTUCL) as standards
6
7 [10,11]. All samples were run in duplicate at two different dilutions. The between- and within-
8
9 run coefficients of variation range 5–10% and results are comparable with ELISA methods
10
11 [12]. SPD determinations were performed using the Biovendor ELISA kit (Biovendor,
12
13 Heidelberg, Germany). Analyses were done in duplicate as recommended by the
14
15 manufacturer.

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19 Total IgE was determined in human serum by a double antibody sandwich ELISA method
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21 (Human IgE ELISA kit, Immunology Consultants Lab; Inc, Newberg, OR). The quantity of
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23 IgE in the samples was interpolated from a standard curve.
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Statistical analyses

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33 All data from CC16 measurements were corrected for diurnal variation according to Helleday
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35 et al 2006 [13] and recalculated to correspond to 7 AM. $CC16(\text{corr}) = CC16 + 0.582 * T -$
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37 $0.032 * T^2$. T is the time after 7.00 AM when the blood sample was taken. Because CC16
38
39 values are highest in the morning [13], corrected CC16 values were somewhat greater than
40
41 measured values.

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44 Statistics: We used repeated measures analyses of variance (Huynh-Feldt corrected) with time
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46 and exposure as within-subject factors and group as between-subject factor. Paired t-test or
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48 Wilcoxon signed rank test was used when comparing exposures to filtered air and pool
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50 environment at baseline (0 hrs) and after exercise (2h). Median IgE values were compared by
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52 the Westenberg-Mood median test. SPSS version 17.0 was used to perform the statistical
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54 analyses. A p-value of 0.05 was considered statistically significant.
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Epidemiological study***Population:***

The epidemiological study group included 1741 persons in the Swedish Census of Population and Housing 1990 who had indicated that they worked at swimming pools. Early 2007 a questionnaire was mailed to them. There was one reminder.

Questionnaire: Questions dealt with time periods in various jobs, time spent in swimming pool environments, various symptoms from the respiratory tract and mucous membranes of the eyes and possible use of medication for asthma. 589 women and 513 men, age 30 ->80 years responded. Among 50 non-responders, interviews were performed via telephone. There was a lower prevalence of asthma and respiratory symptoms among the non-responders, not statistically significant.

In a nested case-control study within this cohort, 44 cases of self reported asthma occurred after the person was hired as a pool worker. 128 age and sex matched controls were selected within the cohort.

Exposure assessment:

Based on information on work titles given by each individual, exposure was classified into three different categories; 0, 1, or 2. 0 stands for no exposure, 1 for low exposure and 2 for high exposure. The exposure level is not an estimate of the concentration of NCl_3 in air but is based on the average time during a workday the individual spent in the pool area. Those within category 0 did not spend any time in a pool area, e.g. a cashier. A person within category 1 did occasionally spend some time in the pool area. A manager of a swimming pool or a technician belongs to this category. Individuals belonging to category 2 were those

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3 spending most of the workday in the pool area, e.g. a swimming teacher, or a swimming pool
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5 worker.
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Comparison data

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12 We obtained data on asthma in 1990 from the study “Respiratory Health in Northern Europe”
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14 (RHINE [14]) via one of the authors of the present paper (BF). As we used the same questions
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16 in the present study as in RHINE, it was possible to derive adequate sex and age stratified
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18 comparison data up to the age of 55.
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Statistics

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28 Fisher’s test was used to test differences between proportions. Conditional logistic regression
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30 was used for analyses in the nested case-control study and logistic regression for analyses of
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32 asthma in relation to years worked in swimming-pool environments. All statistical analyses
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34 were performed using the statistical package R, version 2.9.0 (www.r-project.org). P-values
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36 equal or less than 0.05 were considered statistically significant.
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Ethics

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46 The project was approved by the Regional ethical review board in Umea, Sweden (Dnr 05-
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48 044M) and volunteers provided written informed consent. The study was carried out
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50 according to the declaration of Helsinki.
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54

RESULTS**Air sampling.**

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Experimental exposure (Human exposure study)

The NCl₃ levels during the experimental exposures were

Group A: Mean 0.23 mg/m³ (SD 0.09)

Group B Mean 0.15 mg/m³ (SD 0.04)

Other Swimming pools

NCl₃ concentrations in air at the 10 different indoor swimming pool establishments were between 0.001- 0.77 mg/m³, median 0.18 mg/m³, arithmetic mean (AM) 0.21 mg/m³ (n=129). The AM concentrations of NCl₃ in each of the ten different pool establishments were between 0.09 – 0.32 mg/m³. There was no difference in NCl₃ concentrations during summer compared with winter conditions (results not shown).

Human exposure study***Lung function***

Group A:

Measured FEV₁ volumes among healthy volunteers as well as the difference before and after 2 hours of exposure to pool environment or filtered air are summarized in Table 1. There was a small, statistically significant decrease (p=0.01) in FEV₁ (mean decrease = 0.05 L) after exposure to swimming pool air. After exposure to filtered air there was a slight, not statistically significant increase in FEV₁ (mean increase 0.01 L). When comparing the differences (Δ-values) in FEV₁ before and after exposure to pool environment with the Δ-values for exposure to filtered air in the same individuals, the difference between Δ-values was statistically significant (p=0.01).

FEV_% values among healthy volunteers are also given in table 1. After exposure to pool air, there was a small decrease (0.8 FEV_%) that was marginally statistically significant (p=0.05).

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3 After exposure to filtered air, there was a small (statistically non-significant) increase in
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5 FEV_% values. When the individual differences (Δ -values) of FEV_% before and after exposure
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7 to pool air were compared with the corresponding Δ -values in filtered air, a statistically
8
9 significant difference was demonstrated ($p=0.004$, paired t-test). Airway obstruction is usually
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11 defined as FEV_% below 70 (www.goldcopd.com). Only one value was below 70 (after
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13 exposure) among the healthy volunteers.
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Group B

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18 In table 2, FEV₁ values for the swimming-pool workers are summarized. After exposure to
19
20 pool air there was a small and not statistically significant decrease in FEV₁, 0.01 L. There was
21
22 also a small decrease in FEV₁ after exposure to filtered air (0.05 L, $p=0.054$). When
23
24 considering the FEV_% values for the workers (Table 2) before and after exposure to pool air,
25
26 there was a statistically significant decrease of 1.36% ($p=0.003$). After exposure to filtered air
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28 the small decrease in FEV_% of 0.43% was not statistically significant. Only two FEV_% values
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30 among the pool workers (one before and one after exposure) were below 70.
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Biomarkers of pulmonary epithelial integrity:

Group A

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42 Mean CC16corr values and related standard deviations (SD) in previously unexposed healthy
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44 volunteers, are shown in Figure 1 for 33 of the participants in group A. For the remaining 4
45
46 persons, values were missing and they were therefore excluded from analysis.
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48
49 At baseline (0 hrs), mean CC16corr = 12.6 $\mu\text{g/L}$ before pool exp (0 h) and 10.3 $\mu\text{g/L}$
50
51 immediately before (0 h) exposure to filtered air. This difference ($p=0.018$, paired t-test) is
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53 difficult to explain because the same volunteers were exposed to both pool environment and
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55 filtered air and they were randomly assigned to either exposure.
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Group B

Results are shown in Table 1. The mean CC16corr was 6.5 µg/L before both pool and filtered air exposures.

The difference between groups A and B persisted during and after exposure (0-8 hrs) and is statistically significant ($p < 0.001$ repeated measures analysis of variance on log transformed data). There is also a different change with time. Group A decreases with time and group B increases with time. The difference in trend is statistically significant $p = 0.038$.

The decrease with time in group A during and after exposure to pool environment as well as filtered air is statistically significant ($p < 0.05$, GLM repeated analysis model). There is no statistically significant difference in change with time between pool exposure and filtered air.

For improved analysis, values were converted to their natural logarithms, SDs decreased, providing improved statistical conditions, but no statistically significant effect of exposure could be shown (data not shown).

SPD values, shown in Figure 2, also display a change with time, with lower values with increasing time intervals from initiation of exposure. Considering the log transformed SPD variable, there was a difference ($p < 0.05$) before and after exposure (i.e. SPD values were higher at 0 hrs than at 2 hrs) and there was a further decrease ($p < 0.01$) with time at 2 hrs – 8 hrs (Figure 2). This decrease was similar for exposure to pool air and filtered air. We found no statistically significant changes in SPD values in relation to exposure.

IgE

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3 The median IgE value was 1.0 mg/L in Group A and 0.0 in group B, compared to 3.0 mg/L in
4
5 mild asthmatics (n=18) participating in another study on influence of general air pollution
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7 conducted by one of the authors (BF). Compared to the volunteers in the present study
8
9 (groups A and B), the median value of the asthmatics was statistically significantly higher (p=
10
11 0.002) based on Westenber-Mood median test.
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13

Epidemiological study

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18 There was a statistically significant relationship between the number of hours, during an
19
20 average day, spent in the swimming pool environment and the incidence of acute symptoms
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22 (p<0.01; logistic regression). Frequent symptoms were: dyspnoea (13%), cough (23%), nose
23
24 irritation (29%), throat irritation (24%) and eye irritation (37%).
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30 The prevalence of self reported asthma attacks or medication for asthma was higher (p<0.01;
31
32 Fisher's test) among swimming pool workers in this study (12.3%) compared with the
33
34 reference group 8,1% (RHINE 1999). When considering rates (age and sex adjusted) by
35
36 logistic regression, there was still a higher prevalence among swimming-pool workers, but
37
38 less significant (p=0.11).
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43 In the nested case-control study, the Odds Ratio (OR) for asthma was 2.53 (95% CI 0.89 –
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45 7.19) for persons with exposure level 2 compared with persons exposed to level 0 or 1. After
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47 correction for heredity, the corresponding numbers were: OR 2.31 (95%CI 0.79-6.74).
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49 These values refer to cases of self reported asthma occurring after they started pool work,
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51 compared with controls.
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3 A tendency to a reduced risk of developing asthma in relation to the number of years of work
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5 in swimming-pool environments was indicated among individuals who worked more than one
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7 year and developed asthma after they started to work in such environments. This tendency
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9 was, however not, statistically significant $p=0.07$.
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Discussion

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16 Our observations of statistically significant decreases in FEV_1 and $FEV\%$ in previously non-
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18 exposed volunteers and pool workers after exposure to pool air are the first such observations
19
20 in adults. Carbonelle et al 2002 [4] reported an increase in FEV_1/VC among children and a
21
22 non-statistically significant decrease in adults ($n=13$) after they had attended a chlorinated
23
24 pool. Carbonelle et al 2008 [4] found FEV_1/VC to be unchanged in 11 young adults after
25
26 swimming in a non-chlorinated pool and slightly, but not statistically significantly decreased
27
28 after swimming in a chlorinated pool. The lack of statistically significant decrease may be
29
30 related to the fact that only 11 adults were studied[4], while the statistically significant
31
32 decrease in our study was based on 37 previously unexposed healthy volunteers. Very few
33
34 $FEV\%$ values were below 70 (indicating no clinically significant airway obstruction within
35
36 the study group). The reduction in $FEV\%$ seen after exposure in pool air here, albeit small,
37
38 may be a sign of an obstructive airway effect. In children, Bernard et al 2003 [14] found a
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40 statistically highly significant relationship between cumulative pool attendance during
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42 kindergarten and PEF 15 (post exercise reduction of peak expiratory flow by 15 percent),
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44 providing supportive evidence of airway effects of exposure to chlorinated pool
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46 environments. .
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54 CC16 levels in serum increase when lung epithelium permeability is adversely affected by air
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56 pollutants or other lung toxicants [6, 10,15, 16]. On the other hand, reduced levels of CC16 in
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3 lung lavage fluid occur in several lung disorders, probably due to a decrease in the production
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5 of CC16 as a consequence of a depletion of Clara cells [17]. We found a statistically
6
7 significant difference in the serum level of CC16 between pool workers compared to
8
9 volunteers. This finding is consistent with our previous finding of a lower CC16 value in
10
11 school children frequently attending indoor swimming pools than in those with a low
12
13 attendance at such pools [5]. The difference between workers and previously unexposed
14
15 healthy volunteers like the difference among school children may be due to a depletion of
16
17 Clara cells. We did not find any statistically significant exposure-related changes in
18
19 concentrations of the biomarkers of pulmonary epithelial integrity (CC16 and SPD) after
20
21 exposure to pool air for 2 hours. The lack of such an exposure-related change was probably
22
23 due to the relatively short exposure duration and low exposure level of NCl_3 . Another possible
24
25 explanation is that NCl_3 acts preferentially in the more proximal parts of the respiratory tract,
26
27 inducing a mild constriction of the central airways, but with less interference in the terminal
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29 bronchioles, where the Clara cells are located. In previous studies of volunteers exposed to
30
31 ozone [6], we found both a decrease in FEV_1 and an increase in serum CC16 concentrations
32
33 after exposure.
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40 All CC16 values in the present study were corrected for diurnal variation [12]. In spite of such
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42 correction, there was a statistically significant decrease with time of experiment from 0 h to 8
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44 h in group A (regardless of exposure to NCl_3). This indicates that the real diurnal variation
45
46 exceeded the one assumed in the employed correction calculation. For group B there is an
47
48 opposite trend with time, possibly related to an inadequate correction of the values in this
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50 group. The pool workers were older and had been more exposed to NCl_3 during many years of
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52 work in pool environments. Data on diurnal variation for SPD are not available in the
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3 literature. Our data, with a statistically significant decrease with time between 0 h and 8 h,
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5 indicate that a diurnal variation exists.

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7 The absence of exposure-related effects (after 2 hours exposure) on serum concentrations of
8
9 CC16 and SPD in combination with small, statistically significant decreases in FEV₁ and
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11 FEV_% show that the 2-hour exposure level in this experiment can be regarded as the Lowest-
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13 Observed-Adverse-Effect-Level on the lung for this group of volunteers. It should be borne
14
15 in mind that individuals with increased sensitivity to adverse respiratory effects, like those
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17 with pre-existing asthma, were not included in the present study. Our observation may be of
18
19 use in relation to administrative action in setting exposure limits for NCl₃. To our knowledge,
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21 no health-based limit values for occupational or environmental exposures have yet been set
22
23 for NCl₃. A technical value of 0.2 mg/m³ was recently recommended in Germany [18].
24
25 Bernard et al 2006 [19] showed that serum total IgE was a factor determining the risk of
26
27 adverse pulmonary effects after exposure to pool environments. Serum levels of total IgE in
28
29 the volunteers and workers of our study were lower than among mild asthmatics. The absence
30
31 of an increased level of total serum IgE among the present volunteers indicates that
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33 individuals with possibly increased sensitivity due to increased IgE had been successfully
34
35 excluded. Further studies on persons with elevated serum IgE would be of interest. Another
36
37 group that may suffer respiratory effects at lower air concentrations of NCl₃ is competitive
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39 swimmers because their breathing volumes exceed those of the volunteers in the present
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41 study. Helenius et al 1998 [20] found increased respiratory symptoms and bronchial
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43 responsiveness in elite swimmers.
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51 Our study indicates that employees in Swedish indoor pools are exposed to approximately the
52
53 same level of NCl₃ as employees in France and Belgium. We found median NCl₃
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55 concentrations of 0.18 mg/m³ in ten different premises, while Hery et al 1995 [9] reported
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 0.14-0.91 mg/m³ and Massin et al [3] reported a mean of 0.24 mg/m³ in Public pool
4 environments and 0.67 mg/m³ in establishments with private owners. There are no previous
5 published data on NCl₃ exposure in Swedish indoor pools. The work environment, i.e.
6 ventilation and the use of sodium hypochlorite as disinfectant has probably not changed
7 during the past decades. This makes it reasonable to estimate that pool workers have been
8 exposed to NCl₃ at approximately the same levels as reported in this study.
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18 In the epidemiological part of the present study, we found a statistically significant
19 relationship between the number of hours spent in swimming pool environments and the
20 incidence of symptoms. The workers reported a high incidence of respiratory and mucous
21 irritation symptoms from 13 percent for dyspnoea to 37 percent for eye irritation. These
22 findings are in accordance with previous observations in France [3] and Holland [1].
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32 This study also found a higher prevalence of self-reported asthma in swimming pool workers
33 than in a reference group. This difference remained when adjusted for age and sex, but failed
34 to reach statistical significance (p = 0.11). Our nested case-referent study found an Odds Ratio
35 (OR) for asthma of 2.53 (95% CI 0.89 – 7.19) for workers with more extensive exposure in
36 pool areas (exposure level 2 compared to persons with exposure level 0 or 1). After correction
37 for heredity the corresponding numbers were: OR 2.31 (95% CI 0.79 - 6.74). These values
38 refer to cases of self-reported asthma occurring after they started to work in swimming-pool
39 environments, compared to controls without asthma.
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49 Cases of asthma in pool workers have been reported in the United Kingdom [8], but no
50 epidemiological evidence has been reported. The findings of the present study did not reach
51 statistical significance and provide only limited support for a causal relationship between
52 asthma and work at indoor swimming pools. However, the fact that there was a tendency
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 towards a decreasing risk of asthma in workers with longer work history may indicate a
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5 healthy worker effect due to the irritating properties of NCl_3 in pool environments. A recent
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7 study [21] reported a higher prevalence (4.5%) of new-onset asthma among recreational
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9 swimmers with >320 hours of cumulative pool attendance compared to 0.4% among
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11 swimmers with <320 hours of pool attendance, thus supporting a role for exposure at
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13 chlorinated pools for development of asthma. In children engaged in recreational swimming, a
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15 statistically significant relationship was shown between cumulative attendance at indoor
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17 swimming pools and the probability of developing asthma in those with increased total IgE in
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19 serum [14,19]. Attendance at chlorinated pools before the age of 2 years increased the risk of
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21 bronchiolitis and asthma [22]
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27 The present findings support the previously advanced hypothesis [7, 14, 19,21] that exposures
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29 to NCl_3 levels commonly occurring in indoor swimming pool environments can cause acute
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31 airway and mucosal symptoms as well as changes in lung function and deterioration of
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33 asthma.
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38 **Conclusions:** For the first time in adults, statistically significant decreases in lung function
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40 were found in both previously unexposed subjects and pool-workers after exposure to pool air
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42 containing 0.23 and 0.14 mg/m^3 respectively, of NCl_3 compared to filtered air. The changes in
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44 lung function occurred in adults without any signs of allergy and with low IgE values. In a
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46 cohort of pool workers we found exposure-related acute mucous membrane and respiratory
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48 symptoms. An increased odds ratio for asthma (OR 2.31, 95% CI 0.79-6.74) was indicated in
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50 workers in the highest exposure category compared to lower exposures. Our observations give
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52 support to a previously advanced hypothesis that current exposures to NCl_3 can cause adverse
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 effects on mucous membranes and lungs of humans and contribute to the development of
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5 asthma. Further research in sensitive groups is warranted.
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10 **Data sharing:** There is no additional data available
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13
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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Figures and Tables

Table 1. Healthy volunteers (n=37): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV% (FEV₁x100/forced vital capacity) measured before and after 2h exercise in filtered and pool air respectively. Mean \pm SD. Mean differences (before-after) within parentheses.

| Expiratory volume | Exposure in filtered air | | | Exposure in pool air | | |
|-------------------|--------------------------|-----------------|-------------------------------|----------------------|-----------------|-------------------------------|
| | before | after | mean diff Δ -values | before | after | mean diff Δ -values |
| FEV ₁ | 4.10 \pm 0.85 | 4.11 \pm 0.87 | (-0.01) ^o | 4.14 \pm 0.87 | 4.09 \pm 0.86 | (0.05)** |
| FEV% | 80.5 \pm 5.8 | 80.9 \pm 5.2 | (-0.4) ^o | 80.7 \pm 5.3 | 79.9 \pm 5.3 | (0.8)* |

**FEV₁ significantly lower after exposure to pool air, p = 0.01

*FEV% lower after exposure to pool air, p = 0.05

^odifference not statistically significant

The FEV₁ Δ -values were -0.01 liter/sec in filtered air and 0.05 liter/sec in pool air, difference statistically significant, p = 0.01 (paired t-test).

Paired t-test of the difference in FEV% Δ -value in filtered air (mean -0.4 %) as compared pool s air (mean 0.8 %) was statistically significant, p = 0.004.

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

Table 2. Swimming pool workers (n=14): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV% (FEV₁×100/forced vital capacity) measured before and after 2h exercise in filtered air and pool air respectively. Mean ± SD. Mean differences (before-after) within parentheses.

| Expiratory volume | Exposure in filtered air | | | Exposure in pool air | | |
|-------------------|--------------------------|--------------|------------------------|----------------------|-------------|------------------------|
| | before | after | mean diff Δ -values | before | after | mean diff Δ -values |
| FEV ₁ | 3.56 ± 0.99 | 3.51 ± 0.91 | (0.05) [°] | 3.59 ± 0.93 | 3.57 ± 0.92 | (0.014) [°] |
| FEV% | 78.86 ± 6.3 | 78.43 ± 5.42 | (0.43) [°] | 79.1 ± 4.1 | 77.8 ± 5.1 | (1.36)* |

*FEV% lower after exposure to pool air, p = 0.003 (Wilcoxon signed rank test).

[°] indicates no statistically significant difference

Figure legends

Figure 1: Mean values (µg/L) and SD for CC16corr at various time points before (0h), immediately after exposure (2h) and the following 2 (4h), 4 (6h) and 6 hours (8h). Values are shown for the previously unexposed group of healthy volunteers (A) after exposure in a pool environment, after exposure to filtered air (two upper set of lines and bars). The two lower lines and related bars represent exposure in pool environment and filtered air for Group B, recruited among pool workers with several years exposure to pool environments.

Figure 2: Mean and SD for measured SPD values (µg/L) at various time points (0-8 hours) of the study. Exposure to pool environment or filtered air took place for 2 hours (between 0h and 2h). Group A: previously unexposed healthy volunteers. Group B: pool workers

Fig 1 (separate file)

Fig 2(separate file)

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

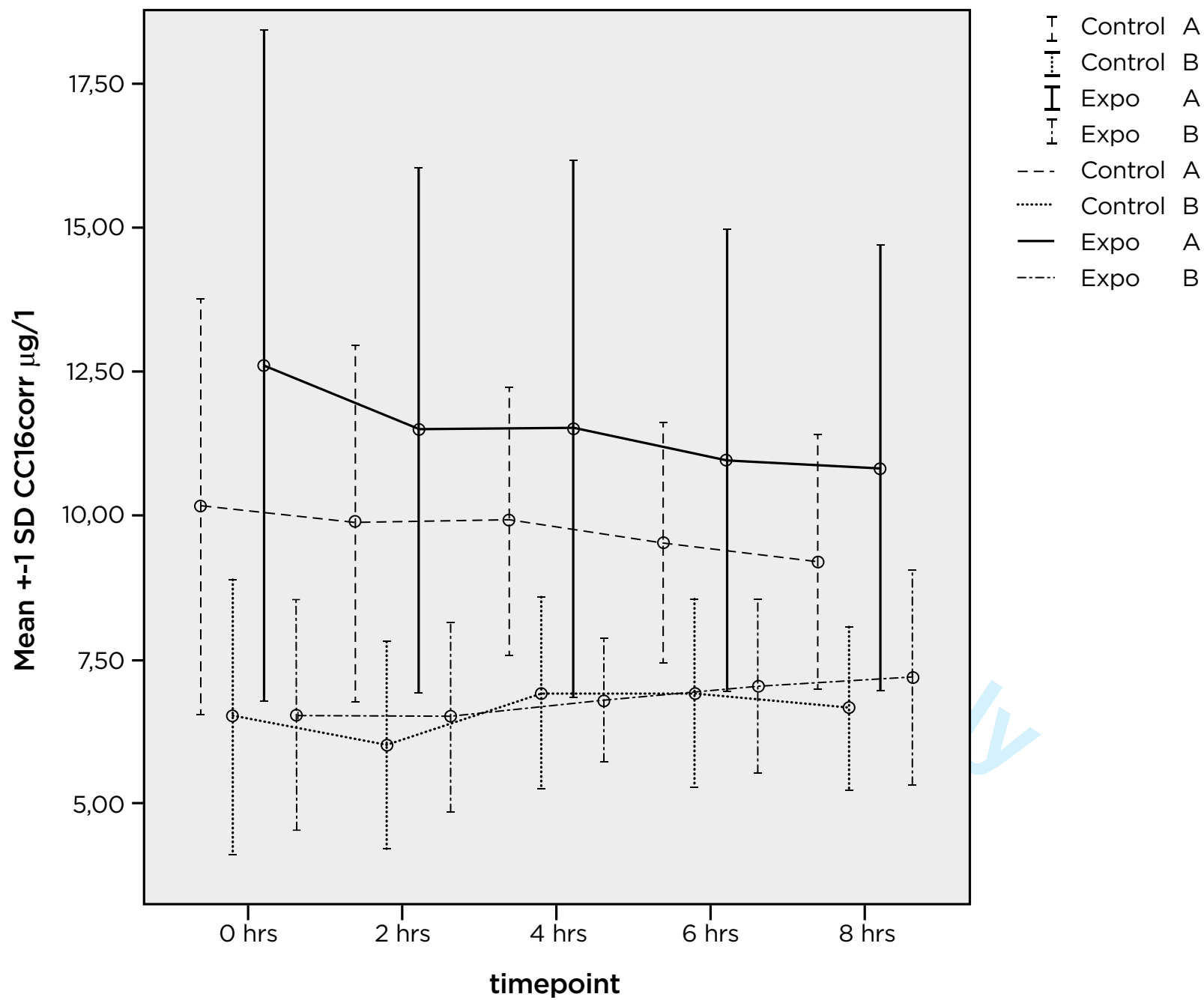
Statement:

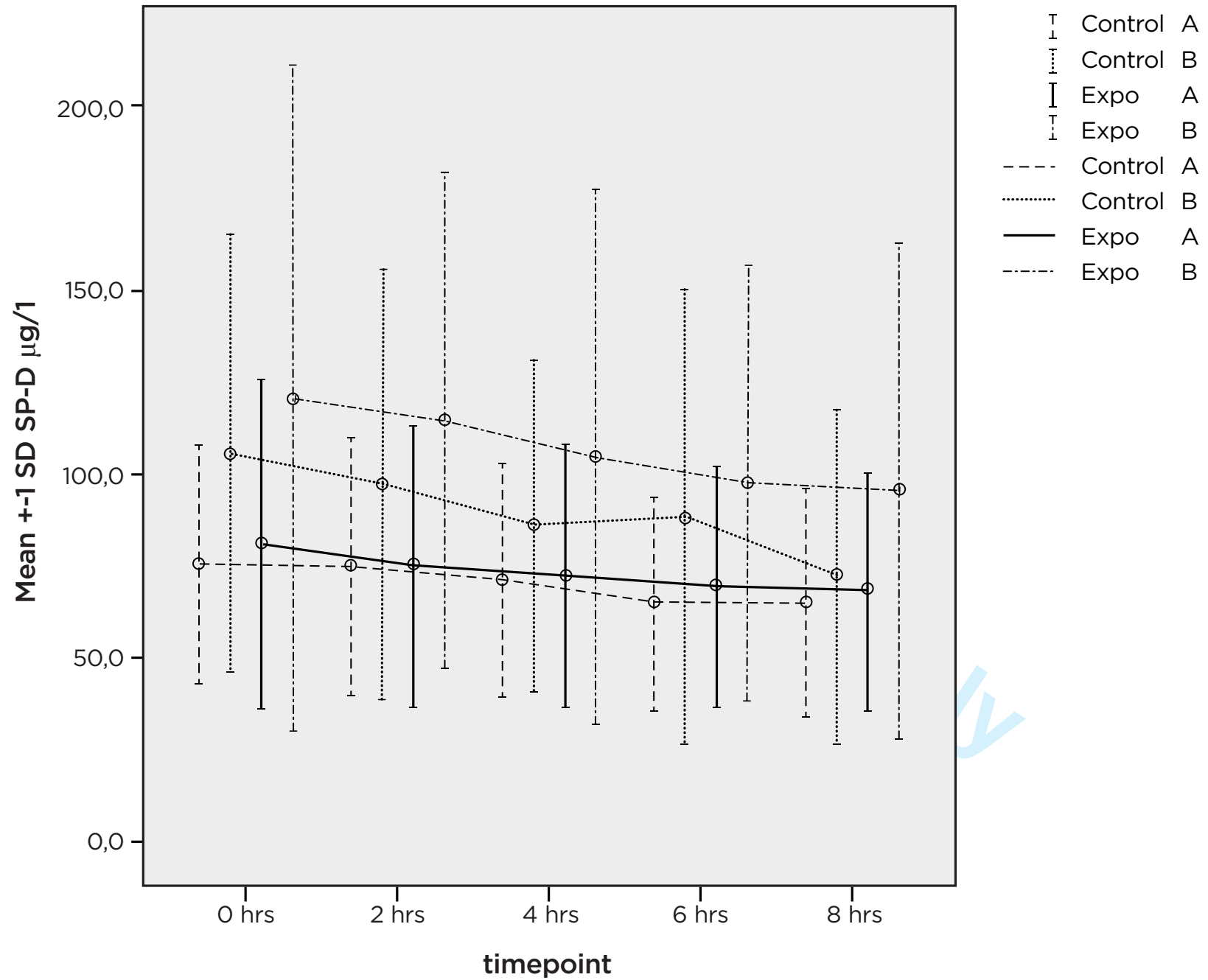
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1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published."





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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|------------------------------|---------|--|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| Introduction | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses |
| Methods | | |
| Study design | 4 | Present key elements of study design early in the paper |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants | 6 | (a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias | 9 | Describe any efforts to address potential sources of bias |
| Study size | 10 | Explain how the study size was arrived at |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses |

Continued on next page

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60**Results**

| | | |
|------------------|-----|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount) |
| Outcome data | 15* | <i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses |

Discussion

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|------------------|----|--|
| Key results | 18 | Summarise key results with reference to study objectives |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results |

Other information

| | | |
|---------|----|---|
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |
|---------|----|---|

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



Lung function in volunteers before and after exposure to trichloramine in indoor pool environments and asthma in a cohort of pool workers

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| | |

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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

20120802

Lung function in volunteers before and after exposure to trichloramine in indoor pool environments and asthma in a cohort of pool workers

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List of abbreviations:

- CC16: Clara Cell protein 16
- FEV₁: Forced Expiratory Volume in 1 second, liters
- FEV_%: FEV₁x100/FVC
- FVC: Forced Vital Capacity, liter
- NCl₃: Nitrogen trichloride or trichloramine
- OR: Odds Ratio
- RHINE: Respiratory Health in Northern Europe
- SPD: Surfactant protein D

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

Summary:**1) Article focus:**

Exposure to trichloramine (NCl₃) in swimming-pool air is known to cause mucous membrane and pulmonary effects, but statistically significant changes in lung function among adults have not been reported.

Epidemiological studies of asthma among pool workers are not available.

2) Key messages:

In this study we found for the first time, statistically significant decreases in lung function in volunteers after exposure to pool air with commonly occurring levels of NCl₃.

We found a tendency towards a higher odds ratio (OR) for asthma in a nested case reference study within a cohort of 1102 pool workers.

Our findings support the notion that current workroom exposures of NCl₃ may contribute to asthma development.

3) Strengths and limitations: This is the first study showing small but statistically significant decreases in lung function after exposure to pool air. This is the first nested Case/Control study in pool workers. It reports an OR for asthma of 2.31 (95% CI 0.79-6.74) among pool workers with the highest exposure (after correction for heredity), but this finding did not reach statistical significance.

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

ABSTRACT

Objectives: Exposure to trichloramine (NCl_3) in indoor swimming pool environments is known to cause mucous membrane irritation, but if it gives rise to changes in lung function or asthma in adults is not known. 1: We determined lung function in volunteers before and after exposure to indoor pool environments 2: We studied the occurrence of respiratory symptoms and asthma in a cohort of pool workers.

Design/Methods/Participants: 1. We studied two groups of volunteers, 37 previously non-exposed healthy persons and 14 pool workers, who performed exercise for two hours in an indoor pool environment. NCl_3 in air was measured during pool exposures and in 10 other pool environments. Filtered air exposures were used as controls. Lung function and biomarkers of pulmonary epithelial integrity were measured before and after exposure. 2. We mailed a questionnaire to 1741 persons who indicated in the Swedish census 1990 that they worked at indoor swimming-pools.

Results: 1. In previously non-exposed volunteers, statistically significant decreases in FEV_1 and $\text{FEV}_\%$ ($p=0.01$ and $p=0.05$ respectively) were found after exposure to pool air (0.23 mg/m^3 of NCl_3). In pool workers, a statistically significant decrease in $\text{FEV}_\%$ ($p=0.003$) was seen (but no significant change of FEV_1) .. In the 10 other pool environments the median NCl_3 concentration was 0.18 mg/m^3 . 2. Our nested Case/Control study in pool workers found an OR for asthma of 2.31 (95% CI 0.79-6.74) among those with the highest exposure. Exposure-related acute mucous membrane and respiratory symptoms were also found.

Conclusions: This is the first study in adults showing statistically significant decreases in lung function after exposure to NCl_3 . An increased OR for asthma among highly exposed pool workers did not reach statistical significance, but the combined evidence supports the notion that current workroom exposures may contribute to asthma development. Further research on sensitive groups is warranted.

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

INTRODUCTION and OBJECTIVES

Mono-, di- and trichloramines are formed following a reaction between ammonia (NH₃) or other nitrogen containing substances present in swimming pool water when hypochlorite is used as a disinfectant. Trichloramine (NCl₃) is the most volatile chloramine and is emitted into the air of indoor swimming pools. Exposure to this substance was the suspected cause of outbreaks of short-incubation ocular and respiratory illness [1,2], but concentrations of NCl₃ in pool environments were not known in these outbreaks. It is known, however, that acute respiratory and eye symptoms may occur among recreational swimmers in relation to measured levels of NCl₃ in pool environments [3] and NCl₃ is considered to be the causative agent.

Only few and inconclusive studies have been performed on lung function among adults after exposures to measured levels of NCl₃ in pool environments [4,5] and additional studies are required.

Clara cell protein 16 (CC16) is an epithelial protective protein in peripheral lung tissue and changes in its serum levels are used as a biomarker of epithelial integrity [6]. It has been shown to be decreased in relation to frequency of pool attendance [7]. However, changes in serum levels of CC16 have not been studied after short term exposure to NCl₃.

Thickett et al 2002 [8] reported three cases of occupational asthma among British pool workers exposed to NCl₃. There is a lack of epidemiological studies on asthma among those working in swimming pool environments.

The objectives of the present study were 1. To perform a controlled human exposure study of

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 lung function and biomarkers of pulmonary epithelial integrity in volunteers before and after
4 exposure to indoor swimming pool environments. 2. To perform an epidemiological study of
5 self-reported asthma and subjective symptoms in a cohort of indoor swimming pool workers
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DESIGN, MATERIALS AND METHODS**Air sampling and determination of NCl_3 :*****Exposure measurements in human exposure study***

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23 In the two pool environments where our study of volunteers and pool-workers took place
24 hypochlorite was used as disinfectant. Air samples were collected in the breathing zone: one
25 sample for each 2-hour exposure, in total 51 samples.
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Determination of NCl_3 at other indoor swimming pools:

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34 Additional determinations of NCl_3 were performed 2004-2008 at 10 different pool
35 establishments (7 conventional ones and 3 “adventure water lands”) in northern Sweden with
36 totally 30 indoor pools. Hypochlorite was used as disinfectant. At each swimming-pool, air
37 was sampled during 3 hours at 3 to 4 different locations in close vicinity of the pool. The
38 equipment was mounted on a stand with the filter at a height of approximately 1.5 meter.
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60 Sampling was performed on three different days during winter and three different days during
summer.

Air collection and Analysis: 1L/min of air was pumped through a filter (quartz filter QM-A
37 mm Whatman International Ltd., Maidstone, England). The filter was soaked in a solution
of sodium carbonate and arsenic trioxide (AsO_3) and dried as presented earlier [9]. When

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 NCl₃ is collected on the filter it is reduced to chloride ion (Cl⁻) [9]. After sampling, the filters
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5 were extracted with 10 ml of ultra-pure water, shaken for 30 minutes and filtered through a 13
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7 mm syringe filter (IC Acrodisc®, PALL). The chlorides were analyzed in a suppressed ion
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9 chromatography system (Triatlon 900 autosampler, Spark, The Netherlands); ICsep AN1,
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11 Anion column (CETAC, Omaha, USA); SCX membrane suppressor column (Sequant, Umeå,
12
13 Sweden); JD-21 conductivity detector (Costech Microanalytical Ltd, Tallin, Estonia)). The
14
15 eluent was 7.5 mM NaOH and the suppressor 5 mM H₂SO₄. Control samples of two known
16
17 chloride concentrations (0.5, 3.0 mg l⁻¹) and at least two blanks were run together with the
18
19 samples in each run. The chloride concentrations in the blanks were subtracted from the
20
21 concentration in the samples. The detection limits of NCl₃ (1.78 and 1.18 µg m⁻³ for 2 h and 3
22
23 h samplings, respectively) were determined as three times the mean standard deviation of the
24
25 amount collected on filters of 10 blanks. The limits of quantification (5.9 µg m⁻³ and 3.9 µg m⁻³
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27 for 2 h and 3 h samplings respectively) were determined as ten times the mean standard
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29 deviation for the same blanks.
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Human exposure study

Study groups:

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41 Group A: 37 healthy subjects (20 men and 17 women, mean age 24.5 years). They were not
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43 regular swimming pool visitors and they had not visited a swimming pool within four weeks
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45 before study start.
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50 Group B: 14 workers at swimming pools (5 men, 9 women, mean age 39.9 years).
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

All participants were non-smokers with normal lung function and had no history of allergy or pre-existing lung disease. Subjects were free of airway infection for ≥ 4 weeks prior to the first exposure and throughout the remainder of the study.

Study design

The study was conducted in a crossover control fashion. Each volunteer was exposed to filtered air in an exposure chamber and on another occasion to an indoor pool environment. In the exposure chamber, located in a separate building away from swimming-pools, incoming air was adjusted to room temperature and filtered through a particle filter. The exposures were performed in random order. Successive exposures were separated by ≥ 2 weeks. The exposures were performed either between 8 AM and 10 AM or between 10 AM and 12 AM. All exposures (pool environment or filtered air) lasted for 2 hours. The study subject was exercising on a bicycle ergometer with moderate exercise (minute ventilation $20 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$), during 15-minutes followed by 15 minutes of rest, i.e. four periods of exercise and four periods of rest.

Lung function:

FVC and forced expiratory volume in 1 sec (FEV_1) was determined using a portable spirometer connected to a computer (KoKo Spirometer and KoKo DigiDoser; Pulmonary Data Service Instrumentation, Inc, Louisville, KY, USA), calibrated in the morning and after every 10th measurement. $\text{FEV}_\%$ was calculated as a percentage of FVC ($\text{FEV}_\% = \text{FEV}_1 \times 100 / \text{FVC}$). Lung function was measured immediately before and after exposure in a room with non-detectable levels of NCl_3 ($< 0.002 \text{ mg NCl}_3/\text{m}^3$) or in a room adjacent to the exposure chamber.

Blood sampling and determination of biomarkers.

We obtained blood samples from the antecubital vein at 0 h and 2 h, i.e. before and after

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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2
3 exposure, and at 4, 6 and 8 hours. Peripheral blood was collected into BD Vacutainer tubes
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5 (BD, Plymouth, UK). Each sample was allowed to clot for 1-2 h at room temperature,
6
7 centrifuged at 3,000xg and serum was transferred to cryotubes and frozen at -80°C. These
8
9 samples were sent to the Industrial Toxicology Unit at the Catholic University of Louvain in
10
11 Brussels (IUTUCL), Belgium for determination of Clara Cell protein 16 (CC16) and
12
13 Surfactant Protein D (SPD). CC16 was determined by latex immunoassay using a rabbit anti-
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15 CC16 antibody (Dakopatts, Glostrup, Denmark) and CC16 purified at (IUTUCL) as standards
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17 [10,11]. All samples were run in duplicate at two different dilutions. The between- and within-
18
19 run coefficients of variation range 5–10% and results are comparable with ELISA methods
20
21 [11]. SPD determinations were performed using the Biovendor ELISA kit (Biovendor,
22
23 Heidelberg, Germany). Analyses were done in duplicate as recommended by the
24
25 manufacturer.
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31 Total IgE was determined in human serum by a double antibody sandwich ELISA method
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33 (Human IgE ELISA kit, Immunology Consultants Lab; Inc, Newberg, OR). The quantity of
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35 IgE in the samples was interpolated from a standard curve.
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Statistical analyses

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43 All data from CC16 measurements were corrected for diurnal variation according to Helleday
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45 et al 2006 [12] and recalculated to correspond to 7 AM. $CC16(\text{corr}) = CC16 + 0.582 * T -$
46
47 $0.032 * T^2$. T is the time after 7.00 AM when the blood sample was taken. Because CC16
48
49 values are highest in the morning [12], corrected CC16 values were somewhat greater than
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51 measured values.
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55 Statistics: We used repeated measures analyses of variance (Huynh-Feldt corrected) with time
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57 and exposure as within-subject factors and group as between-subject factor. Paired t-test or
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 Wilcoxon signed rank test was used when comparing exposures to filtered air and pool
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5 environment at baseline (0 hrs) and after exercise (2h). Median IgE values were compared by
6
7 the Westenberg-Mood median test. SPSS version 17.0 was used to perform the statistical
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9 analyses. A p-value of 0.05 was considered statistically significant.
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Epidemiological study***Population:***

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21 The epidemiological study group included 1741 persons in the Swedish Census of Population
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23 and Housing 1990 who had indicated that they worked at swimming pools. Early 2007 a
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25 questionnaire was mailed to them. There was one reminder.
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Questionnaire: Questions dealt with: year hired as a pool worker, time periods in various
jobs, time spent in swimming pool environments, various symptoms from the respiratory tract
and mucous membranes of the eyes and possible use of medication for asthma 589 women
and 513 men, age 30 ->80 years (mean age 51.2 years,SD 12.0) responded (63 %). Among 50
non-responders, interviews were performed via telephone. There was a lower prevalence of
asthma and respiratory symptoms among the non-responders, not statistically significant.
"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 a person's 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 when the first symptoms of asthma
occurred.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"

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 In a nested case-control study within this cohort, 44 cases of self reported asthma occurred
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5 after the person was hired as a pool worker. 128 age and sex matched controls were selected
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7 within the cohort (mean age 50.5 years SD 10.7).
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Exposure assessment:

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14 Based on information on work titles given by each individual, exposure was classified into
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16 three different categories; 0, 1, or 2. 0 stands for no exposure, 1 for low exposure and 2 for
17
18 high exposure. The exposure level is not an estimate of the concentration of NCl_3 in air but is
19
20 based on the average time during a workday the individual spent in the pool area. Those
21
22 within category 0 did not spend any time in a pool area, e.g. a cashier. A person within
23
24 category 1 did occasionally spend some time in the pool area. A manager of a swimming pool
25
26 or a technician belongs to this category. Individuals belonging to category 2 were those
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28 spending most of the workday in the pool area, e.g. a swimming teacher, or a swimming pool
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30 worker.
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Statistics

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46 Fisher's test was used to test differences between proportions. Conditional logistic regression
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48 was used for analyses in the nested case-control study and logistic regression for analyses of
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50 asthma in relation to years worked in swimming-pool environments. All statistical analyses
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52 were performed using the statistical package R, version 2.9.0 (www.r-project.org). P-values
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54 equal to or less than 0.05 were considered statistically significant.
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

Ethics

The project was approved by the Regional ethical review board in Umea, Sweden (Dnr 05-044M) and volunteers provided written informed consent. The study was carried out according to the declaration of Helsinki.

RESULTS**Air sampling.*****Experimental exposure (Human exposure study)***

The NCl_3 levels during the experimental exposures were

Group A: Mean 0.23 mg/m^3 (SD 0.09)

Group B Mean 0.15 mg/m^3 (SD 0.04)

Other Swimming pools

NCl_3 concentrations in air at the 10 different indoor swimming pool establishments were between $0.001\text{-}0.77 \text{ mg/m}^3$, median 0.18 mg/m^3 , arithmetic mean (AM) 0.21 mg/m^3 ($n=129$). The AM concentrations of NCl_3 in each of the ten different pool establishments were between $0.09\text{--}0.32 \text{ mg/m}^3$. There was no difference in NCl_3 concentrations during summer compared with winter conditions (results not shown).

Human exposure study***Lung function***

Group A:

Measured FEV_1 volumes among healthy volunteers as well as the difference before and after 2 hours of exposure to pool environment or filtered air are summarized in Table 1. There was

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

1
2
3 a small, statistically significant decrease ($p=0.01$) in FEV₁ (mean decrease = 0.05 L) after
4 exposure to swimming pool air. After exposure to filtered air there was a slight, not
5 statistically significant increase in FEV₁ (mean increase 0.01 L). When comparing the
6 differences (Δ -values) in FEV₁ before and after exposure to pool environment with the Δ -
7 values for exposure to filtered air in the same individuals, the difference between Δ -values
8 was statistically significant ($p=0.01$).
9

10
11 FEV_% values among healthy volunteers are also given in table 1. After exposure to pool air,
12 there was a small decrease (0.8 FEV_%) that was marginally statistically significant ($p=0.05$).
13 After exposure to filtered air, there was a small (statistically non-significant) increase in
14 FEV_% values. When the individual differences (Δ -values) of FEV_% before and after exposure
15 to pool air were compared with the corresponding Δ -values in filtered air, a statistically
16 significant difference was demonstrated ($p=0.004$, paired t-test). Airway obstruction is usually
17 defined as FEV_% below 70 (www.goldcopd.com). Only one value was below 70 (after
18 exposure) among the healthy volunteers.
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Group B

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36 In table 2, FEV₁ values for the swimming-pool workers are summarized. After exposure to
37 pool air there was a small and not statistically significant decrease in FEV₁, 0.01 L. There was
38 also a small decrease in FEV₁ after exposure to filtered air (0.05 L, $p=0.054$). When
39 considering the FEV_% values for the workers (Table 2) before and after exposure to pool air,
40 there was a statistically significant decrease of 1.36% ($p=0.003$). After exposure to filtered air
41 the small decrease in FEV_% of 0.43% was not statistically significant. Only two FEV_% values
42 among the pool workers (one before and one after exposure) were below 70. When comparing
43 the Δ -values in filtered air with those in pool air no statistically significant differences were
44 found. The lack of such differences may be partly related to the lower exposure level in group
45 B compared to Group A.
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

Biomarkers of pulmonary epithelial integrity:

Group A

Mean CC16corr values and related standard deviations (SD) in previously unexposed healthy volunteers, are shown in Figure 1 for 33 of the participants in group A. For the remaining 4 persons, values were missing and they were therefore excluded from analysis.

At baseline (0 hrs), mean CC16corr = 12.6 µg/L before pool exp (0 h) and 10.3 µg/L immediately before (0 h) exposure to filtered air. This difference (p=0.018, paired t-test) is difficult to explain because the same volunteers were exposed to both pool environment and filtered air and they were randomly assigned to either exposure.

Group B

Results are shown in Table 1. The mean CC16corr was 6.5 µg/L before both pool and filtered air exposures.

The difference between groups A and B persisted during and after exposure (0-8 hrs) and is statistically significant (p<0.001 repeated measures analysis of variance on log transformed data). There is also a different change with time. Group A decreases with time and group B increases with time. The difference in trend is statistically significant p=0.038.

The decrease with time in group A during and after exposure to pool environment as well as filtered air is statistically significant (p<0.05, GLM repeated analysis model). In Group A and Group B there is no statistically significant difference in change with time between pool exposure and filtered air. For improved analysis, values were converted to their natural logarithms, SDs decreased, providing improved statistical conditions, but no statistically significant effect of exposure could be shown (data not shown).

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SPD values, shown in Figure 2, also display a change with time, with lower values with increasing time intervals from initiation of exposure. Considering the log transformed SPD variable, there was a difference ($p < 0.05$) before and after exposure (i.e. SPD values were higher at 0 hrs than at 2 hrs) and there was a further decrease ($p < 0.01$) with time at 2 hrs – 8 hrs (Figure 2). This decrease was similar for exposure to pool air and filtered air. In groups A and B we found no statistically significant changes in SPD values in relation to exposure.

IgE

The median IgE value was low 1.0 mg/L in Group A and 0.0 in group B.

Epidemiological study

There was a statistically significant relationship between the number of hours, during an average day, spent in the swimming pool environment and the percentage of workers reporting acute symptoms during work ($p < 0.01$; logistic regression). Frequent symptoms were: dyspnoea (13%), cough (23%), nose irritation (29%), throat irritation (24%) and eye irritation (37%).

In the nested case-control study, the Odds Ratio (OR) for asthma was 2.53 (95% CI 0.89 – 7.19) for persons with exposure level 2 (114 controls, 42 cases) compared with persons exposed to level 0 or 1 (14 controls, 2 cases). After correction for heredity, the corresponding numbers were: OR 2.31 (95% CI 0.79-6.74).

These values refer to cases of self reported asthma occurring after they started pool work, compared with controls without asthma.

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 Among individuals who worked more than one year, there was a tendency to a reduced risk of
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5 developing asthma in relation to the number of years of work in swimming-pool
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7 environments. Only asthma cases that occurred after they started to work as pool workers
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9 were considered.. This tendency was, however not, statistically significant $p=0.07$.

Discussion

16 Our observations of statistically significant decreases in FEV_1 and $FEV\%$ in previously non-
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18 exposed volunteers and in $FEV\%$ in pool workers after exposure to pool air are the first such
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20 observations in adults.. Carbonelle et al 2002 [4] reported an increase in FEV_1/VC among
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22 children and a non-statistically significant decrease in adults ($n=13$) after they had attended a
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24 chlorinated pool. Carbonelle et al 2008 [4] found FEV_1/VC to be unchanged in 11 young
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26 adults after swimming in a non-chlorinated pool and slightly, but not statistically significantly
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28 decreased after swimming in a chlorinated pool. The lack of statistically significant decrease
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30 may be related to the fact that only 11 adults were studied [4], while the statistically
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32 significant decrease in our study was based on 37 previously unexposed healthy
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34 volunteers. The findings in volunteers were further supported by statistically significant
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36 differences in Δ -values. In the 14 pool workers, only one measurement of lung function
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38 ($FEV\%$) was statistically significantly decreased and no statistically significant difference was
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40 seen when Δ -values were compared. A possible effect in pool workers at the exposure level of
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42 our study ($0.15\text{mg}/\text{m}^3$) may be considered uncertain. Very few $FEV\%$ values were below 70
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44 (indicating no clinically significant airway obstruction within the study group). The reduction
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46 in $FEV\%$ seen after exposure in pool air here, albeit small, may be a sign of an obstructive
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48 airway effect. In children, Bernard et al 2003 [13] found a statistically highly significant
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50 relationship between cumulative pool attendance during kindergarten and PEF 15 (post
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 exercise reduction of peak expiratory flow by 15 percent), providing supportive evidence of
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5 airway effects of exposure to chlorinated pool environments.
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10 CC16 levels in serum increase when lung epithelium permeability is adversely affected by air
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12 pollutants or other lung toxicants [6, 10,14,15,]. On the other hand, reduced levels of CC16 in
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14 lung lavage fluid occur in several lung disorders, probably due to a decrease in the production
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16 of CC16 as a consequence of a depletion of Clara cells [16]. We found a statistically
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18 significant difference in the serum level of CC16 between pool workers compared to
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20 volunteers. This finding is consistent with our previous finding of a lower CC16 value in
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22 school children frequently attending indoor swimming pools than in those with a low
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24 attendance at such pools [5]. The difference between workers and previously unexposed
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26 healthy volunteers may be due to the older age of the workers but is more likely due to
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28 repeated exposures because a similar difference occurred among school children and all these
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30 differences may be due to a depletion of Clara cells. We did not find any statistically
31
32 significant exposure-related changes in concentrations of the biomarkers of pulmonary
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34 epithelial integrity (CC16 and SPD) after exposure to pool air for 2 hours. The lack of such an
35
36 exposure-related change was probably due to the relatively short exposure duration and low
37
38 exposure level of NCl_3 . Another possible explanation is that NCl_3 acts preferentially in the
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40 more proximal parts of the respiratory tract, inducing a mild constriction of the central
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42 airways, but with less interference in the terminal bronchioles, where the Clara cells are
43
44 located. In previous studies of volunteers exposed to ozone [6], we found both a decrease in
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46 FEV_1 and an increase in serum CC16 concentrations after exposure.
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53 Ideally, all exposures should have been performed at the same hour, because it is known that
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55 CC16 has diurnal variation [12]. However, for practical reasons exposures were started at
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 somewhat different times during the day and all CC16 values in the present study were
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5 corrected for diurnal variation [12]. Such correction is essential, but introduces a certain
6
7 element of uncertainty. In spite of such correction, there was a statistically significant
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9 decrease with time of experiment from 0 h to 8 h in group A (regardless of exposure to NCl₃).
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11 This indicates that the real diurnal variation exceeded the one assumed in the employed
12
13 correction calculation. For group B there is an opposite trend with time, possibly related to an
14
15 inadequate correction of the values in this group. The pool workers were older and had been
16
17 more exposed to NCl₃ during many years of work in pool environments. . Our data on SPD,
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19 with a statistically significant decrease with time between 0 h and 8 h, confirm previously
20
21 reported [17] diurnal variation.
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25 The absence of exposure-related effects (after 2 hours exposure) on serum concentrations of
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27 CC16 and SPD in combination with small, statistically significant decreases in FEV₁ and
28
29 FEV_% show that the 2-hour exposure level in this experiment can be regarded as the Lowest-
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31 Observed-Adverse-Effect-Level on the lung for this group of volunteers. It should be borne
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33 in mind that individuals with increased sensitivity to adverse respiratory effects, like those
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35 with pre-existing asthma, were not included in the present study. Our observation may be of
36
37 use in relation to administrative action in setting exposure limits for NCl₃. To our knowledge,
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39 no health-based limit values for occupational or environmental exposures have yet been set
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41 for NCl₃. A technical value of 0.2 mg/m³ was recently recommended in Germany [18].
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45 Bernard et al 2006 [19] showed that serum total IgE was a factor determining the risk of
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47 adverse pulmonary effects after exposure to pool environments. Serum levels of total IgE in
48
49 the volunteers and workers of our study were low. The absence of an increased level of total
50
51 serum IgE among the present volunteers indicates that individuals with possibly increased
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53 sensitivity due to increased IgE had been successfully excluded. Further studies on persons
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55 with elevated serum IgE would be of interest. Another group that may suffer respiratory
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 effects at lower air concentrations of NCl_3 is competitive swimmers because their breathing
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5 volumes exceed those of the volunteers in the present study. Helenius et al 1998 [20] found
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7 increased respiratory symptoms and bronchial responsiveness in elite swimmers.
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11 Our study indicates that employees in Swedish indoor pools are exposed to approximately the
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13 same level of NCl_3 as employees in France and Belgium. We found median NCl_3
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15 concentrations of 0.18 mg/m^3 (mean 0.21 mg/m^3) in ten different premises, while Hery et al
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17 1995 [9] reported $0.14\text{-}0.91 \text{ mg/m}^3$ and Massin et al [3] reported a mean of 0.24 mg/m^3 in
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19 Public pool environments and 0.67 mg/m^3 in establishments with private owners. There are no
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21 previous published data on NCl_3 exposure in Swedish indoor pools. The work environment,
22
23 i.e. ventilation and the use of sodium hypochlorite as disinfectant has probably not changed
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25 during the past decades. This makes it reasonable to estimate that pool workers have been
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27 exposed to NCl_3 at approximately the same levels as reported in this study.
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34 In the epidemiological part of the present study, we found a statistically significant
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36 relationship between the number of hours spent in swimming pool environments and the
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38 percentage of workers reporting acute symptoms when working. The percentage varied from
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40 13 percent for dyspnoea to 37 percent for eye irritation. These findings are in accordance with
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42 previous observations in France [3] and Holland [1]. These are subjective symptoms reported
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44 in a questionnaire also collecting exposure information and there is a possibility for recall
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46 bias. However similar clear outcomes have been reported also in other studies [1,3].
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52 . Our nested case-referent study found an Odds Ratio (OR) for asthma of 2.53 (95% CI 0.89 –
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54 7.19) for workers with more extensive exposure in pool areas (exposure level 2 compared to
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56 persons with exposure level 0 or 1). After correction for heredity the corresponding numbers
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 were: OR 2.31 (95% CI 0.79 - 6.74). These values refer to cases of self-reported asthma
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5 occurring after they started to work in swimming-pool environments, compared to controls
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7 without asthma.
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10 Cases of asthma in pool workers have been reported in the United Kingdom [8], but no
11
12 epidemiological evidence has been reported. The findings of the present study did not reach
13
14 statistical significance and provide only limited support for a causal relationship between
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16 asthma and work at indoor swimming pools. Individuals who are fit for these type of jobs tend
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18 to exercise more regularly and may notice respiratory symptoms; this may contribute to
19
20 confounding. The fact that there was a tendency towards a decreasing risk of asthma in
21
22 workers with longer work history may indicate a healthy worker effect due to the irritating
23
24 properties of NCl_3 in pool environments. A recent study [21] reported a higher prevalence
25
26 (4.5%) of new-onset asthma among recreational swimmers with >320 hours of cumulative
27
28 pool attendance compared to 0.4% among swimmers with <320 hours of pool attendance,
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30 thus supporting a role for exposure at chlorinated pools for development of asthma. In
31
32 children engaged in recreational swimming, a statistically significant relationship was shown
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34 between cumulative attendance at indoor swimming pools and the probability of developing
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36 asthma in those with increased total IgE in serum [13,19]. Attendance at chlorinated pools
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38 before the age of 2 years increased the risk of bronchiolitis and asthma [22]
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45 The present findings support the previously advanced hypothesis [7, 13, 19,21] that exposures
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47 to NCl_3 levels commonly occurring in indoor swimming pool environments can cause acute
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49 airway and mucosal symptoms as well as changes in lung function and deterioration of
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51 asthma.
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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3 **Conclusions:** For the first time in adults, statistically significant but small decreases in lung
4 function were found in previously unexposed subjects after exposure to pool air containing
5 0.23 mg/m³ of NCl₃ compared to filtered air. The changes in lung function occurred in adults
6 without any signs of allergy and with low IgE values. In a cohort of pool workers we found
7 exposure-related acute mucous membrane and respiratory symptoms. An increased odds ratio
8 for asthma (OR 2.31, 95% CI 0.79-6.74) was indicated in workers in the highest exposure
9 category compared to lower exposures. Our observations give support to a previously
10 advanced hypothesis that current exposures to NCl₃ can cause adverse effects on mucous
11 membranes and lungs of humans and contribute to the development of asthma. Further
12 research in sensitive groups is warranted.
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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Competing Interests Statement

There are no competing interests.

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53 **Figures and Tables** **Table 1.** Healthy volunteers (n=37): FEV₁ (forced expiratory volume,
54 liter during 1 sec) and FEV_% (FEV₁x100/forced vital capacity) measured before and after 2h
55 exercise in filtered air and pool air respectively. Mean \pm SD. Mean differences (before-after)
56 within parentheses.
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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11 **Table 2.** Swimming pool workers (n=14): FEV₁ (forced expiratory volume, liter during 1 sec)
12 and FEV_% (FEV₁×100/forced vital capacity) measured before and after 2h exercise in filtered
13 air and pool air respectively. Mean ± SD. Mean differences (before-after) within parentheses.
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Figure legends

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24 Figure 1: Mean values (µg/L) and SD for CC16corr at various time points before (0h),
25 immediately after exposure (2h) and the following 2 (4h), 4 (6h) and 6 hours (8h).
26 Values are shown for the previously unexposed group of healthy volunteers (A) after
27 exposure in a pool environment, after exposure to filtered air (two upper set of lines and bars).
28 The two lower lines and related bars represent exposure in pool environment and filtered air
29 for Group B, recruited among pool workers with several years exposure to pool environments.
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35 Figure 2: Mean and SD for measured SPD values (µg/L) at various time points (0-8 hours)
36 of the study. Exposure to pool environment or filtered air took place for 2 hours (between 0h
37 and 2h). Group A: previously unexposed healthy volunteers. Group B: pool workers
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42 Fig 1 (separate file)
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published."

LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

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Lung function in volunteers before and after exposure to trichloramine in indoor pool environments and asthma in a cohort of pool workers

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List of abbreviations:

- CC16: Clara Cell protein 16
- FEV₁: Forced Expiratory Volume in 1 second, liters
- FEV_{1%}: FEV₁x100/FVC
- FVC: Forced Vital Capacity, liter
- NCl₃: Nitrogen trichloride or trichloramine
- OR: Odds Ratio
- RHINE: Respiratory Health in Northern Europe
- SPD: Surfactant protein D

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7 **Summary:**
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10 **1) Article focus:**

11 # Exposure to trichloramine (NCl₃) in swimming-pool air is known to cause mucous
12 membrane and pulmonary effects, but statistically significant changes in lung function among
13 adults have not been reported.
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17 # Epidemiological studies of asthma among pool workers are not available.
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20 **2) Key messages:**
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22 # In this study we found for the first time, statistically significant decreases in lung function
23 in volunteers after exposure to pool air with commonly occurring levels of NCl₃.
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26 # We found a tendency towards a higher odds ratio (OR) for asthma in a nested case
27 reference study within a cohort of 1102 pool workers.
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30 # Our findings support the notion that current workroom exposures of NCl₃ may contribute to
31 asthma development.
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34 **3) Strengths and limitations:** This is the first study showing small but statistically significant
35 decreases in lung function after exposure to pool air. This is the first nested Case/Control
36 study in pool workers. It reports an OR for asthma of 2.31 (95% CI 0.79-6.74) among pool
37 workers with the highest exposure (after correction for heredity), but this finding did not reach
38 statistical significance.
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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

ABSTRACT

Objectives: Exposure to trichloramine (NCl_3) in indoor swimming pool environments is known to cause mucous membrane irritation, but if it gives rise to changes in lung function or asthma in adults is not known. 1: We determined lung function in volunteers before and after exposure to indoor pool environments 2: We studied the occurrence of respiratory symptoms and asthma in a cohort of pool workers.

Design/Methods/Participants: 1. We studied two groups of volunteers, 37 previously non-exposed healthy persons and 14 pool workers, who performed exercise for two hours in an indoor pool environment. NCl_3 in air was measured during pool exposures and in 10 other pool environments. Filtered air exposures were used as controls. Lung function and biomarkers of pulmonary epithelial integrity were measured before and after exposure. 2. We mailed a questionnaire to 1741 persons who indicated in the Swedish census 1990 that they worked at indoor swimming-pools.

Results: 1. In previously non-exposed volunteers, statistically significant decreases in FEV_1 and $\text{FEV}_\%$ ($p=0.01$ and $p=0.05$ respectively) were found after exposure to pool air (0.23 mg/m^3 of NCl_3). In pool workers, a statistically significant decrease in $\text{FEV}_\%$ ($p=0.003$) was seen (but no significant change of FEV_1) after exposure to 0.15 mg/m^3 of NCl_3 . In the 10 other pool environments the median NCl_3 concentration was 0.18 mg/m^3 . 2. Our nested Case/Control study in pool workers found an OR for asthma of 2.31 (95% CI 0.79-6.74) among those with the highest exposure. Exposure-related acute mucous membrane and respiratory symptoms were also found.

Conclusions: This is the first study in adults showing statistically significant decreases in lung function after exposure to NCl_3 . An increased OR for asthma among highly exposed pool workers did not reach statistical significance, but the combined evidence supports the

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LUNG FUNCTION and ASTHMA in POOL ENVIRONMENTS

notion that current workroom exposures may contribute to asthma development. Further research on sensitive groups is warranted.

INTRODUCTION and OBJECTIVES

Mono-, di- and trichloramines are formed following a reaction between ammonia (NH_3) or other nitrogen containing substances present in swimming pool water when hypochlorite is used as a disinfectant. Trichloramine (NCl_3) is the most volatile chloramine and is emitted into the air of indoor swimming pools. Exposure to this substance was the suspected cause of outbreaks of short-incubation ocular and respiratory illness [1,2], but concentrations of NCl_3 in pool environments were not known in these outbreaks. It is known, however, that acute respiratory and eye symptoms may occur among recreational swimmers in relation to measured levels of NCl_3 in pool environments [3] [and \$\text{NCl}_3\$ is considered to be the causative agent.](#)

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Only few and inconclusive studies have been performed on lung function among adults after exposures to measured levels of NCl_3 in pool environments [4,5] and additional studies are required.

Clara cell protein 16 (CC16) is an epithelial protective protein in peripheral lung tissue and changes in its serum levels are used as a biomarker of epithelial integrity [6]. It has been shown to be decreased in relation to frequency of pool attendance [7]. However, changes in serum levels of CC16 have not been studied after short term exposure to NCl_3 .

Thickett et al 2002 [8] reported three cases of occupational asthma among British pool workers exposed to NCl_3 . There is a lack of epidemiological studies on asthma among those working in swimming pool environments.

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The objectives of the present study were 1. To perform a controlled human exposure study of lung function and biomarkers of pulmonary epithelial integrity in volunteers before and after exposure to indoor swimming pool environments. 2. To perform an epidemiological study of self-reported asthma and subjective symptoms in a cohort of indoor swimming pool workers

DESIGN, MATERIALS AND METHODS

Air sampling and determination of NCl_3 :

Exposure measurements in human exposure study

In the two pool environments where our study of volunteers and pool-workers took place hypochlorite was used as disinfectant. Air samples were collected in the breathing zone: one sample for each 2-hour exposure, in total 51 samples.

Determination of NCl_3 at other indoor swimming pools:

Additional determinations of NCl_3 were performed 2004-2008 at 10 different pool establishments (7 conventional ones and 3 "adventure water lands") in northern Sweden with totally 30 indoor pools. Hypochlorite was used as disinfectant. At each swimming-pool, air was sampled during 3 hours at 3 to 4 different locations in close vicinity of the pool. The equipment was mounted on a stand with the filter at a height of approximately 1.5 meter. Sampling was performed on three different days during winter and three different days during summer.

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Air collection and Analysis: 1L/min of air was pumped through a filter (quartz filter QM-A 37 mm Whatman International Ltd., Maidstone, England). The filter was soaked in a solution of sodium carbonate and arsenic trioxide (AsO_3) and dried as presented earlier [9]. When NCl_3 is collected on the filter it is reduced to chloride ion (Cl^-) [9]. After sampling, the filters were extracted with 10 ml of ultra-pure water, shaken for 30 minutes and filtered through a 13 mm syringe filter (IC Acrodisc®, PALL). The chlorides were analyzed in a suppressed ion chromatography system (Triatlon 900 autosampler, Spark, The Netherlands); IC Sep AN1, Anion column (CETAC, Omaha, USA); SCX membrane suppressor column (Sequant, Umeå, Sweden); JD-21 conductivity detector (Costech Microanalytical Ltd, Tallin, Estonia)). The eluent was 7.5 mM NaOH and the suppressor 5 mM H_2SO_4 . Control samples of two known chloride concentrations (0.5 , 3.0 mg l^{-1}) and at least two blanks were run together with the samples in each run. The chloride concentrations in the blanks were subtracted from the concentration in the samples. The detection limits of NCl_3 (1.78 and $1.18 \text{ } \mu\text{g m}^{-3}$ for 2 h and 3 h samplings, respectively) were determined as three times the mean standard deviation of the amount collected on filters of 10 blanks. The limits of quantification ($5.9 \text{ } \mu\text{g m}^{-3}$ and $3.9 \text{ } \mu\text{g m}^{-3}$ for 2 h and 3 h samplings respectively) were determined as ten times the mean standard deviation for the same blanks.

Human exposure study

Study groups:

Group A: 37 healthy subjects (20 men and 17 women, mean age 24.5 years). They were not regular swimming pool visitors and they had not visited a swimming pool within four weeks before study start.

Group B: 14 workers at swimming pools (5 men, 9 women, mean age 39.9 years).

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All participants were non-smokers with normal lung function and had no history of allergy or pre-existing lung disease. Subjects were free of airway infection for ≥ 4 weeks prior to the first exposure and throughout the remainder of the study.

Study design

The study was conducted in a crossover control fashion. Each volunteer was exposed to filtered air in an exposure chamber and on another occasion to an indoor pool environment. In the exposure chamber, located in a separate building away from swimming-pools, incoming air was adjusted to room temperature and filtered through a particle filter. The exposures were performed in random order. Successive exposures were separated by ≥ 2 weeks. The exposures [were performed either between 8AM and 10 AM or between 10 Am and 12 AM. All exposures](#) (pool environment or filtered air) lasted for 2 hours. The study subject was exercising on a bicycle ergometer with moderate exercise (minute ventilation $20 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$), during 15-minutes followed by 15 minutes of rest, i.e. four periods of exercise and four periods of rest.

Lung function:

FVC and forced expiratory volume in 1 sec (FEV_1) was determined using a portable spirometer connected to a computer (KoKo Spirometer and KoKo DigiDoser; Pulmonary Data Service Instrumentation, Inc, Louisville, KY, USA), calibrated in the morning and after every 10th measurement. $\text{FEV}_\%$ was calculated as a percentage of FVC ($\text{FEV}_\% = \text{FEV}_1 \times 100 / \text{FVC}$). Lung function was measured immediately before and after exposure in a room with non-detectable levels of NCl_3 ($< 0.002 \text{ mg NCl}_3/\text{m}^3$) or in a room adjacent to the exposure chamber.

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Blood sampling and determination of biomarkers.

We obtained blood samples from the antecubital vein at 0 h and 2 h, i.e. before and after exposure, and at 4, 6 and 8 hours. Peripheral blood was collected into BD Vacutainer tubes (BD, Plymouth, UK). Each sample was allowed to clot for 1-2 h at room temperature, centrifuged at 3,000xg and serum transferred to cryotubes and frozen at -80°C. These samples were sent to the Industrial Toxicology Unit at the Catholic University of Louvain in Brussels (IUTUCL), Belgium for determination of Clara Cell protein 16 (CC16) and Surfactant Protein D (SPD). CC16 was determined by latex immunoassay using a rabbit anti-CC16 antibody (Dakopatts, Glostrup, Denmark) and CC16 purified at (IUTUCL) as standards [10,11]. All samples were run in duplicate at two different dilutions. The between- and within-run coefficients of variation range 5–10% and results are comparable with ELISA methods [112]. SPD determinations were performed using the Biovendor ELISA kit (Biovendor, Heidelberg, Germany). Analyses were done in duplicate as recommended by the manufacturer.

Total IgE was determined in human serum by a double antibody sandwich ELISA method (Human IgE ELISA kit, Immunology Consultants Lab; Inc, Newberg, OR). The quantity of IgE in the samples was interpolated from a standard curve.

Statistical analyses

All data from CC16 measurements were corrected for diurnal variation according to Helleday et al 2006 [123] and recalculated to correspond to 7 AM. $CC16(\text{corr}) = CC16 + 0.582 * T - 0.032 * T^2$. T is the time after 7.00 AM when the blood sample was taken. Because CC16 values are highest in the morning [123], corrected CC16 values were somewhat greater than measured values.

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Statistics: We used repeated measures analyses of variance (Huynh-Feldt corrected) with time and exposure as within-subject factors and group as between-subject factor. Paired t-test or Wilcoxon signed rank test was used when comparing exposures to filtered air and pool environment at baseline (0 hrs) and after exercise (2h). Median IgE values were compared by the Westenberg-Mood median test. SPSS version 17.0 was used to perform the statistical analyses. A p-value of 0.05 was considered statistically significant.

Epidemiological study

Population:

The epidemiological study group included 1741 persons in the Swedish Census of Population and Housing 1990 who had indicated that they worked at swimming pools. Early 2007 a questionnaire was mailed to them. There was one reminder.

Questionnaire: Questions dealt with: [year hired as a pool worker](#), -time periods in various jobs, time spent in swimming pool environments, various symptoms from the respiratory tract and mucous membranes of the eyes and possible use of medication for asthma- 589 women and 513 men, age 30 ->80 years ([mean age 51.2 years,SD 12.0](#)) responded ([63 %](#)). Among 50 non-responders, interviews were performed via telephone. There was a lower prevalence of asthma and respiratory symptoms among the non-responders,- not statistically significant.

["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 a person's 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 when the first symptoms of asthma occurred.Under the general heading "Acute symptoms when working in a swimming-pool](#)

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[environment” there was a question “How large a part of a working day did you usually spend in the swimming pool environment Hours”](#)

In a nested case-control study within this cohort, 44 cases of self reported asthma occurred after the person was hired as a pool worker. 128 age and sex matched controls were selected within the cohort ([mean age 50.5 years SD 10.7](#)).

Exposure assessment:

Based on information on work titles given by each individual, exposure was classified into three different categories; 0, 1, or 2. 0 stands for no exposure, 1 for low exposure and 2 for high exposure. The exposure level is not an estimate of the concentration of NCl_3 in air but is based on the average time during a workday the individual spent in the pool area. Those within category 0 did not spend any time in a pool area, e.g. a cashier. A person within category 1 did occasionally spend some time in the pool area. A manager of a swimming pool or a technician belongs to this category. Individuals belonging to category 2 were those spending most of the workday in the pool area, e.g. a swimming teacher, or a swimming pool worker.

Comparison data

~~We obtained data on asthma in 1990 from the study “Respiratory Health in Northern Europe” (RHINE [14]) via one of the authors of the present paper (BF). As we used the same questions in the present study as in RHINE, it was possible to derive adequate sex and age stratified comparison data up to the age of 55.~~

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Statistics

Fisher's test was used to test differences between proportions. Conditional logistic regression was used for analyses in the nested case-control study and logistic regression for analyses of asthma in relation to years worked in swimming-pool environments. All statistical analyses were performed using the statistical package R, version 2.9.0 (www.r-project.org). P-values equal to or less than 0.05 were considered statistically significant.

Ethics

The project was approved by the Regional ethical review board in Umea, Sweden (Dnr 05-044M) and volunteers provided written informed consent. The study was carried out according to the declaration of Helsinki.

RESULTS

Air sampling.

Experimental exposure (Human exposure study)

The NCl_3 levels during the experimental exposures were

Group A: Mean 0.23 mg/m^3 (SD 0.09)

Group B Mean 0.15 mg/m^3 (SD 0.04)

Other Swimming pools

NCl_3 concentrations in air at the 10 different indoor swimming pool establishments were

between $0.001\text{-}0.77 \text{ mg/m}^3$, median 0.18 mg/m^3 , arithmetic mean (AM) 0.21 mg/m^3

($n=129$). The AM concentrations of NCl_3 in each of the ten different pool establishments were

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between 0.09 – 0.32 mg/m³. There was no difference in NCl₃ concentrations during summer compared with winter conditions (results not shown).

Human exposure study

Lung function

Group A:

Measured FEV₁ volumes among healthy volunteers as well as the difference before and after 2 hours of exposure to pool environment or filtered air are summarized in Table 1. There was a small, statistically significant decrease (p=0.01) in FEV₁ (mean decrease = 0.05 L) after exposure to swimming pool air. After exposure to filtered air there was a slight, not statistically significant increase in FEV₁ (mean increase 0.01 L). When comparing the differences (Δ-values) in FEV₁ before and after exposure to pool environment with the Δ-values for exposure to filtered air in the same individuals, the difference between Δ-values was statistically significant (p=0.01).

FEV₁ values among healthy volunteers are also given in table 1. After exposure to pool air, there was a small decrease (0.8 FEV₁) that was marginally statistically significant (p=0.05). After exposure to filtered air, there was a small (statistically non-significant) increase in FEV₁ values. When the individual differences (Δ-values) of FEV₁ before and after exposure to pool air were compared with the corresponding Δ-values in filtered air, a statistically significant difference was demonstrated (p=0.004, paired t-test). Airway obstruction is usually defined as FEV₁ below 70 (www.goldcopd.com). Only one value was below 70 (after exposure) among the healthy volunteers.

Group B

In table 2, FEV₁ values for the swimming-pool workers are summarized. After exposure to pool air there was a small and not statistically significant decrease in FEV₁, 0.01 L. There was

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also a small decrease in FEV₁ after exposure to filtered air (0.05 L, p=0.054). When considering the FEV_% values for the workers (Table 2) before and after exposure to pool air, there was a statistically significant decrease of 1.36% (p=0.003). After exposure to filtered air the small decrease in FEV_% of 0.43% was not statistically significant. Only two FEV_% values among the pool workers (one before and one after exposure) were below 70. [When comparing the Δ-values in filtered air with those in pool air no statistically significant differences were found. The lack of such differences may be partly related to the lower exposure level in group B compared to Group A.](#)

Biomarkers of pulmonary epithelial integrity:

Group A

Mean CC16corr values and related standard deviations (SD) in previously unexposed healthy volunteers, are shown in Figure 1 for 33 of the participants in group A. For the remaining 4 persons, values were missing and they were therefore excluded from analysis.

At baseline (0 hrs), mean CC16corr = 12.6 µg/L before pool exp (0 h) and 10.3 µg/L immediately before (0 h) exposure to filtered air. This difference (p=0.018, paired t-test) is difficult to explain because the same volunteers were exposed to both pool environment and filtered air and they were randomly assigned to either exposure.

Group B

Results are shown in Table 1. The mean CC16corr was 6.5 µg/L before both pool and filtered air exposures.

The difference between groups A and B persisted during and after exposure (0-8 hrs) and is statistically significant (p<0.001 repeated measures analysis of variance on log transformed

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data). There is also a different change with time. Group A decreases with time and group B increases with time. The difference in trend is statistically significant $p=0.038$.

The decrease with time in group A during and after exposure to pool environment as well as filtered air is statistically significant ($p<0.05$, GLM repeated analysis model). [In Group A and Group B](#) there is no statistically significant difference in change with time between pool exposure and filtered air. For improved analysis, values were converted to their natural logarithms, SDs decreased, providing improved statistical conditions, but no statistically significant effect of exposure could be shown (data not shown).

SPD values, shown in Figure 2, also display a change with time, with lower values with increasing time intervals from initiation of exposure. Considering the log transformed SPD variable, there was a difference ($p<0.05$) before and after exposure (i.e. SPD values were higher at 0 hrs than at 2 hrs) and there was a further decrease ($p<0.01$) with time at 2 hrs – 8 hrs (Figure 2). This decrease was similar for exposure to pool air and filtered air. [In groups A and B](#) we found no statistically significant changes in SPD values in relation to exposure.

IgE

The median IgE value was [low](#) 1.0 mg/L in Group A and 0.0 in group B, ~~compared to 3.0 mg/L in mild asthmatics (n=18) participating in another study on influence of general air pollution conducted by one of the authors (BF). Compared to the volunteers in the present study (groups A and B), the median value of the asthmatics was statistically significantly higher ($p=0.002$) based on Westenberg-Mood median test.~~

Epidemiological study

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There was a statistically significant relationship between the number of hours, during an average day, spent in the swimming pool environment and the [percentage of workers reporting incidence of acute symptoms during work](#) ($p < 0.01$; logistic regression). Frequent symptoms were: dyspnoea (13%), cough (23%), nose irritation (29%), throat irritation (24%) and eye irritation (37%).

~~The prevalence of self reported asthma attacks or medication for asthma was higher ($p < 0.01$; Fisher's test) among swimming pool workers in this study (12.3%) compared with the reference group 8.1% (RHINE 1999). When considering rates (age and sex adjusted) by logistic regression, there was still a higher prevalence among swimming pool workers, but less significant ($p = 0.11$).~~

In the nested case-control study, the Odds Ratio (OR) for asthma was 2.53 (95% CI 0.89 – 7.19) for persons with exposure level 2 ([114 controls, 42 cases](#)) compared with persons exposed to level 0 or 1 ([14 controls, 2 cases](#)). After correction for heredity, the corresponding numbers were: OR 2.31 (95% CI 0.79-6.74).

These values refer to cases of self reported asthma occurring after they started pool work, compared with controls [without asthma](#).

~~Among individuals who worked more than one year, there was a~~ tendency to a reduced risk of developing asthma in relation to the number of years of work in swimming-pool environments. ~~was indicated among individuals who worked more than one year and d~~ [Only asthma cases that occurred developed asthma](#) after they started to work ~~in as pool workers were considered, such environments~~. This tendency was, however not, statistically significant $p = 0.07$.

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Discussion

Our observations of statistically significant decreases in FEV₁ and FEV% in previously non-exposed volunteers and in FEV% in pool workers after exposure to pool air are the first such observations in adults. Carbonelle et al 2002 [4] reported an increase in FEV₁/VC among children and a non-statistically significant decrease in adults (n=13) after they had attended a chlorinated pool. Carbonelle et al 2008 [4] found FEV₁/VC to be unchanged in 11 young adults after swimming in a non-chlorinated pool and slightly, but not statistically significantly decreased after swimming in a chlorinated pool. The lack of statistically significant decrease may be related to the fact that only 11 adults were studied [4], while the statistically significant decrease in our study was based on 37 previously unexposed healthy volunteers. The findings in volunteers were further supported by statistically significant differences in Δ-values. In the 14 pool workers, only one measurement of lung function (FEV%) was statistically significantly decreased and no statistically significant difference was seen when Δ-values were compared. A possible effect in pool workers at the exposure level of our study (0.15mg/m³) may be considered uncertain. Very few FEV% values were below 70 (indicating no clinically significant airway obstruction within the study group). The reduction in FEV% seen after exposure in pool air here, albeit small, may be a sign of an obstructive airway effect. In children, Bernard et al 2003 [143] found a statistically highly significant relationship between cumulative pool attendance during kindergarten and PEF 15 (post exercise reduction of peak expiratory flow by 15 percent), providing supportive evidence of airway effects of exposure to chlorinated pool environments. —

CC16 levels in serum increase when lung epithelium permeability is adversely affected by air pollutants or other lung toxicants [6, 10, 14, 15, 16]. On the other hand, reduced levels of CC16

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in lung lavage fluid occur in several lung disorders, probably due to a decrease in the production of CC16 as a consequence of a depletion of Clara cells [16+7]. We found a statistically significant difference in the serum level of CC16 between pool workers compared to volunteers. This finding is consistent with our previous finding of a lower CC16 value in school children frequently attending indoor swimming pools than in those with a low attendance at such pools [5]. The difference between workers and previously unexposed healthy volunteers may be due to the older age of the workers but is more likely due to repeated exposures because a similar ~~like the~~ difference occurred among school children and all these differences may be due to a depletion of Clara cells. We did not find any statistically significant exposure-related changes in concentrations of the biomarkers of pulmonary epithelial integrity (CC16 and SPD) after exposure to pool air for 2 hours. The lack of such an exposure-related change was probably due to the relatively short exposure duration and low exposure level of NCl_3 . Another possible explanation is that NCl_3 acts preferentially in the more proximal parts of the respiratory tract, inducing a mild constriction of the central airways, but with less interference in the terminal bronchioles, where the Clara cells are located. In previous studies of volunteers exposed to ozone [6], we found both a decrease in FEV_1 and an increase in serum CC16 concentrations after exposure.

Ideally, all exposures should have been performed at the same hour, because it is known that CC16 has diurnal variation [12]. However, for practical reasons exposures were started at somewhat different times during the day and aAll CC16 values in the present study were corrected for diurnal variation [12].Such correction is essential, but introduces a certain element of uncertainty. In spite of such correction, there was a statistically significant decrease with time of experiment from 0 h to 8 h in group A (regardless of exposure to NCl_3). This indicates that the real diurnal variation exceeded the one assumed in the employed

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correction calculation. For group B there is an opposite trend with time, possibly related to an inadequate correction of the values in this group. The pool workers were older and had been more exposed to NCl_3 during many years of work in pool environments. ~~Data on diurnal variation for SPD are not available in the literature.~~ Our data on SPD, with a statistically significant decrease with time between 0 h and 8 h, confirm previously reported [17] indicate that a diurnal variation exists.

The absence of exposure-related effects (after 2 hours exposure) on serum concentrations of CC16 and SPD in combination with small, statistically significant decreases in FEV_1 and $\text{FEV}_\%$ show that the 2-hour exposure level in this experiment can be regarded as the Lowest-Observed-Adverse-Effect-Level on the lung for this group of volunteers. It should be borne in mind that individuals with increased sensitivity to adverse respiratory effects, like those with pre-existing asthma, were not included in the present study. Our observation may be of use in relation to administrative action in setting exposure limits for NCl_3 . To our knowledge, no health-based limit values for occupational or environmental exposures have yet been set for NCl_3 . A technical value of 0.2 mg/m^3 was recently recommended in Germany [18]. Bernard et al 2006 [19] showed that serum total IgE was a factor determining the risk of adverse pulmonary effects after exposure to pool environments. Serum levels of total IgE in the volunteers and workers of our study were ~~lower than among mild asthmatics.~~ The absence of an increased level of total serum IgE among the present volunteers indicates that individuals with possibly increased sensitivity due to increased IgE had been successfully excluded. Further studies on persons with elevated serum IgE would be of interest. Another group that may suffer respiratory effects at lower air concentrations of NCl_3 is competitive swimmers because their breathing volumes exceed those of the volunteers in the present study. Helenius et al 1998 [20] found increased respiratory symptoms and bronchial responsiveness in elite swimmers.

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Our study indicates that employees in Swedish indoor pools are exposed to approximately the same level of NCl_3 as employees in France and Belgium. We found median NCl_3 concentrations of 0.18 mg/m^3 (mean 0.21 mg/m^3) in ten different premises, while Hery et al 1995 [9] reported $0.14\text{-}0.91 \text{ mg/m}^3$ and Massin et al [3] reported a mean of 0.24 mg/m^3 in Public pool environments and 0.67 mg/m^3 in establishments with private owners. There are no previous published data on NCl_3 exposure in Swedish indoor pools. The work environment, i.e. ventilation and the use of sodium hypochlorite as disinfectant has probably not changed during the past decades. This makes it reasonable to estimate that pool workers have been exposed to NCl_3 at approximately the same levels as reported in this study.

In the epidemiological part of the present study, we found a statistically significant relationship between the number of hours spent in swimming pool environments and the ~~percentage of workers reporting acute incidence of symptoms when working.~~ The ~~percentage varied workers reported a high incidence of respiratory and mucous irritation symptoms~~ from 13 percent for dyspnoea to 37 percent for eye irritation. These findings are in accordance with previous observations in France [3] and Holland [1]. ~~These are subjective symptoms reported in a questionnaire also collecting exposure information and there is a possibility for recall bias. However similar clear outcomes have been reported also in other studies [1,3].~~

~~This study also found a higher prevalence of self reported asthma in swimming pool workers than in a reference group. This difference remained when adjusted for age and sex, but failed to reach statistical significance ($p = 0.11$).~~ Our nested case-referent study found an Odds Ratio (OR) for asthma of 2.53 (95% CI 0.89 – 7.19) for workers with more extensive exposure in pool areas (exposure level 2 compared to persons with exposure level 0 or 1). After correction

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for heredity the corresponding numbers were: OR 2.31 (95% CI 0.79 - 6.74). These values refer to cases of self-reported asthma occurring after they started to work in swimming-pool environments, compared to controls without asthma.

Cases of asthma in pool workers have been reported in the United Kingdom [8], but no epidemiological evidence has been reported. The findings of the present study did not reach statistical significance and provide only limited support for a causal relationship between asthma and work at indoor swimming pools [Individuals who are fit for these type of jobs tend to exercise more regularly and may notice respiratory symptoms; this may contribute to confounding.](#) ~~T-However,~~ the fact that there was a tendency towards a decreasing risk of asthma in workers with longer work history may indicate a healthy worker effect due to the irritating properties of NCl_3 in pool environments. ~~A~~ recent study [21] reported a higher prevalence (4.5%) of new-onset asthma among recreational swimmers with >320 hours of cumulative pool attendance compared to 0.4% among swimmers with <320 hours of pool attendance, thus supporting a role for exposure at chlorinated pools for development of asthma. In children engaged in recreational swimming, a statistically significant relationship was shown between cumulative attendance at indoor swimming pools and the probability of developing asthma in those with increased total IgE in serum [13,14,19]. Attendance at chlorinated pools before the age of 2 years increased the risk of bronchiolitis and asthma [22]

The present findings support the previously advanced hypothesis [7, 13,14, 19,21] that exposures to NCl_3 levels commonly occurring in indoor swimming pool environments can cause acute airway and mucosal symptoms as well as changes in lung function and deterioration of asthma.

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Conclusions: For the first time in adults, statistically significant but small decreases in lung function were found in both previously unexposed subjects and pool workers after exposure to pool air containing 0.23 and 0.14 mg/m³ respectively, of NCl₃ compared to filtered air. The changes in lung function occurred in adults without any signs of allergy and with low IgE values. In a cohort of pool workers we found exposure-related acute mucous membrane and respiratory symptoms. An increased odds ratio for asthma (OR 2.31, 95% CI 0.79-6.74) was indicated in workers in the highest exposure category compared to lower exposures. Our observations give support to a previously advanced hypothesis that current exposures to NCl₃ can cause adverse effects on mucous membranes and lungs of humans and contribute to the development of asthma. Further research in sensitive groups is warranted.

Data sharing: There is no additional data available

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Figures and Tables

Table 1. Healthy volunteers (n=37): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV₁% (FEV₁ × 100/forced vital capacity) measured before and after 2h exercise in filtered air and pool air respectively. Mean ± SD. Mean differences (before-after) within parentheses.

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Table 1. Healthy volunteers (n=37): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV₁% (FEV₁ × 100/forced vital capacity) measured before and after 2h exercise in filtered and pool air respectively. Mean ± SD. Mean differences (before after) within parentheses.

| Expiratory volume | Exposure in filtered air | | | Exposure in pool air | | |
|--------------------|--------------------------|-------------|-----------------------|----------------------|-------------|-----------------------|
| | before | after | mean diff Δ values | before | after | mean diff Δ values |
| FEV ₁ | 4.10 ± 0.85 | 4.11 ± 0.87 | (-0.01) ^o | 4.14 ± 0.87 | 4.09 ± 0.86 | (-0.05)** |
| FEV ₁ % | 80.5 ± 5.8 | 80.9 ± 5.2 | (-0.4) ^o | 80.7 ± 5.3 | 79.9 ± 5.3 | (-0.8)* |

**FEV₁ significantly lower after exposure to pool air, p = 0.01

*FEV₁% lower after exposure to pool air, p = 0.05

^odifference not statistically significant

The FEV₁ Δ values were -0.01 liter/sec in filtered air and 0.05 liter/sec in pool air, difference statistically significant, p = 0.01 (paired t test).

Paired t test of the difference in FEV₁% Δ value in filtered air (mean -0.4 %) as compared pool's air (mean 0.8 %) was statistically significant, p = 0.004.

Table 2. Swimming-pool workers (n=14): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV₁% (FEV₁ × 100/forced vital capacity) measured before and after 2h exercise in filtered air and pool air respectively. Mean ± SD. Mean differences (before after) within parentheses.

| Expiratory volume | Exposure in filtered air | | | Exposure in pool air | | |
|-------------------|--------------------------|-------|-----------------------|----------------------|-------|-----------------------|
| | before | after | mean diff Δ values | before | after | mean diff Δ values |

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|------------------------|------------------------|-------------------------|-------------------------------|------------------------|------------------------|--------------------------------|
| FEV₁ | 3.56 ± 0.99 | 3.51 ± 0.91 | (0.05)[°] | 3.59 ± 0.93 | 3.57 ± 0.92 | (0.014)[°] |
| FEV% | 78.86 ± 6.3 | 78.43 ± 5.42 | (0.43)[°] | 79.1 ± 4.1 | 77.8 ± 5.1 | (1.36)* |

*FEV% lower after exposure to pool air, $p = 0.003$ (Wilcoxon signed rank test).

[°] indicates no statistically significant difference

Table 2. Swimming pool workers (n=14): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV% (FEV₁ × 100 / forced vital capacity) measured before and after 2h exercise in filtered air and pool air respectively. Mean ± SD. Mean differences (before-after) within parentheses.

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Figure legends

Figure 1: Mean values (µg/L) and SD for CC16corr at various time points before (0h), immediately after exposure (2h) and the following 2 (4h), 4 (6h) and 6 hours (8h). Values are shown for the previously unexposed group of healthy volunteers (A) after exposure in a pool environment, after exposure to filtered air (two upper set of lines and bars). The two lower lines and related bars represent exposure in pool environment and filtered air for Group B, recruited among pool workers with several years exposure to pool environments.

Figure 2: Mean and SD for measured SPD values (µg/L) at various time points (0-8 hours) of the study. Exposure to pool environment or filtered air took place for 2 hours (between 0h and 2h). Group A: previously unexposed healthy volunteers. Group B: pool workers

Fig 1 (separate file)

Fig 2(separate file)

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24 **Contributorship statement:**

25 "The contributions of the authors are as follows: G.Nordberg 1,2,3; N-G.Lundstrom 1,2,3;
26 B.Forsberg1,2,3; A.Hagenbjork-Gustafsson1,2,3; B. J-son Lagerkvist1,2,3; J. Nilsson1,2,3;
27 M.Svensson1,2,3; A.Blomberg1, 2,3; L. Nilsson1,2,3; A. Bernard1,2,3; X. Dumont1,2,3;
28 H.Bertilsson1,2,3 and K. Eriksson1,2,3.

29 1) substantial contributions to conception and design, acquisition of data, or analysis and
30 interpretation of data; 2) drafting the article or revising it critically for important intellectual
31 content; and 3) final approval of the version to be published."
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Table 1. Healthy volunteers (n=37): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV% (FEV₁ × 100 / forced vital capacity) measured before and after 2h exercise in filtered air and pool air respectively. Mean ± SD. Mean differences (before-after) within parentheses.

| Expiratory volume | Exposure in filtered air | | | Exposure in pool air | | | Difference in changes [‡] |
|------------------------|--------------------------|-------------|------------------------|----------------------|-------------|------------------------|------------------------------------|
| | before | after | mean diff Δ -values | before | after | mean diff Δ -values | |
| FEV₁ | 4.10 ± 0.85 | 4.11 ± 0.87 | (-0.01) [°] | 4.14 ± 0.87 | 4.09 ± 0.86 | (0.05) ** | p=0.01 |
| FEV% | 80.5 ± 5.8 | 80.9 ± 5.2 | (-0.4) [°] | 80.7 ± 5.3 | 79.9 ± 5.3 | (0.8)* | p=0.004 |

** FEV₁ significantly lower after exposure in pool air, p=0.01

*FEV% lower after exposure to pool air, p = 0.05

[°] indicates no statistically significant difference

[‡] statistical significance of difference between Δ -values in filtered air and in pool air

Table 2. Swimming pool workers (n=14): FEV₁ (forced expiratory volume, liter during 1 sec) and FEV% (FEV₁x100/forced vital capacity) measured before and after 2h exercise in filtered air and pool air respectively. Mean \pm SD. Mean differences (before-after) within parentheses.

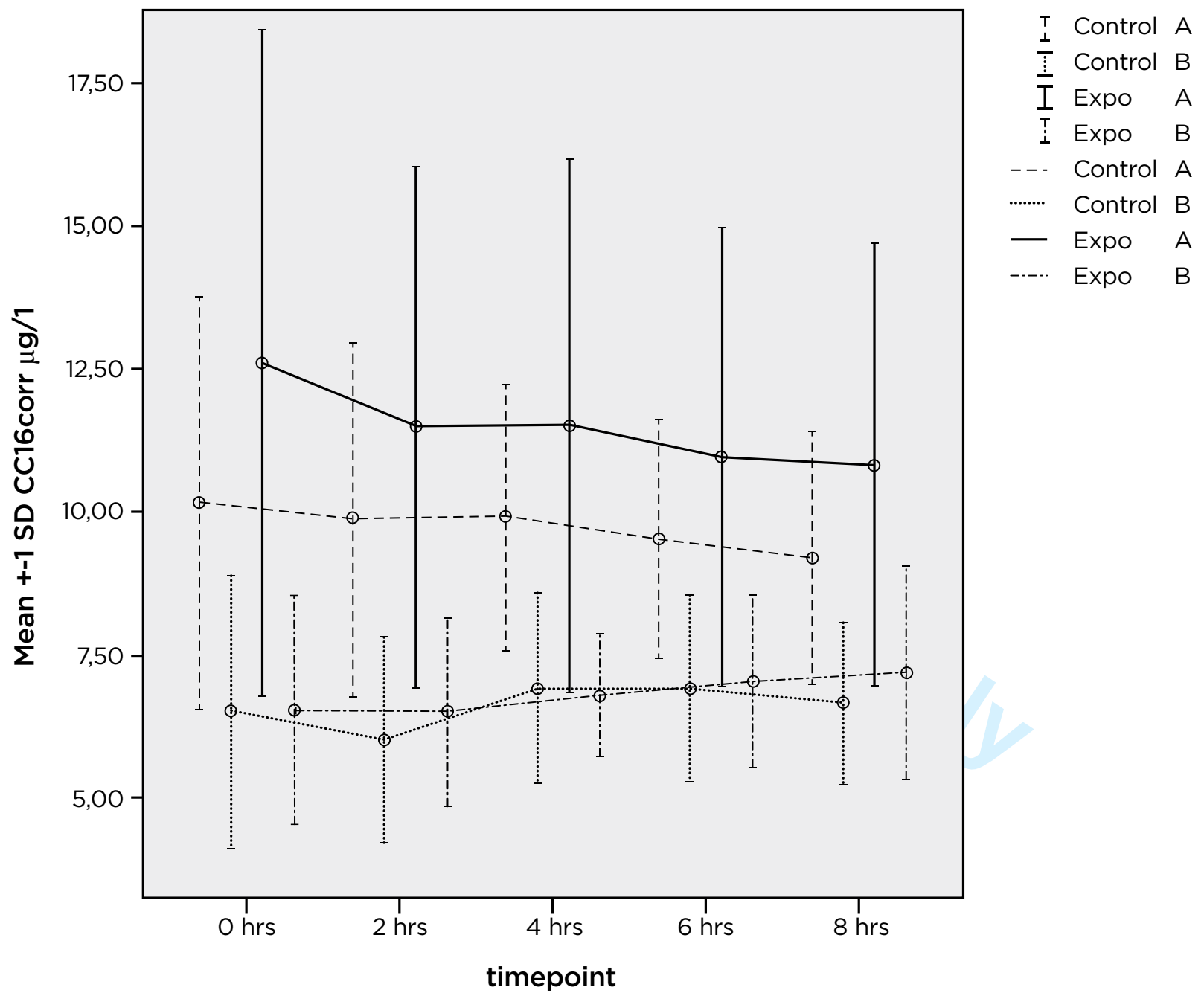
| Expiratory volume | Exposure in filtered air | | | Exposure in pool air | | | Difference in changes [‡] |
|-------------------|--------------------------|------------------|-------------------------------|----------------------|-----------------|-------------------------------|------------------------------------|
| | before | after | mean diff Δ -values | before | after | mean diff Δ -values | |
| FEV ₁ | 3.56 \pm 0.99 | 3.51 \pm 0.91 | (0.05) [°] | 3.59 \pm 0.93 | 3.57 \pm 0.92 | (0.014) [°] | Non-significant |
| FEV% | 78.86 \pm 6.3 | 78.43 \pm 5.42 | (0.43) [°] | 79.1 \pm 4.1 | 77.8 \pm 5.1 | (1.36)* | Non-significant |

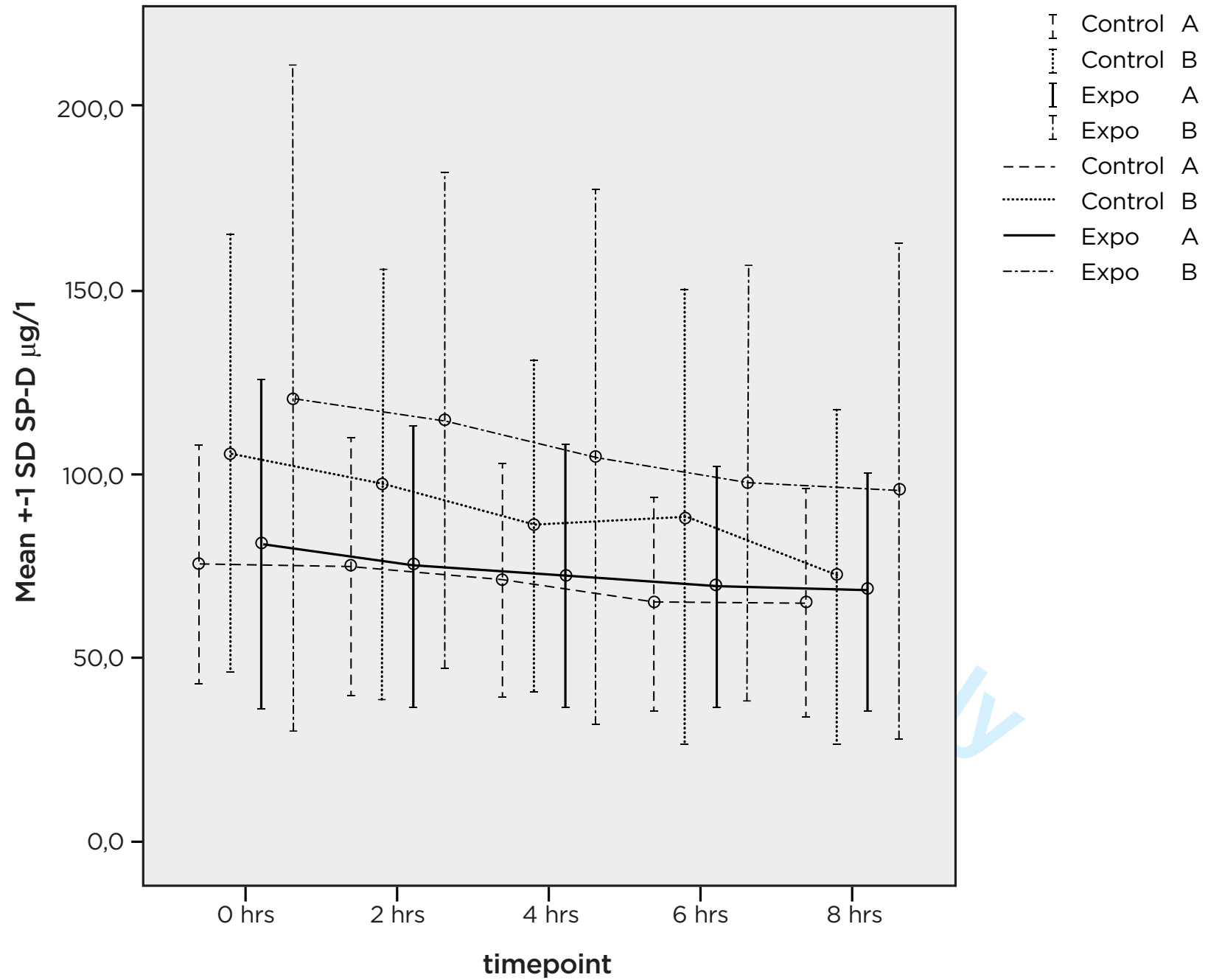
*FEV% lower after exposure to pool air, p = 0.003 (Wilcoxon signed rank test).

[°] indicates no statistically significant difference

[‡]Statistical significance of difference between Δ -values in filtered air and pool air

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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|------------------------------|---------|--|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| Introduction | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses |
| Methods | | |
| Study design | 4 | Present key elements of study design early in the paper |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants | 6 | (a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias | 9 | Describe any efforts to address potential sources of bias |
| Study size | 10 | Explain how the study size was arrived at |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses |

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Results

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| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount) |
| Outcome data | 15* | <i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses |

Discussion

| | | |
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| Key results | 18 | Summarise key results with reference to study objectives |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results |

Other information

| | | |
|---------|----|---|
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |
|---------|----|---|

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.