Eur Respir J 2000; 16: 626–632
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European Respiratory Journal
ISSN 0903-1936

Nasal inflammatory and respiratory parameters in human volunteers during and after repeated exposure to chlorine

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Nasal inflammatory and respiratory parameters in human volunteers during and after repeated exposure to chlorine. R.P.F. Schins, H. Emmen, L. Hoogendijk, P.J.A. Borm. ©ERS Journals Ltd 2000.

ABSTRACT: The objectives of this study were: 1) to determine if chlorine exposure at low levels induces nasal effects in humans as it does in rodents; and 2) to establish a possible occurrence of respiratory effects in human volunteers exposed to chlorine vapour at concentrations of 0, 0.1, 0.3 and 0.5 ppm.

The study was conducted in a double-blind fashion in 8 male volunteers using a repeated measures design, with randomly selected exposure sequences. Subjects were exposed for 6 h·day^{-1} on 3 consecutive days to each of the 4 exposure conditions. In nasal lavage, interleukin-8 (IL-8), albumin, total cell number, and percentages of neutrophils, lymphocytes, monocytes, eosinophils, and epithelial cells were determined. The lung function parameters that were analysed included forced vital capacity (FVC), forced expiratory volume in first second (FEV1), FEV1/FVC ratio, and maximal mid expiratory flow (MMEF). Data analysis was limited to 7 subjects since one volunteer decided to stop participating for reasons not related to the study.

Nasal lavage measurements did not support an inflammatory response or irritant effects on the nasal epithelium. For FVC, FEV1, and FEV1/FVC, no significant differences were found. MMEF was significantly different between the 0 and 0.5 ppm exposure, but this was attributed to an unexplained shift in baseline values during control (0 ppm) exposure.

The present data does not support an inflammatory effect in the nose nor shows changes in respiratory function at repeated exposure up to 0.5 ppm. This discrepancy with previous data in rodents can be attributed at least in part to differences in respiratory tract airflow characteristics.

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Keywords: Chlorine human volunteer study
inflammation
interleukin-8
lung function
nasal lavage

Received: July 15 1999
Accepted after revision June 19 2000

This study was supported by EUROCHLOR Brussels (Belgium).

Chlorine (Cl_2) gas is potentially irritating to the mucous membranes of the eyes and the respiratory tract. It is widely used in the manufacture of plastics, various organic and inorganic chemicals, in the paper industry and as a disinfectant. Acute effects of high chlorine exposure are well known in laboratory animals and humans. Data for human risk assessment of inhaled chlorine at low concentrations are provided by several chronic inhalation studies in rodents and primates [1–3]. Exposure to 2.3 ppm (6.8 mg·m^{-3}) chlorine for 6 h·day^{-1}, 5 days·week^{-1} for 1 yr resulted in eye irritation as well as mild focal hyperplasia and cilia loss in the nasal passages and trachea of Rhesus monkeys [1]. At lower concentrations (0.5 and 0.1 ppm) minimal nasal hyperplasia was observed and its clinical relevance was questioned by the authors. Exposure of rats and mice to 0, 0.4, 1.0 and 2.5 ppm Cl_2 (up to 24 months) [2] and re-examination of the previous Rhesus monkey tissue samples [3] showed that Cl_2 induced lesions were confined to the respiratory tract. At equivalent airborne concentrations (~2.5 ppm), responses were less severe in monkeys but extended more distally to involve the trachea, while in rodents lesions were confined to the nose. The difference between lesion distribution in rodents and monkeys can be attributed at least in part to airflow driven regional dosimetry patterns [3].

There is a paucity of human data on the effect of chlorine on the upper respiratory tract. In contrast, effects on the lower airways have been reported. In humans exposed to 0.5, 1.0 and 2.0 ppm no difference was found at the 0.5 ppm level in forced vital capacity (FVC) and forced expiratory volume in one second (FEV1), and with respect to increased subjective irritation [4]. Similar 8 h exposure at 0.5 ppm produced no significant pulmonary function changes and less subjective irritation. At 1.0 ppm, six of the 14 subjects showed increased mucous secretion from the nose and increased mucus in the hypopharynx. In another human study ROTMAN *et al.* [5], found only non-consistent changes at 0.5 ppm. At 1.0 ppm, changes were observed in FVC, FEV1, peak expiratory flow (PEF), forced expiratory flow rate at 25 and 50% of the vital capacity (FEF_{25} and FEF_{50}, respectively) and airway resistance. Most of the results had returned to normal by the next day.

Considering the previous data, the aim of the present study was to investigate the effects of short-term (6 h·day^{-1}, 3 days·week^{-1}) chlorine exposure (0, 0.1, 0.3 and 0.5 ppm) on both nasal epithelium and the lower respiratory tract in man. This study focused on (nasal) inflammatory effects since inflammation is considered to be an intermediate to chronic airway effects [6, 7] and recent work in mice, rats

and monkeys [3] has revealed the presence of eosinophils in a dose-response fashion after a one-or-two-yr exposure of rodents to chlorine (up to 2.3 ppm). The inflammatory effects were assessed by counting the total number of cells and the proportion of neutrophils, eosinophils, lymphocytes, monocytes and epithelial cell in nasal lavage. In addition, albumin and interleukin-8 (IL-8) were measured in nasal lavage fluid (NALF). Albumin is an indicator for epithelial permeability and IL-8 represents a sensitive biomarker of an inflammatory response [8]. To test for functional effects on the airways, flow-volume curves were used to measure lung function. FVC, FEV_1, MMEF and FEV_1/FVC are used to indicate obstruction of the large and small airways. Nasal lavage and lung function measurements were carried out before and after each exposure and one and four days after exposure. Lung function was also measured two weeks after the last exposure.

Methods

Study design

Testing was conducted in 8 subjects using a repeated measures design. Subjects complying with all study selection criteria were exposed on three consecutive days, 6 h·day^{-1}, to four conditions: 0, 0.1, 0.3 and 0.5 ppm chlorine (fig. 1). The exposure periods were spaced eleven days apart. Subjects were exposed in two groups of four (A and B), based on the availability of the subjects and by ballot. The exposure sequences were assigned by lot, and are shown in figure 1. The exposure to the test substance and the effect measurements were conducted in a double-blind fashion, *i.e*. neither the subject nor the co-investigators were aware of exposure conditions. The persons involved in chlorine generation were not involved in the effect measurement procedures.

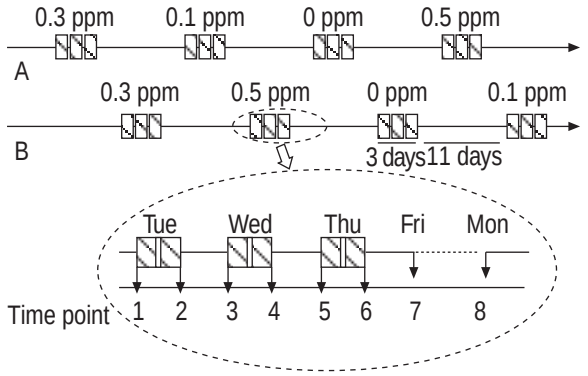

Fig. 1. – Study design of this human volunteer study, showing the consecutive exposure of 2 groups (A, B) of 4 volunteers to different concentrations of chlorine, for three consecutive days. After each exposure week, an exposure free-week was given to the subjects, which allowed for the study of recovery in the previously exposed group (A), while exposing the other group (B). Within each exposure period, subjects were exposed for 3 consecutive days 6 h·day^{-1} starting on Tuesday morning: nasal lavage and lung function measurements were performed at 8 time-points (1–8) for each exposure period, resulting in a total of 32 measurement time points for each subject. Exposure related data were evaluated by comparing values measured in the control (0 ppm) week with those during chlorine exposure weeks. As such, values measured during individual time points (*i.e*. 1–8) as well as changes in parameters over different time intervals (*e.g*. change in value during 6 h exposure on Tuesday, *i.e*. 2 minus 1) were statistically analysed. In addition, mean values of different time points within an exposure week were also combined (*e.g*. mean value of measurement at the three daily post-exposure time points 2, 4, and 6).

Inclusion and exclusion of subjects

For inclusion in the study, each volunteer had to meet the following criteria: 1) be male and Caucasian, 2) 20–50 yrs of age, 3) healthy as determined by medical and laboratory examination, 4) have normal lung function (*i.e*. FEV_1>90%; FVC>80%) [9], 5) an interleukin-6 (IL-6) concentration in NALF under detection limit (<20 pg·mL^{-1}), 6) skin calliper: body fat volume <30%, 7) Dutch as their native language, and 8) have given written informed consent.

Subjects were excluded when one of the following exclusion criteria was met: 1) a history of medical/surgical disease that may significantly affect the outcome, 2) hay-fever or rhinitis based on anamnesis and NALF, 3) abnormal respiratory impedance values (*i.e*. frequency dependence of resistance<0; resonant frequency >15Hz) [10], 4) a history of alcohol, amphetamine, cocaine, barbiturate, or other drug abuse, 5) participated in a clinical study within 3 months of present study, 6) presently using any chronic medication, 7) using more than 28 alcoholic beverages a week, 8) show evidence of liver or kidney dysfunction, 9) an employee of the TNO research institute or their first or second removed relatives, 10) be claustrophobic, 11) be a smoker, 12) having a cold, the week before or during the study, 13) be a regular swimmer, 14) regularly in contact with chlorine, bleaching agent or known respiratory irritants.

Exposure

The subjects were exposed to the test material for 6 h·day^{-1} (9:00–12:00 h and 12:30–15:30 h), on 3 consecutive days·week^{-1} over alternate weeks for a total period of eight weeks (fig. 1). Subject exposures were carried out in an air-conditioned exposure chamber (13.6 m^{-3}). The number of air changes was slightly more than 2·h^{-1}. The temperature range was 21.5–23.4°C, and the relative humidity range was 37–50%. The inhalation equipment was designed to expose the subjects to a continuous supply of fresh test atmosphere. To generate the test atmosphere, the test material was passed from the gas cylinder, which was kept just outside the exposure room, *via* a special reducing valve, stainless tubing and a mass flow controller to a mixing chamber where it was mixed with nitrogen. The nitrogen was also delivered *via* a reducing valve and a mass flow controller. Using a third mass flow controller, a small, filtered part of the generated mixture was passed to the exposure room where it was mixed with clean air. The main part of the mixture was passed to the exhaust using a constant pressure controller.

Nasal lavage

Five mL of sterile phosphate buffered saline solution (37°C) was delivered into each nostril and allowed to remain there for 10 s, during which the volunteers should hold their breath. During the morning sessions and during the 1-day and 4-days after exposure the left nostril was

used for lavage, while in the afternoon sessions the right nostril was chosen to avoid possible wash-out effects due to repetitive washing of one nostril within a single day [11]. The lavage fluid was processed as described previously [12]. Albumin was measured in NALF aliquots by an automated micro-assay using immunoturbidometry with Beckman reagents (Array, Beckman Inc. Mijdrecht, the Netherlands). IL-8 and IL-6 (used an exclusion criterion) were determined with specific enzyme-linked immunosorbent assays (ELISA) as described previously [13], with a detection limit of 20 $pg \cdot mL^{-1}$ for both assays. Cytospin preparations were scored by two cytologists: if present, 300 cells were counted and damaged cells excluded. If less than 20 cells were present, no differential cell count was performed. Total cell number was determined by using a haemocytometer.

Lung function measurements

Lung function was evaluated using both the effort-dependent (FEV_1, FVC, FEV_1/FVC) and effort-independent (MMEF) parameters from forced-expiratory manoeuvres recorded using a portable Jaeger Masterscope (Breda, the Netherlands) equipped with a pneumatograph. All flow-volume values were related to European community for Steel and Coal (ECSC) reference values for individual diagnosis [9]. All subjects were measured by the same operator (except the check-out measurement) and using the same apparatus. The spirometer was calibrated at 4 h intervals on ambient conditions (pressure, temperature) and volume. Data were evaluated according to the ECSC and American Thoracic Society (ATS) criteria [9, 14].

Statistical evaluations

For all effect parameters, the distribution characteristics were examined using the K-S- Lilliefors and Shapiro-Wilks tests. Since the effect parameters lacked normal distribution, non-parametric tests were used. First, the pre-exposure baseline values "over-time" (*i.e.* the mean scores of the Tuesday morning measurements grouped by study period) were tested using the Friedman′s test (fig. 1). In addition, data were evaluated with respect to the mean scores of post-exposure (Monday-morning) measurements. A p-value of less than 0.05 was considered to be statistically significant.

Exposure related data were evaluated using the Wilcoxon matched-pairs tests. Control values (i.*e.* data from the zero exposure condition) were compared with mean values of the three exposure conditions (0.1, 0.3, and 0.5 ppm) combined. This test was applied as a first indication of a possible difference between exposure to Cl_2 and control (0 ppm). To identify differences between all four exposure conditions, the Friedman′s test was applied. Correcting for the number of planned post-hoc comparisons (0.5 *versus* 0 ppm; 0.3 *versus* 0 ppm; 0.1 *versus* 0 ppm), a value of $p<0.017$ was considered to be statistically significant. In case of a significant finding, additional Wilcoxon-tests ($p<0.05$) were carried out to compare all exposure group data to the control condition. Finally, the Cochran-MantelHaenzel (non-zero) statistics were calculated to examine the association between scores on the effect parameters and the Cl_2 exposure levels. It is noted that a significant result in this case, does not necessarily indicate a linear trend. For statistical analyses SPSS (version 6.0 and 7.0) and the SAS software package (version 6.12; Heidelberg, Germany) were used.

Results

General and exposure characteristics

The demographic and (baseline) lung function characteristics of the 8 subjects are shown in table 1. The main reasons for the exclusion of other screened subjects were: deviant impedance values [10], detectable IL-6 in nasal lavage (>20 $pg \cdot mL^{-1}$) and claustrophobia. The concentration of Cl_2 was recorded continuously and calculated at 10 min intervals during the exposure sessions. The mean results of these determinations are listed in table 2 and reveal a high degree of compliance with the desired concentrations, and no difference between the two exposure sessions (A and B).

Adverse events/respiratory symptoms

At the end of each session the well-being of all subjects was checked by a physician. These adverse events are listed in table 3. None of the registered adverse events were judged to be treatment-related *per se*. In 6 cases the relation to exposure was considered as "not", 18 cases were judged as "unlikely" (*i.e.* relation to exposure is unlikely, but not impossible), while in 9 cases the relation to Cl_2 exposure was judged "possible" (*i.e.* relation is not likely, but may exist). None of these adverse events was reason to undertake action with regard to the protocol. However, by the end of the study it appeared that one of the subjects (NR. 8) had a thyroid carcinoma (follicular)

Table 1. – Individual demographics and baseline lung function from subjects included in the study

Subject	Protocol A/B	Age yrs	Weight kg	Height cm	FEV_1* $L \cdot s^{-1}$	FVC* L	MMEF*	Alcohol**
1	A	25	76.0	188.3	5.26±0.07	6.66±0.09	4.43±0.16	15–21
2	A	22	69.3	177.5	4.83±0.05	6.00±0.14	4.37±0.07	<1
3	A	23	77.3	189.6	4.63±0.04	5.56±0.03	4.41±0.28	8–14
4	A	35	89.0	188.7	6.67±0.34	7.78±0.19	6.90±0.91	1–7
5	B	22	83.1	188.3	5.03±0.05	6.45±0.15	4.08±0.18	1–7
6	B	22	63.9	182.0	4.17±0.05	5.38±0.04	3.48±0.14	22–28
7	B	22	85.9	188.8	5.09±0.18	6.38±0.19	4.47±0.31	1–7
8	B	35	91.1	188.2	4.97±0.08	6.10±0.12	4.64±0.37	0
Mean***					5.10±0.78	6.32±0.80	4.59±1.00	

Data are presented as mean%plusmn;SD. *: Mean±SD of baseline-week, determinations on Tuesday pre-exposure (n=4) in 4 different week; **: number of alcoholic beverages per week; ***: subject number 8 excluded (n=7).

Table 2. – Chlorine concentrations in the test atmosphere for both exposure group protocols

Target concentration ppm[a]	Day	Group A	Group B
0.1	1	0.11±0.01 (n=37)	0.10±0.01 (n=37)
	2	0.11±0.00 (n=37)	0.10±0.00 (n=37)
	3	0.11±0.00 (n-37)	0.09±0.02 (n=37)
0.3	1	0.29±0.02 (n=36)	0.31±0.01 (n=36)
	2	0.30±0.01 (n=37)	0.30±0.01 (n=30)
	3	0.30±0.01 (n=37)	0.30±0.01 (n=37)
0.5	1	0.50±0.03 (n=37)	0.53±0.04 (n=36)
	2	0.49±0.03 (n=37)	0.52±0.02 (n=37)
	3	0.50±0.03 (n=37)	0.52±0.02 (n=37)

Data are presented as mean±SD. [a]: Sequence of exposures in group A: 0.3 ppm, 0.1 ppm, 0 ppm. 0.5 ppm; group B: 0.3 ppm, 0.5 ppm, 0 ppm, 0.1 ppm. n: number of subjects.

which was judged as not treatment related. The subject decided not to participate any further in the study and although he completed nearly all the tests, this subject was excluded from further statistical evaluations.

Effects on nasal lavage parameters

Nasal lavage data are shown in table 4. The table shows the mean values of pre-post changes within the three consecutive exposure days, as well as the mean values of the Friday and Monday postexposure measurements, for all four different exposure conditions. No differences were observed in baseline values of any of the nasal lavage parameters over time. This testing was done by comparing the mean scores of the Tuesday morning measurements grouped by study period (Friedman′s test, $p<0.05$). In addition no significant time-effect was noted with respect to the mean scores of the Monday morning (post-exposure) nasal lavage parameters.

Albumin and IL-8 values (table 4) were all within range of previous studies in the laboratory [12]. Significant differences were found between the control group and the combined exposure conditions (0.1, 0.3 and 0.5 ppm) for albumin over the time interval 2–1 (*i.e.* Tuesday post-exposure minus pre-exposure) (table 4), and for IL-8 over the time interval 3–1 (*i.e.* Wednesday pre-exposure minus Tuesday pre-exposure) (not shown). For IL-8 levels, the Friedman′s analyses revealed no significant differences between all four exposure conditions and no association between exposure levels and IL-8. On the other hand, albumin data revealed significant differences ($p<0.017$) for two time points. However, these are explained by a difference between 0 and 0.1 ppm Cl_2 (post-hoc Wilcoxon testing, table 5). Also correlation analysis (Cochrane-Mantel-Haenzel) indicated that albumin levels were related to exposure at several time points, but these findings were not consistent with the results of the post-hoc tests (table 5).

For the total cell counts a significant difference was found between the control conditions and the combined exposure conditions at time interval 4–1 (data not shown). Friedman analysis revealed a significant difference in total cells between all exposure conditions for the time interval 7–1. At this time interval, however, the "post hoc" Wilcoxon tests only indicated a significance between the 0.1 ppm and control conditions and not at higher exposure (table 5). Moreover, no significant correlations were observed between total cell counts and exposure levels.

Differential cell counts revealed several significant differences, that should be interpreted with caution. With regard to neutrophil percentages, significant differences between control and combined exposure conditions were found at several time points/intervals. However, Friedman analysis revealed no difference between all exposure conditions that would forward additional post-hoc testing (*i.e.* $p<0.017$ table 5). Cochran-Mantel-Haenzel analysis suggests a decreasing trend for the percentage neutrophils along with increasing Cl_2 exposure at time points/intervals 4, 4–1, 4–3, and (2, 4, 6)–1. However, it should be emphasized that changes in neutrophil percentage are interdependent with changes in other cell types, and that neutrophil percentages were found not to be significantly different at any of the exposure levels *versus* the control (zero) condition. With the exception of one time point (*i.e.* 4) no significant difference in the percentage of epithelial cells was found in control *versus* combined exposure conditions. Although the Cochran-Mantel-Haenzel statistic indicated an association between exposure level and percentage epithelial cells at 5 different occasions, the required statistical significance ($p<0.017$) was not attained in a Friedman′s test comparing all four exposure conditions. Monocyte percentages were not significantly different between the various exposure periods. Further detailed statistical analysis provides no indication for a positive or negative dose-response association with the Cl_2 exposure level. Although a significantly higher percentage of monocytes was found at pre-exposure time-points at 0.3 ppm compared to zero exposure (table 5), a

Table 3. – Overview of adverse events in relation to its likeliness to be associated to chlorine exposure, judged as "Impossible", "Unlikely", and "Possible", before breaking the treatment code

Impossible	Unlikely	Possible
Rhinorrhea (1)[a]; coughing (1)[a]; odontolithiasis (3); irritating sensation of the tongue (8); inguimal pain (8); thyroid carcinoma (8)	Headache (2); common cold (2, 4); light headed feeling (3, 6); rhinorrhea (3, 8); coughing (4); nasal twang (4); coughing after nasal lavage (4); fatigue (5, 8); sneezing (5); mental dullness (6); diarrhoea (6); nasal congestion (6); rhinitis (8); enlargement of lymph nodes (8); nausea (8); pain in region thyroidea (8); sleeping problem (8); concentration impairment (8)	Sinus tension (2); eye irritation (2, 3, 5, 6, 8); coughing (2, 8); nose congestion (6, 7); dry throat (6); dry mouth (6); irritation throat (8); expiratory wheeze (5); mucus production in nasal cavity (8)

[a]: Adverse event reported 1 week after second exposure. The subject number is given between brackets.

Table 4. – Mean or mean changes±SD in 7 subjects for nasal lavage and lung function parameters in the control and exposure conditions

Parameter	Concentration ppm	Tuesday Post-Pre 2 minus 1*	Wednesday Post-Pre 4 minus 3*	Thursday Post-Pre 6 minus 5*	Friday 7	Monday (week after) 8
Nasal lavage						
IL-8 pg·mL^{-1}	0	-279±45	-57±374	-300±190	852±408	855±438
	0.1	-123±176	-476±287	-315±290	988±738	916±366
	0.3	-1±577	-244±367	-180±389	979±774	723±508
	0.5	-123±233	-171±190	-270±156	499±151	545±306
Albumin mg·mL^{-1}	0	-8.9±14.4$^{\#}$	8.4±27.6	-22.1±30.5	35.0±30.3	44.9±51.7
	0.1	-3.2±10.7	-18.7±12.0	-9.9±8.9	21.4±12.4	17.0±4.9
	0.3	9.2±24.1	-0.3±5.6	0.8±7.4	51.7±104.8	35.9±53.7
	0.5	4.3±6.1	0.9±4.9	-4.7±4.5	15.9±14.2	18.8±15.7
Cells $\times 10^3$	0	1.6±7.9	-87.0±257.3	-25.8±46.9	33.4±36.8	42.0±48.7
	0.1	-7.5±11.9	-32.4±28.3	-14.9±26.5	15.5±18.8	30.6±35.5
	0.3	19.1±68.4	-28.3±65.7	-3.4±19.1	74.0±98.5	33.4±50.8
	0.5	0.4±5.5	-189.9±493.4	-7.6±18.7	26.5±58.3	27.1±21.5
Neutrophils %	0	-0.8±24.4	7.8±7.5	6.7±16.8	83.9±14.5	83.8±10.9
	0.1	1.5±17.5	-5.8±8.1	7.0±23.4	72.3±24.3	80.4±13.0
	0.3	-1.0±14.5	4.3±26.5	8.5±8.4	85.5±7.0	86.9±9.5
	0.5	-13.3±13.1	-12.5±15.5	9.6±13.2	78.4±12.3	79.9±7.1
Lymphocytes %	0	7.4±13.1	0.3±3.4	1.6±1.2	3.5±4.0	4.1±6.4
	0.1	0.5±4.2	2.3±3.1	3.8±5.3	5.6±8.7	3.6±5.6
	0.3	4.9±5.7	7.8±13.1	-2.1±7.1	7.0±8.6	2.5±3.1
	0.5	2.1±3.4	-2.7±6.5	0.9±2.5	4.5±4.0	6.1±5.3
Eosinophils %	0	0.8±1.2	-0.3±1.3	-0.5±0.3	0.3±0.4	0.1±0.1
	0.1	0.1±0.6	0.3±0.3	-0.8±2.6	0.3±0.5	0.3±0.5
	0.3	-4.5±11.8	-0.1±0.4	-4.5±11.7	0.2±0.3	0.2±0.2
	0.5	-0.3±0.9	-0.2±0.7	0.0±1.1	4.3±8.7	2.1±0.2
Monocytes %	0	1.5±1.8	0.6±1.0	0.4±0.6	0.3±0.4	0.4±0.4
	0.1	0.6±0.6	0.4±0.9	0.6±1.2	0.3±0.3	0.3±0.4
	0.3	1.5±2.7	0.4±1.8	0.6±2.8	2.2±2.1	0.7±0.8
	0.5	0.4±0.3	0.6±1.8	0.0±0.2	0.5±0.7	0.4±0.4
Epithelial	0	-8.9±14.8	-7.8±7.6	-7.6±18.1	11.9±15.2	11.7±10.9
	0.1	-2.7±16.3	2.8±6.5	-10.7±21.5	21.5±26.5	15.3±13.8
	0.3	-0.9±6.7	-3.8±19.5	-2.6±6.6	5.1±5.5	9.6±10.3
	0.5	11.2±14.5	14.9±14.4	8.7±14.2	12.3±2.3	11.5±8.9
Lung function						
FVC L	0	-0.13±0.10$^{\#}$	-0.02±0.15	-0.14±0.11	6.36±0.84	6.34±0.90
	0.1	-0.01±0.22	0.00±0.12	0.00±0.07	6.33±0.95	6.24±0.87
	0.3	0.03±0.13	-0.06±0.20	-0.01±0.09	6.30±0.80	6.34±0.92
	0.5	0.06±0.25	-0.15±0.43	0.02±0.11	6.38±0.90	6.37±0.92
FEV_1 L	0	0.00±0.08	0.13±0.24	0.03±0.17	5.10±0.73	5.08±0.77
	0.1	0.05±0.18	0.05±0.14	-0.03±0.08	5.01±1.00	5.05±0.91
	0.3	-0.03±0.19	-0.13±0.24	0.01±0.06	5.09±0.86	5.08±0.93
	0.5	0.05±0.15	-0.11±0.33	0.06±0.13	5.12±0.94	5.17±0.89
MMEF L·s^{-1}	0	0.39±0.31$^{\#}$	0.40±0.61	0.40±0.27	4.51±0.74	4.44±0.88
	0.1	0.19±0.35	0.20±0.19	0.01±0.28	4.52±1.59	4.52±1.38
	0.3	0.00±0.52	-0.19±0.51	0.11±0.17	4.60±1.27	4.51±1.25
	0.5	0.19±0.16	-0.16±0.32	0.18±0.38	4.60±1.31	4.71±1.16
FEV_1/FVC	0	0.01±0.01	0.02±0.03	0.02±0.02	0.80±0.03	0.80±0.04
	0.1	0.01±0.01	0.02±0.01	0.00±0.01	0.80±0.05	0.81±0.06
	0.3	-0.01±0.02	-0.01±0.03	0.00±0.01	0.81±0.04	0.80±0.04
	0.5	0.00±0.01	0.00±0.01	0.01±0.02	0.80±0.04	0.81±0.04

$^{\#}$: significant different from mean change calculated from the three exposure conditions (0.1, 0.3, 0.5 ppm) (Wilcoxon, $p<0.05$); *: daily changes for the three consecutive exposure days.

biological explanation for such a finding is not present. Since the absolute monocyte counts are rather low, this difference is considered to be caused by chance or minor variability in visual cytological scoring. Although eosinophils and lymphocytes were well detectable in nasal lavage, there was no or little statistical proof that these cell types are affected by Cl_2 exposure. No significant differences between different study periods were observed. Although an association between cell percentage and exposure was found at an incidental test point, comparison to findings with other cell types suggests that this is merely a matter of chance.

Effects on lung function parameters

With the exception of MMEF no differences were observed in baseline (*i.e.* Tuesday morning) values of lung function parameters over time. No significant time-effect

Table 5. – Results of post-hoc Wilcoxon tests comparing the exposure conditions and the control conditions

Time point or time interval	Parameter	Exposure used in comparison* ppm	p-value
Wednesday pre-exposure	Albumin	0.5	NS
		0.3	NS
		0.1	0.034 (↑)
Mean of Tuesday-, Wednesday-, and Thursday pre-exposures	Monocytes	0.5	NS
		0.3	0.018 (↑)
		0.1	SS
Thursday post-exposure minus Tuesday pre-exposure	MMEF	0.5	0.018 (↓)
		0.3	0.018 (↓)
		0.1	0.043 (↓)
Friday minus Tuesday pre-exposure	Cells	0.5	NS
		0.3	NS
		0.1	0.018 (↓)
Wednesday post-exposure minus Wednesday pre-exposure	Albumin	0.5	NS
		0.3	NS
		0.1	0.018 (↓)
Mean of Tuesday-, Wednesday-, and Thursday post-exposures minus Tuesday pre-exposure	MMEF	0.5	0.018 (↓)
		0.3	0.018 (↓)
		0.1	NS
Mean of Tuesday-, Wednesday-, and Thursday post-exposures minus Tuesday pre-exposure and Monday (week after)	MMEF	0.5	0.018 (↓)
		0.3	NS
		0.1	NS

Results are shown only for those parameters and time points that are significantly different in Friedman's four group test at $p<0.017$ (based on three planned comparisons *i.e.* control (0 ppm) *versus* 0.5 ppm, *versus* 0.3 ppm, and *versus* 0.1 ppm.) *: *Post-hoc* comparison *versus* control. NS: not significant; ↑: increase; ↓: decrease.

was noted with respect to the mean scores of the Monday morning (post-exposure) lung function parameters, *i.e.* this time also including MMEF values.

Several significant differences for FVC (*e.g.* time point 2, time interval 2–1), FEV_1 (*e.g.* time point 2, intervals 4–1, 7–1) and FVC/FEV_1 (*e.g.* time point 1, intervals 4–1, 6–1) were observed comparing different time-points or time intervals using the Wilcoxon matched pairs test. However, none of these differences "survived" more rigid statistical testing comparing the four exposure conditions using Friedman's test. The differences in MMEF, however, remained statistically significant (*e.g.* time interval 6–1) although changes are smaller than individual physiological variations. As already stated, comparison (Tuesday) pre-exposure MMEF revealed a statistically significant difference between the four study periods. Also post-hoc Wilcoxon analyses (table 5) indicated a difference between various periods, and was caused by the fact that MMEF values in the third period (zero exposure in both protocols) were significantly lower (4.29 L) than in other periods (0.1 ppm: 4.59 L; 0.3 ppm: 4.79 L; 0.5 ppm: 4.79 L). To further interpret this finding, one subjects (NR 04) with a high intra-subject variation in MMEF and clearly deviant from others was excluded from statistical analysis and testing was done using repeated measures ANOVA (data not shown). This analysis confirmed the baseline drift as a cause of the observed difference in MMEF.

Discussion

This study was carried out to evaluate the effects of short-term exposure to chlorine (0, 0.1, 0.3 and 0.5 ppm, 6 $h \cdot day^{-1}$, 3 consecutive days) on the respiratory tract in humans. Lung and nasal lavage parameters were determined to study the possible effects on airway obstruction in the large and small airways and to determine nasal inflammatory responses and the onset of epithelial irritation/damage.

Although previous chronic animal studies [2, 3] have demonstrated the presence of submucosal eosinophils in nasal airways passages in a dose-response fashion, the authors were not able to demonstrate an inflammatory or irritating effect using several markers in nasal lavage. No increase in eosinophil percentage or any other cell type, or albumin and IL-8 was detected after a 3 day exposure of up to 0.5 ppm. In finding explanations for this difference, it should be recognized that airflow characteristics play a major role in the distribution of lesions and interspecies differences. In addition, it should be mentioned that the high intra- and inter-individual variation coefficient of the biomarkers in nasal washings [8, 12], in the current design would require a minimal change of 80% (albumin, IL-8) or 150% for total cells to be detected as statistically significant when tested with parametric statistics. Previously IL-8 but not total cell number was found to be increased in NALF of workers exposed to high concentrations of endotoxin in cotton dust [12]. However, total cell counts were found to be affected in subjects chronically exposed to wood dust, grain dust, swine dust, to ozone, or after challenge with phtalic anhydride or formaldehyde [15–21].

Occasionally, changes in percentages of lymphocytes and monocytes were detected. However, given the relatively small number of cells counted for these cell types, data should be interpreted cautiously and it is concluded that these percentages are not affected by chlorine exposure. Analysis of the percentage of neutrophils, the most abundant cell type in nasal lavage [8, 18], indicated a

reduction of this cell type in response to Cl_2 exposure. However, the observed differences were not statistically significant at any of the exposure levels compared to sham exposure. On the other hand, the percentage of epithelial cells tended to increase with exposure. The responses of neutrophils and epithelial cells could be an antiparallel phenomena caused by the same denominator and exposure. However, in total cell counts epithelial cells were not included and changes in total cell counts were not related to exposure. Therefore, it is likely that the observed effect on epithelial cells may reflect the absolute number of cells.

In the current design, exposing humans up to 0.5 ppm Cl_2 several lung function differences were observed, which were no longer significant after more rigid statistical testing. However, a consistent statistical effect on MMEF was observed, caused by an unexplained baseline drift in MMEF values during the two consecutive control weeks. Previous human studies on lung function after short-term chlorine exposure [4, 5] found no or non-consistent changes in FVC, FEV_1 and FEF_{50} after 8 h exposure to 0.5 ppm chlorine. At 1.0 and 2.0 ppm Cl2 exposure was significant but reversible effects were noted on various indices derived from flow-volume curves. Interestingly, where in this study changes in MMEF seem to be caused by a drift in baseline values, ROTMAN *et al*. [5] found a decrease in FEF_{50} and an increase in airway resistance (R_{aw}) after 8 h exposure to 0.5 ppm of Cl_2. No drift in exogenous conditions (climate, air pollution) were found in the National registry data that could explain this baseline change in the two (consecutive) control weeks. No other medical cause or inclusion/exclusion criteria could have caused such a difference. Subjects that were hyperreactive or having small airway obstruction were excluded by impedance measurements [10] and high IL-6 in nasal lavage fluid. Although the treatment code was blinded for subjects and key investigators, both groups must have been able to detect the difference between existent or non-existent (sham) exposure. Whether this may have affected MMEF specifically is unlikely.

To conclude, no adverse effects of chlorine exposure to nasal and respiratory parameters were found at repeated exposure of human volunteers to chlorine (6 h, 3 days) up to 0.5 ppm. A statistically significant effect on maximal mid expiratory flow was found at 0.5 ppm chlorine, but this was due to a significant but unexplained drift in baseline (control exposure) values. Nasal lavage data did not indicate an inflammatory response or irritant effects on the nasal epithelium.

Acknowledgements. The authors would like to thank C. Balleux, J. de Gerlache for their contributions and the study monitors C. Braun and L. Bloemen. We also thank those that contributed to technical parts of the study, J. Arts and S. Spoor for inhalation techniques and H. de Vries for medical supervision. Finally, we thank all test subjects for their patience and compliance in the completion of this trial.

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