Int Arch Occup Environ Health (2005) 78: 189–197
DOI 10.1007/s00420-004-0586-2

ORIGINAL ARTICLE

D. Steiner · S. Jeggli · A. Tschopp · A. Bernard
A. Oppliger · S. Hilfiker · P. Hotz

Clara cell protein and surfactant protein B in garbage collectors and in wastewater workers exposed to bioaerosols

Received: 20 February 2004 / Accepted: 2 October 2004 / Published online: 16 March 2005

Abstract ***Objectives***: Inhalation of bioaerosols has been hypothesised to cause "toxic pneumonitis" that should increase lung epithelial permeability at the bronchioloalveolar level. Serum Clara cell protein (CC16) and serum surfactant protein B (SPB) have been proposed as sensitive markers of lung epithelial injury. This study was aimed at looking for increased lung epithelial permeability by determining CC16 and SPB in workers exposed to bioaerosols from wastewater or garbage. ***Methods***: Subjects (778 wastewater, garbage and control workers; participation 61%) underwent a medical examination, lung function tests [American Thoracic Society (ATS) criteria], and determination of CC16 and SPB. Symptoms of endotoxin exposure and several potential confounders (age, gender, smoking, kidney function, obesity) were looked for. Results were examined with multiple linear or logistic regression. ***Results***: Exposure to bioaerosols increased CC16 concentration in the wastewater workers. No effect of exposure on SPB was found. No clue to work-related respiratory diseases was found. ***Conclusions***: The increase in CC16 in serum supports the hypothesis that bioaerosols cause subclinical "toxic pneumonitis", even at low exposure.

D. Steiner · S. Jeggli · S. Hilfiker · P. Hotz (✉)
Occupational and Environmental Medicine Unit,
Sumatrastrasse 30,
8006 Zurich, Switzerland
E-mail: photz@ifspm.unizh.ch
Fax: +41-1-6344986

A. Tschopp
Department of Biostatistics, University of Zurich,
Zurich, Switzerland

A. Bernard
Unit of Toxicology, Catholic University of Louvain,
Brussels, Belgium

A. Oppliger
Institute of Occupational Health Sciences,
University of Lausanne,
Lausanne, Switzerland

Keywords Pulmonary surfactant-associated protein B · Clara cell protein · Spirometry · Sewage · Garbage

Introduction

Investigations conducted in workers exposed to wastewater or garbage have reported four main groups of symptoms or diseases (Bunger et al. 2000) attributed to contact with or inhalation of bioaerosols: irritative symptoms (skin, mucous membranes), toxic effects (organic dust toxic syndrome, impairment of lung function, gastrointestinal symptoms), infections, and allergies. Whereas infections and allergies do not seem to represent a frequent problem, toxic and irritative effects have been repeatedly found (Thorn et al. 2002; Yang et al. 2001; Bunger et al. 2000; Friis et al. 1999; Rylander 1999; Thorn et al. 1998; Ivens et al. 1997; Zuskin et al. 1996; Poulsen et al. 1995; Sigsgaard et al. 1994; Zuskin et al. 1993; Malmros et al. 1992; Nethercott and Holness 1988; Rylander et al. 1977; Rufener-Press et al. 1976).

Bioaerosols, i.e. "aerosols containing biologically active agents, e.g. microorganisms and their metabolites and toxins" (Poulsen et al. 1995), are emitted in the air when biologically contaminated wastes are handled. Endotoxin is one important component of bioaerosols and is assumed to cause a non-specific inflammation of the airways and alveoli with, at an advanced stage, a decrease in lung function. According to Rylander (2002; Rylander and Malmberg 1992), this non-specific inflammation is the physiopathological process common to several diseases, such as organic dust toxic syndrome (ODTS), mycotoxicosis, or grain fever, and comprises toxic alveolitis (toxic pneumonitis), which releases cytokines into the circulation. Those cytokines would, in turn, be responsible for general symptoms. If this hypothesis is right, the permeability of the lung epithelial barrier should be increased by the inflammatory process. Thus, a leak of lung proteins such as Clara cell protein

(CC16) or surfactant protein B (SPB) into the circulation should occur. A description of the physiopathological mechanisms linking inflammation and pneumoprotein leak has been given by Hermans and Bernard (1999).

The first purpose of the study was to examine whether the hypothesis of increased permeability of the lung epithelial barrier could be confirmed in workers handling wastes by using two pneumoproteins, CC16 and SPB, as indicators of the integrity of this barrier. The other purpose was to assess the current working conditions of garbage collectors. Only one study, which had suggested an increased risk of pulmonary damage, has been performed in Swiss garbage collectors (Rufener-Press et al. 1976).

Subjects and methods

All examinations were conducted in the Canton of Zurich between June 2000 and July 2002. They took place in the frame of a compulsory assessment of occupational risks required by Swiss law. All workers were informed about the purpose of the risk assessment and gave written consent. The study protocol was approved by the Swiss National Insurance Fund for Occupational Disease.

The study was planned as a prospective cohort study. Results presented here relate to the baseline examination some preliminary results of which have already been presented (Steiner et al. 2003). For reasons described elsewhere (Jeggli et al. 2004), it was attempted to have a population of 150 garbage collectors, 300 wastewater workers and 150 control workers. From the results reported by Bernard et al. (1994), a mean CC16 serum concentration of 16 μg/l, a standard deviation (SD) of 5 μg/l, and a clinically relevant difference of 4 μg/l were assumed. Thus, the power would be 90% in a group comprised of 50 control and 50 garbage workers at a level of significance of 5%.

Those eligible were municipal manual workers from the Canton of Zurich. All workers exposed to garbage dust from the two largest cities and all workers exposed to wastewater in the Canton of Zurich had the opportunity to participate, whereas the groups of control subjects were approached one by one and were asked to participate, until enough control subjects had entered the study according to power calculations.

Garbage collectors ($n = 86$; participation rate 28%) and workers from wastewater plants ($n = 355$; participation rate 90%) were compared with control subjects comprising gardeners ($n = 197$; participation rate 76%), workers maintaining waterways ($n = 52$; participation rate 79%), public transport workers ($n = 25$; participation rate 15%) and forestry workers ($n = 63$; participation rate 93%). Overall, 778 subjects entered the study (participation rate 61%). The participation of garbage workers remained low in one plant, although they were given the opportunity three times to participate in the medical examination.

All garbage collectors collected mixed household waste, in plastic bags or containers, and metal and bulky waste. Collection of household waste has been carried out mostly once (seldom twice) a week for a few years (formerly twice a week). Bags are loaded or containers emptied into a compactor truck by loaders. Most drivers remain in the cab and do not load (no function shift). Some of the garbage collectors also collect garden waste. Carcasses, glass, and paper are collected by garbage workers in one of both towns only. The work of the wastewater workers has already been described (Jeggli et al. (2004). Gardeners maintain the green areas of the city of Zurich (parks, graveyards, flower production, etc.). Workers maintaining waterways have jobs like gardeners in the summer and forestry workers in the winter. Public transport workers maintain tramways, buses and railways (welding, painting, metalworking, etc.). Forestry workers maintain the forests. The main problems in this group are physical work and hand–arm transmitted vibrations.

To minimise a recall bias, we attempted not to draw attention to work-related symptoms when explaining the study purpose. Procedures for collecting clinical data (check list, coding, quality control) have been described elsewhere (Jeggli et al. 2004). Current symptoms are defined as having occurred during the 4 weeks prior to the clinical examination if not stated otherwise. Work-related symptoms are defined as those brought about by a specific task, occurring during a specific task in co-workers as well, and without other cause. Fever, chills, fatigue, diarrhoea, and headache were defined, a priori, as general symptoms possibly due to toxic pneumonitis (Rylander 1999). Arthralgias were not included, because they may also result from ergonomic factors in garbage collectors. The questions about and definitions of respiratory symptoms and asthma (Appendix) were taken from the SAPALDIA study (Zemp et al. 1999; Leuenberger et al. 1998; Ackermann-Liebrich et al. 1991). We assessed smoking by using questions proposed by the European Community of Steel and Coal (revision 1967) and pack-years calculated or subjects classified into never, ex-, and current smokers either of cigarettes or of a pipe and/or cigars and/or cigarillos. The socio-economic level was defined by the highest education level attained by the subjects at the age of 20 years, with three levels (no apprenticeship/apprenticeship/university). Duration of exposure to garbage dust was estimated in years for the whole working life. In accordance with Ivens et al. (1997) and Nielsen et al. (1995), drivers were considered as not exposed if they did not load garbage. Exposure to wastewater was assessed as described by Jeggli et al. (2004). Work as a farmer was defined according to the Swiss census (codes 111.01 and 111.02; Meier 1996). Height and weight were measured with the subjects' shoes and jackets removed. Body mass index (BMI) is equal to weight/height^2 (in kilogrammes per square metre).

Spirometry was carried out with two Microlab spirometers (MicroLab ML 3300 and 3500; Micro Medical

Ltd., Kent, England) calibrated daily with a 3 l syringe (mostly at three different flow rates) and yearly by the manufacturer's local representative. Measurements were performed while the subjects were in a sitting position, and acceptability and reproducibility were assessed independently by two physicians in accordance with the criteria of the American Thoracic Society (ATS) 1995). Predicted values of forced vital capacity (FVC%) and forced expiratory volume in the first second (FEV_1%) were calculated according to Quanjer et al. (1993) as were the fifth percentile of FVC, FEV_1, and FEV_1/FVC.

Blood was taken without the subjects having fasted (Vacutainer, ref. 368441), kept at 4°C, and centrifuged within 7 h. Serum was kept at −20°C. Serum creatinine (S-creatinine) was determined with a Technicon RA 1000 (Technicon RA systems, 1989/1994). CC16 was determined by latex immunoassay after pre-treatment to avoid possible interference by complement, chylomicrons, and rheumatoid factor. All samples were analysed in duplicate at two different dilutions. Detection limit is 0.5 μg/l, average analytical recovery is 95%, intra-assay and inter-assay coefficients of variation range from 5% to 10% and correlation with a fluorescence enzyme immunoassay, using monoclonal antibodies, is 0.92 (Hermans et al. 1998). SPB was determined by enzyme-linked immunosorbent assay (Doyle et al. 1997) and results expressed in arbitrary units defined by a reference serum pool. All analyses were done in batches comprising samples from exposed and control workers. The laboratory did not know the exposure status. CC16 and SPB analyses were available for 720 and 732 subjects, respectively, because of blood sampling refusals and technical reasons.

Preliminary measures of exposure were carried out, and the details will be presented elsewhere (Oppliger et al. 2005). Briefly summarised, 11 wastewater treatment plants employing 2–117 workers were selected to represent various work conditions (small and large plants, plants with and without symptoms of exposure to bioaerosols). Airborne endotoxin was measured (by Limulus amoebocyte lysate assay) indoors and outdoors in winter and in summer by stationary sampling (over 4 h). Moreover, personal samples were taken during specific tasks that the workers associated with work-related diarrhoea, such as spraying off deposits from tank walls or grids.

Data analyses

The normality of the distribution was tested, and, if necessary, logarithmic transformations done or non-parametric tests used. Linear multiple regression models were laid down before the beginning of the study. In these models the dependent variables were FVC (in litres), FEV_1 (in litres per second), or FEV_1/FVC, and pneumoproteins. Independent variables were age (in years), gender (0: male; 1: female), height in metres (not included for FEV_1/FVC), pack-years, time elapsed since cessation of smoking (in years), asthma and/or symptoms of bronchitis (0: absent, 1: present), BMI, S-creatinine (for pneumoproteins only) and exposure as a wastewater worker, a garbage collector, and/or as a farmer. In some further analyses, the variables asthma or symptoms of bronchitis were not lumped together. Exposure was either a continuous (years) or a dichotomous (having never/ever been exposed; coded 0/1, respectively) variable. Workers with previous exposure only are lumped with the currently exposed workers if not stated otherwise. As a decreasing effect of exposure to endotoxin with increasing duration of exposure has been described (Rylander 2002), the number of days at work since the last day on leave was considered in exploratory analyses of serum pneumoprotein determinants. Collinearity and residuals of the final models were examined. The P value of the goodness-of-fit statistics of logistic regression models was always greater than 0.15. All calculations were done with SAS statistical software (version 6.12; SAS Institute, Cary, N.C., USA).

Results

The main characteristics of the workers are summarised in Table 1. There were 369 workers without previous or current exposure to bioaerosols from wastewater or garbage, 325 with exposure to wastewater aerosols but no garbage dust (15 with only former exposure), and 84 with exposure to garbage dust (currently or formerly). In the latter group 16 subjects were currently exposed to both garbage and wastewater and had nearly the same duration of exposure to bioaerosols from both garbage (median 11 years; 0.5–22.5 years) and wastewater (median 11 years; 0.5–21 years). Exposure shifts from garbage to sewage or conversely had occurred in eight subjects. Twenty-six subjects had only some former exposure, and median time elapsed since the end of exposure was 11 years (1 month to 31 years). Fifteen drivers did not leave the cab and had never been garbage collectors. Thus, they were considered as controls. Garbage collectors had a history of farming more often than the controls or wastewater workers (Table 1; $P = 0.008$, χ^2 test) but the length of exposure did not differ significantly between the three groups ($P = 0.9$; Kruskal–Wallis test). Three hundred and ten subjects had no exposure as a wastewater or garbage worker or as a farmer.

With respect to gender, age, and nationality (Swiss vs foreigners), no statistically relevant differences ($P > 0.1$) were found between participating and non-participating garbage collectors. Non-participating wastewater workers were slightly older (median age 50 years vs 46 years). By contrast, non-participating controls were older (47 years vs 41 years) and more often foreigners (53% vs 36%).

Endotoxin concentrations measured in wastewater plants by stationary samplers, both in winter and summer, ($n = 22$) ranged from 1.5 to 158 endotoxin units/m^3

Table 1 Characteristics of the study population. Values are median (5th to 95th percentile) or number (percent)

Characteristic	Control workers ($n=369$)	Wastewater workers ($n=325$)	Garbage workers ($n=84$)
Age (years)	41 (22–58)	47 (30–61)	44 (28–57)
Gender (male)	341 (92)	324 (99)	83 (99)
Education level			
Low	69 (19)	37 (11)	37 (44)
Middle	282 (77)	284 (88)	47 (56)
High	14 (4)	4 (1)	0
Swiss nationality	298 (81)	295 (91)	51 (61)
Smoking			
Never smoker	157 (43)	103 (32)	17 (21)
Ex-smoker	85 (23)	94 (29)	26 (31)
Current smoker	126 (34)	128 (39)	40 (48)
Pack-years (in smokers only)	15 (1–63)	20 (1–61)	20 (1–65)
Time since giving up smoking (years)[a]	10 (0–32)	15 (1–33)	6 (1–30)
Alcohol			
No/only socially	244 (66)	211 (65)	60 (71)
Daily	123 (34)	113 (35)	24 (29)
Height (m)	1.75 (1.63–1.86)	1.76 (1.65–1.86)	1.73 (1.64–1.86)
Weight (kg)	78 (60–102)	81 (65–110)	81 (63–102)
BMI (kg/m^2)	25 (21–32)	27 (22–34)	26 (22–33)
Symptoms of bronchitis[b]	57 (16)	33 (10)	16 (19)
Dyspnoea on exertion[b]	51 (14)	26 (8)	3 (4)
Ever asthma[b]	39 (11)	23 (7)	5 (6)
Current asthma[b]	14 (4)	7 (2)	1 (1)
Previous pneumonia	13 (4)	17 (5)	1 (1)
Duration of exposure[c]	0	10 (1–28)	9 (1–21)
Previous job change	31 (8)	37 (11)	9 (11)
Farming	59 (16)	32 (10)	18 (21)

[a]Number of former smokers: 85, 94, and 26, respectively
[b]Definition: see methods
[c]Details: see text

(EU/m^3) and concentrations above 100 EU/m^3 (104, 137, 158) were found only three times. From 15 measurements conducted with personal samplers during jobs suspected of causing work-related diarrhoea, concentrations above 100 EU/m^3 were found five times, whereas control measures ($n=11$) ranged between 0.1 and 21.4 EU/m^3.

After the exclusion of subjects with previous exposure only, work-related diarrhoea during the past month was reported by six wastewater workers and one control. Prevalence of non-work related diarrhoea was approximately 4.4 % in all three groups. Previous clinical history also suggested an increased prevalence of work-related diarrhoea in wastewater workers (17 wastewater workers but no garbage collectors and no control workers). Prevalence of work-related nausea during the past month was 0.5%, 1%, and 3% ($n=2$, $n=3$, and $n=2$) in control, wastewater workers and garbage collectors, respectively. However, no clustering of the work-related general symptoms during the past month [(work-related fever or chill ($n=2$), abnormal work-related fatigue ($n=23$), or work-related headache ($n=8$)] was found. Work-related irritation of conjunctiva, nose or throat ($n=15$) did not occur more often in the exposed subgroups. Too few subjects reported work-related cough or expectoration for us to be able to perform a statistical analysis ($n<6$).

It could be hypothesised that work relatedness of respiratory symptoms disappears with increasing duration of exposure because symptoms become chronic and persist after the end of exposure. Thus, work-related and non-work related symptoms of bronchitis were lumped together and examined in the whole group of currently and formerly exposed workers by logistic regression. The odds ratio (OR) for symptoms of bronchitis was clearly increased by smoking, whereas having ever been exposed to wastewater decreased it (OR 0.40; 95%CI 0.25–0.64). However, the effect of exposure duration was of borderline significance (OR for one exposure year 0.96; 95% CI 0.94–0.99). No statistically significant effect of garbage exposure was found ($P>0.1$). No significant differences in the prevalence of asthma (neither ever nor current) were found between the three subgroups (Table 1; $P>0.15$, χ^2 test). Three workers were diagnosed with hypersensitivity pneumonitis or ODTS, but no association with exposure to wastewater or garbage existed. The prevalence of infectious pneumonia (Table 1) did not differ significantly across the three groups ($P=0.2$; χ^2 test), and FVC%, FEV_1% and FEV_1/FVC were comparable in people with and without a history of pneumonia ($P>0.3$; Wilcoxon two-sample test).

With regard to lung function tests, 31 workers (4%), whose recordings did not meet acceptability criteria, were excluded. The prevalence ($n=54$; 7.3%) of recordings that did not meet reproducibility criteria was comparable in control, wastewater, and garbage workers ($P=0.8$; χ^2 test), and FVC % and FEV_1 % were

comparable in workers with and without reproducible recordings as well ($P = 0.2$ and $P = 0.8$, respectively; *t*-test). Workers with only former exposure to bioaerosols (garbage and/or wastewater) had FVC%, FEV_1% and FEV_1/FVC very similar to those still currently exposed ($P > 0.2$; Wilcoxon two-sample test).

The results of spirometric measurements and determinations of CC16 and SPB are presented in Table 2, according to smoking status.

In the whole group, CC16 (median 9.3 μg/l; 5th–95th percentile 4.2–19.0) was weakly associated with S-creatinine, measured FVC and FEV_1, and FEV_1/FVC (Spearman's $r = 0.07$–0.11; $0.003 < P < 0.07$). No association between low or high (below the 5th percentile or above the 95th percentile) CC16 concentrations and endotoxin exposure (as indicated by work-related diarrhoea) was found, but the analysis was limited by the small number of cases. In the whole group, SPB (median 0.79; 5th and 95th percentile 0.26–1.82 arbitrary units) did not correlate with S-creatinine, measured FVC or FEV_1, and FEV_1/FVC ($0.08 < P < 1.0$).

The results of multiple linear regression are summarised in Table 3. Further analyses with multiple regression showed that duration of exposure as a garbage collector, as a wastewater worker, or as a farmer had no significant effect ($P > 0.14$) on FEV_1. With respect to FEV_1/FVC, having ever been exposed to wastewater decreased the FEV_1/FVC (Table 3), with a dose–effect relationship (multiple regression coefficient for 1 year of exposure to wastewater was −0.08; standard error = 0.03; $P = 0.01$). In contrast, duration of exposure as a farmer or a garbage collector was not significant ($P > 0.2$). With regard to FVC, having ever been exposed to wastewater was associated with an increased FVC (multiple regression coefficient = 0.16; standard error = 0.05; $P = 0.0004$), but duration of exposure to wastewater had no significant effect ($P = 0.12$). There were too few workers with FEV_1 or FEV_1/FVC under the 5th percentile for meaningful logistic regressions to be performed.

As for CC16, the effect of smoking was consistently highly significant and decreased the CC16 concentration. Having ever been exposed to wastewater (Table 3) and duration of exposure to wastewater (partial correlation coefficient 0.05 for 1 exposure year; standard error 0.02; $P = 0.04$) increased the CC16 concentration, whereas exposure as a garbage collector or a farmer had no significant effect ($P > 0.1$). However, the adjusted r^2 was always low (at most 6%). The results were not changed when we used the number of daily cigarettes or smoking categories instead of pack-years, included measured FVC or excluded workers with only previous exposure to bioaerosols. When the number of days at work since the last day on leave was included in the regression model, it did not improve the regression equation either. However, only six workers currently exposed to bioaerosols had spent more than 2 weeks away from work. Some further exploratory analyses did not give clues to further possible predictor variables and confirm the effect of smoking and bioaerosols from wastewater. Having ever had asthma and current asthma were never significant ($P > 0.05$) in multiple regression.

In univariable analyses SPB concentration was higher in current smokers (Table 2) than in never or ex-smokers ($P = 0.04$; Kruskal–Wallis test). No association with asthma (ever or current) appeared. In multivariable analyses the full model was not significant ($P = 0.08$;

Table 2 Lung function, CC16, and SPB results. Values are median and 5th and 95th percentile. Thirty-one spirometry recordings did not meet acceptability criteria and were excluded, and some subjects had missing values for FEV_1 and/or FVC. For SPB and CC16 some determinations are missing (see methods). *P* level of significance of the differences between occupational subgroups in the same smoking category (one-way analysis of variance), *n* subgroup size

Parameter	Control workers	Wastewater workers	Garbage workers	*P*
FVC (percent predicted)				
Never smokers	98.1 (78.9–123.5) $n = 148$	105.2 (83.6–122.9) $n = 98$	97.7 (85.3–120.3) $n = 16$	0.009
Ex-smokers	99.4 (78.3–119.5) $n = 80$	101.0 (87.7–118.6) $n = 86$	103.1 (88.8–120.3) $n = 22$	0.7
Current smokers	99.3 (81.7–118.5) $n = 121$	102.3 (83.1–121.7) $n = 121$	96.0 (73.7–116.4) $n = 38$	0.007
FEV1 (percent predicted)				
Never smokers	98.5 (78.9–117.9) $n = 151$	102.6 (81.2–124.5) $n = 99$	100.2 (82.2–115.0) $n = 17$	0.05
Ex-smokers	98.6 (72.2–117.9) $n = 81$	101.3 (79.6–119.7) $n = 90$	100.9 (77.8–119.5) $n = 22$	0.6
Current smokers	96.4 (74.5–115.8) $n = 120$	98.6 (78.4–117.3) $n = 121$	91.5 (62.6–124.7) $n = 38$	0.004
FEV_1/FVC (FEV_1 in % of FVC)				
Never smokers	82.5 (71.7–90.3) $n = 148$	81.0 (71.1–88.9) $n = 98$	82.8 (74.5–88.6) $n = 16$	0.07
Ex-smokers	80.7 (70.8–88.7) $n = 80$	81.0 (67.9–88.5) $n = 86$	79.5 (72.1–85.6) $n = 22$	0.7
Current smokers	80.4 (69.2–88.8) $n = 120$	79.4 (65.5–86.3) $n = 120$	80.8 (49.6–88.5) $n = 37$	0.3
CC16 (μg/l)				
Never smokers	9.4 (4.3–18.0) $n = 143$	11.0 (5.6–23.0) $n = 98$	11.3 (5.6–21.0)$n = 17$	0.01
Ex-smokers	9.4 (4.7–20.9) $n = 79$	10.2 (5.8–20.2) $n = 85$	9.7 (4.9–18.5) $n = 24$	0.5
Current smokers	8.1 (2.5–16.9) $n = 115$	8.7 (3.8–18.1) $n = 120$	7.7 (1.6–21.6) $n = 37$	0.2
SPB				
Never smokers	0.75 (0.24–1.60) $n = 147$	0.74 (0.25–1.88) $n = 99$	0.58 (0.22–1.49) $n = 17$	0.3
Ex-smokers	0.87 (0.25–1.93) $n = 78$	0.68 (0.18–1.98) $n = 86$	0.58 (0.32–1.01) $n = 24$	0.02
Current smokers	0.87 (0.29–1.80) $n = 118$	0.87 (0.28–1.96) $n = 123$	0.83 (0.30–2.31) $n = 38$	1.0

Table 3 Multiple linear regression: partial regression coefficients and *P* levels. Values indicate the partial regression coefficient with the corresponding significance level. *NA* not applicable

Parameter	FEV_1 (l/s)	FEV_1/FVC	CC16 (μg/l)	Log SPB (arbitrary units)
Intercept	−2.11 (0.0001)	82.0 (0.0001)	6.54 (0.2)	0.11 (0.7)
Gender (male = 0; female = 1)	−0.46 (0.0001)	1.83 (0.11)	−0.19 (0.9)	−0.09 (0.14)
Age (years)	−0.02 (0.0001)	−0.14 (0.0001)	0.03 (0.11)	0.002 (0.15)
Height (m)	4.04 (0.0001)	NA	1.65 (0.5)	−0.16 (0.3)
Pack-years[a] (number)	−0.006 (0.0001)	−0.07 (0.0001)	−0.04 (0.0005)	0.003 (0.0008)
Time since ceased smoking (years)	0.002 (0.4)	0.04 (0.2)	0.02 (0.5)	−0.0007 (0.6)
BMI (kg/m^2)	−0.01 (0.02)	0.21 (0.0005)	−0.11 (0.03)	−0.004 (0.2)
Asthma and/or symptoms of bronchitis (no = 0; yes = 1)	−0.10 (0.06)	−1.53 (0.01)	−0.15 (0.8)	0.02 (0.5)
Creatinine (μmol/l)	NA	NA	0.02 (0.11)	0.0007 (0.4)
Exposure to bioaerosols from				
Wastewater[b] (no = 0; yes = 1)	0.09 (0.02)	−1.03 (0.03)	1.01 (0.009)	−0.05 (0.02)
Garbage[b] (no = 0; yes = 1)	−0.09 (0.15)	−0.77 (0.3)	0.75 (0.2)	−0.06 (0.07)
Farming[b] (no = 0; yes = 1)	−0.03 (0.6)	−1.21 (0.06)	−0.72 (0.2)	−0.03 (0.3)
Adjusted r^2	0.45	0.14	0.03	0.03

[a]Smoking: in statistical analyses relating to SPB, number of cigarettes smoked daily were used instead of pack-years (see text)
[b]Exposure defined as never (0)/ever (1); models with duration of exposure: see text

F test). Exploratory analyses using number of daily cigarettes instead of pack-years found a positive association with smoking and a negative association with having ever been exposed to wastewater (Table 3). However, duration of exposure as a wastewater worker, garbage collector or farmer was without effect ($P > 0.4$ for all three exposure indicators), whereas the effect of daily number of cigarettes remained significant ($P = 0.003$). Adjusted r^2 was low (< 4%). The inclusion of the number of days at work since the last day on leave did not improve the regression equation, and the effect of duration of exposure was not improved after exclusion of the workers with only former exposure.

Regression using the CC16/SPB ratio as an endpoint (Robin et al. 2002) did not disclose unexpected findings (details not shown). Firstly, job change prior to current job because of any health problem was associated with respiratory symptoms and function (Table 4). Three sources of bias were further examined. However, no differences in the prevalence of previous job change were found between control, wastewater, and garbage workers (Table 1; $P = 0.4$; χ^2 test). Between the subgroups of garbage collectors, wastewater workers, and control workers that had previously changed job, FVC%, FEV_1%, and FEV_1/FVC did not differ significantly ($0.07 < P < 0.7$). Secondly, participants from the two plants with low participation rates ($n = 83$) may have differed from the whole population of these plants and biased the results. Thus, the main regression analyses were carried out again after exclusion of those two plants. Having ever been exposed to garbage decreased SPB more clearly in this model (Table 5). However, in the model using duration of exposure to garbage, the effect of garbage was not confirmed ($P = 0.2$). Thirdly, the large dispersion of the CC16 and SPB concentrations may suggest that the criteria defining a ''healthy'' lung

Table 4 Characteristics of workers with previous job change. Values are median (5th to 95th percentile) or number (percent). *P* level of statistical significance calculated with χ^2 test, Fisher's exact test, *t* test, or Wilcoxon two-sample test, as appropriate

Characteristic	Previous job change		
	No ($n = 696$)	Yes ($n = 77$)	*P*
Symptoms of chronic bronchitis	86 (12)	20 (26)	0.002
Ever asthma	51 (7)	15 (19)	0.002
Current asthma	16 (2)	5 (7)	0.05
FVC%	101 (81–122)	98 (78–118)	0.08
FEV_1%	99 (78–119)	96 (74–115)	0.03
FEV_1/FVC	81 (69–89)	79 (70–86)	0.1
CC16 (μg/l)	9.3 (4.2–19.0)	8.7 (2.5–17.5)	0.3
SPB (arbitrary units)	0.79 (0.25–1.80)	0.72 (0.28–1.94)	1.0
Pack (years)	18 (1–60)	23 (4–65)	0.04
Current smoking	256 (37)	36 (47)	0.1
BMI≥30 kg/m^2	99 (14)	17 (22)	0.1
Age (years)	44 (23–59)	46 (30–56)	0.6
Lowest education level	127 (18)	13 (17)	0.8
Exposure to garbage (years)[a]	9 (0.5–27) ($n = 75$)	9 (5–14) ($n = 9$)	0.7
Exposure to wastewater (years)[a]	11 (0.5–27) ($n = 306$)	10 (1–24) ($n = 41$)	0.6

[a]Calculations were restricted to workers exposed to garbage and wastewater, respectively

Table 5 Multiple linear regression: partial regression coefficients and *P* levels in the restricted population. Values indicate the partial regression coefficient with the corresponding significance level. Both plants with low participation rate excluded ($n = 83$). *NA* not applicable

Parameter	FEV_1 (l/s)	FEV_1/FVC	CC16 (μg/l)	Log SPB (arbitrary units)
Intercept	−1.97 (0.0004)	82.8 (0.0001)	5.96 (0.3)	0.001 (1.0)
Gender (male = 0; female = 1)	−0.46 (0.0001)	1.58 (0.18)	−0.35 (0.8)	−0.07 (0.20)
Age (years)	−0.02 (0.0001)	−0.14 (0.0001)	0.04 (0.07)	0.001 (0.2)
Height (m)	3.94 (0.0001)	NA	2.09 (0.5)	−0.07 (0.7)
Pack-years [a] (number)	−0.006 (0.0001)	−0.07 (0.0001)	−0.04 (0.0005)	0.003 (0.002)
Time since ceased smoking (years)	0.002 (0.5)	0.05 (0.13)	0.03 (0.3)	−0.0006 (0.7)
BMI (kg/m^2)	−0.01 (0.05)	0.18 (0.004)	−0.09 (0.12)	−0.005 (0.11)
Asthma and/or symptoms of bronchitis (no = 0; yes = 1)	−0.11 (0.06)	−1.64 (0.01)	−0.07 (0.9)	0.01 (0.8)
Creatinine (μmol/l)	NA	NA	0.01 (0.3)	0.0007 (0.4)
Exposure to bioaerosols from				
Wastewater[b] (no = 0; yes = 1)	0.08(0.04)	−1.13 (0.02)	0.92 (0.03)	−0.04 (0.11)
Garbage[b] (no = 0; yes = 1)	−0.10 (0.2)	−0.90 (0.3)	0.95 (0.2)	−0.12 (0.006)
Farming[b] (no = 0; yes = 1)	−0.03 (0.6)	−0.98 (0.15)	−1.21 (0.04)	−0.04 (0.3)
Adjusted R^2	0.43	0.14	0.03	0.03

[a]Smoking: in statistical analyses relating to SPB, number of cigarettes smoked daily were used instead of pack-years (see text)
[b]Exposure defined as never (0)/ever (1); models with duration of exposure: see text

were not strict enough. If this were the case, subjects with subclinical disease could have distorted the results. However, in a highly selected subgroup of never smokers without cough, expectoration, dyspnoea, wheezing, any asthma, history of lung disease, and with measured FVC, FEV_1, and FEV_1/FVC≥5th percentile and normal S-creatinine (< 141 μmol/l), CC16 and SPB concentrations hardly changed (CC16, median 10.1; 5th and 95th percentile 4.4 and 20.1 μg/l; $n = 154$; SPB, median 0.74; 5th and 95th percentile 0.23 and 1.67 arbitrary units; $n = 166$).

Discussion

This study examined the effect of bioaerosols from wastewater or garbage on lung epithelial permeability in the frame of an occupational risk assessment. Effects on lung epithelial permeability and results of the risk assessment will be discussed in turn.

The effect of exposure to wastewater and garbage dust on lung epithelial permeability is described in a large population for the first time. An increased CC16 concentration was already visible in never smokers, although no clinically relevant spirometric differences appeared. Multiple regression analyses confirmed this finding.

Whereas a positive association between exposure to bioaerosols from wastewater and increased CC16 concentration was found, no statistically significant effects were found in garbage collectors. Moreover, the explained variance was very small, and the serum SPB concentration was not consistently affected. On the whole, this raises the possibility of this association not being causal. Chance, bias and confounding are possible explanations.

With regard to chance, the hypothesis is an a priori one that is biologically plausible. Indeed, bioaerosols that cause ODTS are supposed to act through inflammation at the alveolar level (Arsalane et al. 2000; Rylander 2002; Rylander and Malmberg 1992; Lecours et al. 1986). Therefore, a mere chance finding is rather unlikely.

Several sources of bias were looked for but could not easily explain the findings for the following reasons: (1) CC16 was determined without knowing the exposure status, (2) samples from control and exposed workers were analysed in the same series, (3) the exclusion of workers with only former exposure did not change the results, (4) no preferential selection of workers with previous job change into one particular exposure group could be demonstrated, (5) the careful assessment of occupational history made systematic misclassifications unlikely, (6) exclusion of the subjects from both plants with low participation did not change the results, and (7) participating control workers were younger and more often of Swiss origin, which might be associated with still unknown factors that have confounded the analyses. However, several univariable and multivariable analyses were conducted and failed to identify such factors. A lack of specificity of CC16 or SPB is unlikely to explain these findings (Robin et al. 2002; Hermans and Bernard 1999; Hermans et al. 1998). Indeed, glomerular filtration rate, the main non-specific factor capable of increasing the CC16 or SPB concentrations, was considered in the analyses.

As these results came from the baseline examination of a prospective cohort study, we will determine CC16 concentration again at the end of the follow-up study to see whether the present results can be confirmed.

The effect of exposure on CC16 was not considerable. However, this may reflect an exposure level that was rather low in wastewater workers and very low in garbage collectors. Indeed, effects of endotoxin exposure are dose dependent, and endotoxin may affect deep lung structures, as evidenced by a decrease in carbon monoxide diffusion before clinical symptoms or spirometric

changes are apparent (Jagielo et al. 1996; Herbert et al. 1992; Rylander et al. 1989). The preliminary measurements of endotoxin found concentrations exceeding 100 EU/m^3 at several plants, which is above the no-effect level for airway inflammation but below the concentration causing fatigue, fever or chills (Rylander 2002). Therefore, if this dose–response relationship is correct, inflammatory effects with a leak of CC16 are to be expected without spirometric changes or general symptoms. According to Arsalane et al. (2000), endotoxin inhalation can damage the Clara cells and, hence, decrease the serum CC16, but this occurs at far higher exposure levels.

CC16 concentration may be a fairly stable subject characteristic (Blomberg et al. 2003). Thus, the serum concentration might be predictive of the risk of later changes in lung symptoms or function. This hypothesis will be tested in the follow-up study of this population.

The doubtful effect of exposure on SPB concentration cannot be easily explained. SPB is a small molecule and an essential component of surfactant (Whitsett and Weaver 2002). Its circulating level is assumed to reflect the integrity of the alveolocapillary barrier, at least in patients with acute lung injury (Doyle et al. 1998). SPB concentration increases in smokers (Robin et al. 2002) and after exposure to irritants from swimming pools (Carbonnelle et al. 2002). Therefore, SPB was expected to increase after exposure to bioaerosols as well. Decreased SPB expression might be a tentative explanation. Indeed, depressed SPB expression was found in mice after they had inhaled endotoxin (Ingenito et al. 2001). However, exposure levels were far above those encountered in the present study. As SPB is secreted by type II epithelial cells and not by Clara cells, it might reflect different pathological processes than CC16. However, this explanation is presently speculative.

With regard to the risk assessment, clues to an increased prevalence of work-related respiratory symptoms, infectious pneumonia, asthma, hypersensitivity pneumonitis, or ODTS were found neither in the garbage collectors nor in the wastewater workers. Likewise, no clinically significant decrease in FVC, FEV_1, or FEV_1/FVC was found. Thus, there is some heterogeneity between the conclusions of studies on this topic (Thorn et al. 2002; Yang et al. 2001; Bunger et al. 2000; Friis et al. 1999; Rylander 1999; Thorn et al. 1998; Ivens et al. 1997; Zuskin et al. 1996; Poulsen et al. 1995; Sigsgaard et al. 1994; Zuskin et al. 1993; Malmros et al. 1992; Nethercott and Holness 1988; Rylander et al. 1977; Rufener-Press et al. 1976). Both true differences in exposure and methodological factors explain these apparently contradictory findings. For example, the only previous Swiss study (Rufener-Press et al. 1976) reported decreased lung function in Swiss garbage workers and increased risk of chronic bronchitis. However, their population also comprised road sweepers, they measured the peak expiratory flow rate only, and a multiple regression analysis was not carried out. Furthermore, an improvement in working conditions is likely to have occurred in the past 30 years.

In conclusion, this study found increased CC16 concentration in workers exposed to bioaerosols and was reassuring about occupationally induced lung diseases or respiratory symptoms. The increased CC16 concentration is compatible with the hypothesis that bioaerosols cause subclinical alveolitis. Whether this biochemical change is real, and whether it is of clinical value, will be examined in the course of the follow-up study of these workers.

Acknowledgments We are very grateful to Mr. X. Dumont, Dr. C. Glas, Mrs. N. Koelliker, and Dr. S. Heinrich for their skilful assistance, and we thank, very much, the workers, the heads of the plants, and the head of the sewage plants of the Canton of Zurich for their constant support in organising and conducting the study. The Swiss National Accident Insurance Fund (SUVA) supported part of the study. There was no conflict of interest.

Appendix

Definition of symptoms

Symptoms of bronchitis: positive response to one of the questions "Do you usually cough during the day, or at night?" and/or "Do you usually bring up any phlegm from your chest during the day, or at night?"

Dyspnoea on exertion: positive answer to the question "Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?"

Ever asthma: positive response to both questions "Have you ever had asthma" and "Was this confirmed by a doctor?"

Current asthma: positive answer to at least one of the two following questions as well: "Are you currently taking any medicine for asthma" or "Have you had an attack of asthma in the past 12 months"?

References

Ackermann-Liebrich U, Domenighetti G, Filliger P, Keller-Wossidlo H, Kunzli N, Leuenberger P, Medici T, Tschopp JM, Wuthrich B, Zellweger JP (1991) SAPALDIA-Fragebogen. Swiss study on air pollution and lung diseases in adults. Department of Social and Preventive Medicine, Basel

American Thoracic Society (1995) Standardization of spirometry—1994 update. Am J Respir Crit Care Med 152:1107–1136

Arsalane K, Broeckaert F, Knoops B, Wiedig M, Toubeau G, Bernard A (2000) Clara cell specific protein (CC16) expression after acute lung inflammation induced by intratracheal lipopolysaccharide administration. Am J Respir Crit Care Med 161:1624–1630

Bernard AM, Gonzalez-Lorenzo JM, Siles E, Trujillano G, Lauwerys R (1994) Early decrease of serum Clara cell protein in silica-exposed workers. Eur Resp J 7:1932–1937

Blomberg A, Mudway I, Svensson M, Hagenbjork-Gustafsson A, Thomasson L, Helleday R, Dumont X, Forsberg B, Nordberg G, Bernard A (2003) Clara cell protein as a biomarker for ozone-induced lung injury in humans. Eur Resp J 22:883–888

Bunger J, Antlauf-Lammers M, Schulz TG, Westphal GA, Muller MM, Ruhnau P, Hallier E (2000) Health complaints and immunological markers of exposure to bioaerosols among biowaste collectors and compost workers. Occup Environ Med 57:458–464

Carbonnelle S, Francaux M, Doyle I, Dumont X, Deburbure C, Morel G, Michel O, Bernard A (2002) Changes in serum pneumoproteins caused by short-term exposures to nitrogen trichloride in indoor chlorinated swimming pools. Biomarkers 7:464–478

Doyle IR, Bersten AD, Nicholas TE (1997) Surfactant proteins-A and -B are elevated in plasma of patients with acute respiratory failure. Am J Respir Crit Care Med 156:1217–1229

Doyle IR, Hermans C, Bernard A, Nicholas TE, Bersten AD (1998) Clearance of Clara cell secretory protein 16 (CC16) and surfactant proteins A and B from blood in acute respiratory failure. Am J Respir Crit Care Med 158:1528–1535

Friis L, Norback D, Edling C (1999) Self-reported asthma and respiratory symptoms in sewage workers. J Occup Health 41:87–90

Herbert A, Carvalheiro M, Rubenowitz E, Bake B, Rylander R (1992) Reduction of alveolar–capillary diffusion after inhalation of endotoxin in normal subjects. Chest 102:1095–1098

Hermans C, Bernard A (1999) Lung epithelium-specific proteins—characteristics and potential applications as markers. Am J Respir Crit Care Med 159:646–678

Hermans C, Aly O, Nyberg BI, Peterson C, Bernard A (1998) Determinants of Clara cell protein (CC16) concentration in serum: a reassessment with two different immunoassays. Clin Chim Acta 272:101–110

Ingenito EP, Mora R, Cullivan M, Marzan Y, Haley K, Mark L, Sonna LA (2001) Decreased surfactant protein-B expression and surfactant dysfunction in a murine model of acute lung injury. Am J Respir Cell Mol Biol 25:35–44

Ivens UI, Ebbehoj N, Poulsen OM, Skov T (1997) Season, equipment, and job function related to gastrointestinal problems in waste collectors. Occup Environ Med 54:861–867

Jagielo PJ, Thorne PS, Watt JL, Frees KL, Quinn TJ, Schwartz DA (1996) Grain dust and endotoxin inhalation challenges produce similar inflammatory responses in normal subjects. Chest 110:263–270

Jeggli S, Steiner D, Joller H, Tschopp A, Steffen R, Hotz P (2004) Hepatitis E, Helicobacter pylori, and gastrointestinal symptoms in workers exposed to waste water. Occup Environ Med 61:622–627

Lecours R, Laviolette M, Cormier Y (1986) Bronchoalveolar lavage in pulmonary mycotoxicosis (organic dust toxic syndrome). Thorax 41:924–926

Leuenberger P, Kunzli N, Ackermann-Liebrich U, Schindler C, Bolognini G, Bongard JP, Brandli O, Defila C, Domenighetti G, Karrer W, Keller R, Medici T, Monn C, Perruchoud AP, Schoni M, Tschopp JM, Villiger B, Wuthrich B, Zellweger JP, Le groupe SAPALDIA (1998) Etude suisse sur la pollution de l'air et les maladies respiratoires chez l'adulte (SAPALDIA). Schweiz Med Wochenschr 128:150–161

Malmros P, Sigsgaard T, Bach B (1992) Occupational health problems due to garbage sorting. Waste Manage Res 10:227–234

Meier U, Bundesamt fur Statistik (1996) Verzeichnis der personlichen Berufe. Bundesamt fur Statistik, Bern

Nethercott JR, Holness DL (1988) Health status of a group of sewage treatment workers in Toronto, Canada. Am Ind Hyg Assoc J 49:346–350

Nielsen EM, Nielsen BH, Breum NO (1995) Occupational bioaerosol exposure during collection of household waste. Ann Agric Environ Med 2:53–59

Oppliger A, Hilfiker S, Vu Duc T (2005) Influence of seasons and sampling strategy on assessment of bioaerosols in sewage treatment plants in Switzerland. Ann Occup Hyg (in press)

Poulsen OM, Breum NO, Ebbehoj N, Hansen AM, Ivens UI, van Lelieveld D, Malmros P, Matthiasen L, Nielsen BH, Moller Nielsen E, Schibye B, Skov T, Stenbaek EI, Wilkins CK (1995) Collection of domestic waste. Review of occupational health problems and their possible causes. Sci Total Environ 170:1–19

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC (1993) Lung volumes and forced ventilatory flows. Report working party. Standardization of lung function tests. European Community for Steel and Coal. Eur Resp J 6 [Suppl 16]:5–40

Robin M, Dong P, Hermans C, Bernard A, Bersten AD, Doyle IR (2002) Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. Eur Resp J 20:1152–1161

Rufener-Press C, Bahy M, Voinier B, Rey P (1976) Bronchite chronique et facteurs de risque chez les employes de la voirie a Geneve. Rev Epidemiol Sante Publique 24:141–150

Rylander R (1999) Health effects among workers in sewage treatment plants. Occup Environ Med 56:354–357

Rylander R (2002) Endotoxin in the environment—exposure and effects. J Endotoxin Res 8:241–252

Rylander R, Malmberg P (1992) Non-infectious fever: inhalation fever or toxic alveolitis? Br J Ind Med 49:296

Rylander R, Andersson K, Belin L, Berglund G, Bergstroem R, Hanson L, Lundholm M, Mattsby I (1977) Studies on humans exposed to airborne sewage sludge. Schweiz Med Wochenschr 107:182–184

Rylander R, Bake B, Fischer JJ, Helander IM (1989) Pulmonary function and symptoms after inhalation of endotoxin. Am Rev Respir Dis 140:981–986

Sigsgaard T, Malmros P, Nersting L, Petersen C (1994) Respiratory disorders and atopy in Danish refuse workers. Am J Respir Crit Care Med 149:1407–1412

Steiner D, Jeggli S, Bernard A, Hotz P (2003) Lungenerkrankungen bei mullstaubexponierten Personen. Schweiz Rundsch Med Prax 92:436–440

Thorn J, Beijer L, Rylander R (1998) Airways inflammation and glucan exposure among household waste collectors. Am J Ind Med 33:463–470

Thorn J, Beijer L, Rylander R (2002) Work related symptoms among sewage workers: a nationwide survey in Sweden. Occup Environ Med 59:562–566

Whitsett JA, Weaver TE (2002) Mechanisms of disease: hydrophobic surfactant proteins in lung function and disease. N Engl J Med 347:2141–2148

Yang CY, Chang WT, Chuang HY, Tsai SS, Wu TN, Sung FC (2001) Adverse health effects among household waste collectors in Taiwan. Environ Res 85:195–199

Zemp E, Elsasser S, Schindler C, Kunzli N, Perruchoud AP, Domenighetti G, Medici T, Ackermannliebrich U, Leuenberger P, Monn C, Bolognini G, Bongard JP, Brandli O, Karrer W, Keller R, Schoni MH, Tschopp JM, Villiger B, Zellweger JP (1999) Long-term ambient air pollution and respiratory symptoms in adults (SAPALDIA study). Am J Respir Crit Care Med 159:1257–1266

Zuskin E, Mustajbegovic J, Schachter EN (1993) Respiratory function in sewage workers. Am J Ind Med 23:751–761

Zuskin E, Mustajbegovic J, Schachter EN, Kern J, Pavicic D, Budak A (1996) Airway function and respiratory symptoms in sanitation workers. J Occup Environ Med 38:522–527