

IMMUNOGLOBULINS AND PEAK EXPIRATORY FLOW MEASUREMENTS IN WASTE COLLECTORS IN RELATION TO BIOAEROSOL EXPOSURE*

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Abstract: To study the effect of exposure to low levels of bioaerosols on PEF-monitoring and antibody responses, five groups of waste collectors from different areas in Denmark were investigated. The five groups handled different kinds of waste using different kinds of organisation logistics and collection equipment. One group worked with a new, experimental system ("System 2000"), which involved source separation of waste into compostable and residual fractions. Waste collectors exposed to "high" (maximum of 72 EU/m³) levels of endotoxins had significantly ($p < 0.001$) higher serum concentrations of IgG than waste collectors exposed to "low" levels of endotoxins. Working with "System 2000" resulted in significantly higher serum concentrations of IgG ($p < 0.001$) and IgA ($p < 0.01$). Peak flow variability appeared to be significantly affected by exposure to *Aspergillus fumigatus*. It is concluded that the changes in peak flow variability and the elevated concentrations of immunoglobulins may be used as indicators of sub-clinical effects of relatively low exposure to organic dust. Assessment of peak flow variability (or serum IgG concentrations) may be used in surveillance systems to prevent clinical symptoms caused by organic dust exposure.

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INTRODUCTION

Handling of waste from urban areas and especially activities related to recycling of waste have been shown to create health problems. There is evidence that workers in waste recycling facilities have an elevated risk of occupational health problems related to bioaerosol exposure, including symptoms of organic dust toxic syndrome (ODTS), gastrointestinal and pulmonary problems [14]. Previous research at our institute has documented that handling of waste in general is associated with relatively low level exposure to organic dust, endotoxins and microorganisms [1], below that

which is known to cause acute respiratory symptoms. However, we have shown that waste collectors working with certain combinations of equipment, types of waste and work routines resulting in relatively high bioaerosol exposure have a significantly increased prevalence of self reported symptoms related to bronchitis [7].

To investigate the effect of bioaerosol exposure on lung function, peak expiratory flow (PEF) can be used. PEF measurement and PEF variability are regarded as good indicators of pulmonary function both in persons with occupational and non-occupational exposure to dust of biological origin (e.g. pollen, hair, moulds). PEF variability less than 15-20% is regarded as normal [4]. On

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Table 1. Size (n) of subgroups of waste collectors and characteristics of the different waste collection systems.

System (size)	Waste type	Storage and collection equipment	Collection frequency
I (n = 5)	Garden waste, paper and bulky waste	Garden waste in plastic sacks; platform trucks and compactor trucks (low loading)	monthly
II (n = 8)	Source separated household waste (biodegradable and residual fractions)	Sacks on platform trucks or in compactor trucks; bins in compactor trucks (low loading)	14 days
III (n = 30)	Mixed household waste	Containers and bins in compactor trucks with low loading	1-2 times weekly
IV (n = 14)	Mixed household waste	Containers and bins in compactor trucks with low loading	1-2 times weekly
V (n = 15) "System 2000"	Source separated household waste (biodegradable fraction, residual fractions, paper)	Containers and two-compartment containers in compactor trucks with high loading. Paper in compactor trucks with low loading	2 times weekly, 14 days, or monthly (paper)

the other hand Sigsgaard *et al.* [16] found an increased proportion of waste recycling workers with more than 20% variability in peak flow rates.

In addition to pulmonary problems, exposure to bioaerosols from household waste seems also to involve agents eliciting an inflammatory response. Inhalation of microorganisms may cause non-infectious symptoms and diseases by the activation of non-immune defence mechanisms and by causing immune responses [12]. Antibody formation is an important part in the immune response and antibody levels may be related to exposure levels of microorganisms [5].

However, knowledge is lacking concerning immune responses among waste collectors exposed to bioaerosols. Furthermore, to our knowledge an evidence of clinical or sub-clinical signs of impaired lung function among waste collectors has not been reported. Therefore a study was undertaken to evaluate PEF-monitoring and antibody response in a group of waste collectors, with no prior history of pulmonary symptoms.

MATERIALS AND METHODS

Subjects. The study population included a total of 72 waste collectors, 21-54 years of age, in five groups from different areas in Denmark. The five groups handled different kinds of waste (mixed household waste, biodegradable household waste etc.) using different kinds of organisation logistics and collection equipment (Tab. 1). One of the groups collected garden waste in plastic sacks and bulky waste and was included in the study due to complaints of mucous membrane irritation. Another group worked with a new, experimental system ("System 2000"), which involves source separation of waste into compostable and residual fractions, that was collected in different types of containers with different time intervals (up to 14 days) using special trucks with high loading of the waste.

Questionnaire and peak expiratory flow measurements. All waste collectors received a self administered respiratory symptoms questionnaire and/or were given a

clinical examination and interview with focus on lung function. 63 workers returned the questionnaire.

Peak flow (PEF) monitoring was performed as self administered measurements after detailed instruction, using a calibrated Mini-Wright peak-flow meter. A minimum of 4 daily measurements were made for two weeks, with the best of 3 repeated measurements recorded. 50 workers completed PEF measurements. The percentage of daily PEF variability (% PEF_{var}) was calculated as (daily maximum PEF value - daily minimum PEF value) / maximum PEF value. The average variability for the recording period was calculated from the daily variability.

Blood samples. Blood samples were drawn from 72 waste collectors in the morning prior to work. The blood was left to clot for 2 hours at room temperature and subsequently centrifuged for 10 minutes at 3000 rpm. Serum was analysed for concentrations of IgG, IgA and IgE.

Rocket immuno-electrophoresis was used to determine serum concentrations of IgG [10]. Polyclonal rabbit anti human IgG (Dako, Denmark) was mixed with 1% agarose gel in electrophoresis buffer (Tris/veronal buffer, pH 8.6, I = 0.02) and poured onto a glass plate (10 × 20 cm). For the application holes a gel puncher was used. 5 µl of serum samples, standards and controls (diluted in PBS, pH = 7.3) was applied to the holes. The electrophoresis was performed overnight at 2 V/cm in electrophoresis buffer. After electrophoresis the plates were pressed, washed in 0.15 M NaCl, pressed and washed in distilled water, pressed and dried. The plates were stained in Coomassie brilliant blue R250. The concentration of total IgG was quantified by measurement of the heights of the precipitates.

An enzyme linked immunosorbent assay (ELISA), as previously described [6], was used to determine the concentrations of IgA. Microtiter plates (Nunc, Denmark) were coated with anti-IgA (Dako, Denmark) overnight at 4°C. The plates were washed 3 times with PBS (0.05% Tween 20, pH 7.3) and incubated with serum samples (diluted in PBS) for 1 hour. Then plates were washed again and subsequently incubated with peroxidase (HRP) conjugated antibodies (Dako, Denmark) diluted in PBS.

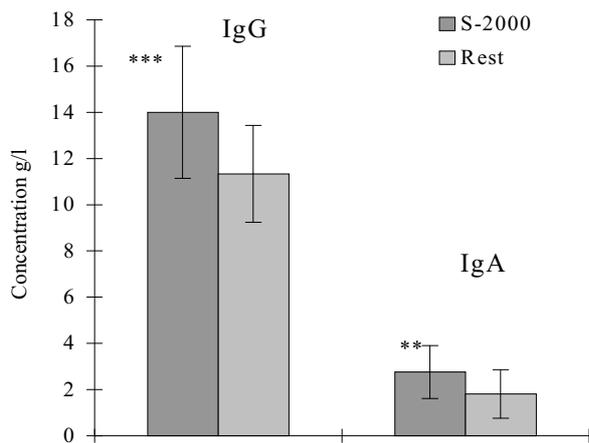


Figure 1. Serum concentrations of IgG and IgA for the group of waste collectors working with "System 2000" (n = 15) and all others (Rest, n = 48) (***: p < 0.001, **: p < 0.01).

The plates were washed and reactivity was detected using 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) as the chromogene and H₂O₂ as the substrate. The plates were measured at 405 nm in an ELISA reader.

Serum IgE concentrations were determined by enzyme fortified chemiluminescent assay analysed on Immulite® (Diagnostic Products Cooperation, USA). WHO 2nd IRP 75/502 (Diagnostic Product Research) was used as reference for the method. The analyses were performed at a commercial laboratory, Medi-Lab a.s., Copenhagen, Denmark.

Exposure assessment. The waste collectors' exposure to bioaerosols during work was measured by personal sampling equipment as described earlier [2]. Exposure to bioaerosols was recorded in the same weeks when blood sampling, peak flow monitoring and the symptom survey were carried out.

Bioaerosol exposure was recorded during collection of different types of waste (mixed household waste,

biodegradable household waste, garden waste, source separated waste, recyclable paper, bulk waste) with different combinations of storage equipment (bins, sacks and containers) and collection equipment (compactor trucks with high or low loading, platform trucks).

Dust samples were analysed as described by Palmgren *et al.* [15] and Nielsen *et al.* [13] for ordinary microbiological parameters and exposure levels were calculated as concentrations per 1 m³ of air: total number of microorganisms (cells/m³), viable counts of fungi and bacteria (cfu/m³), and endotoxins (endotoxin units, EU/m³).

The waste collectors were ranked according to the average exposure level recorded for the particular combination of waste type, storage and collection equipment the individual waste collector utilised during the study. Information on job function was also taken into account. The study population was then divided into "high" and "low" exposure groups based on the exposure ranking for the different microbiological parameters. The limits between "high" and "low" exposures were as follows: 4 × 10⁵ cells/m³ for total microorganisms, 1 × 10⁵ cfu/m³ for viable fungi, 2 × 10³ cfu/m³ for *Aspergillus fumigatus*, 2 × 10⁴ cfu/m³ for viable bacteria, and 10 EU/m³ for endotoxin.

Statistics. The non-parametric Mann-Whitney test was used to evaluate the possible association of exposure with serum concentrations of IgG, IgA, IgE and the PEF using Minitab (version 10X). Fisher-exact test was used to test differences in health status according to level of exposure.

RESULTS

Collection systems. The 15 waste collectors working with "System 2000" had significantly higher serum concentrations of IgG (p < 0.001) and IgA (p < 0.01) compared to all the other waste collectors participating in this survey (Fig. 1). Out of these 15 workers, 2 were exposed to "low" levels of endotoxins and 13 to "high" levels and there was no difference in mean immunoglobulin

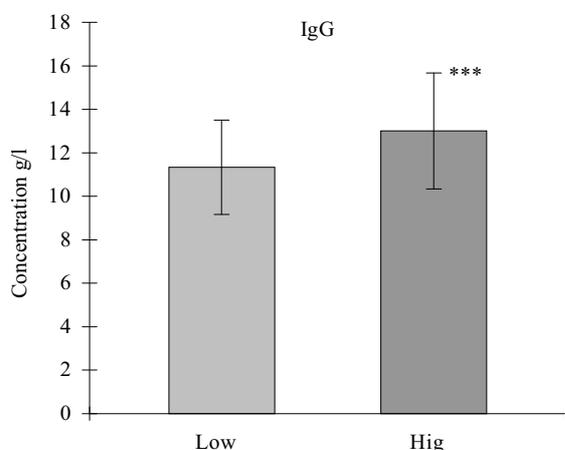


Figure 2. Serum concentrations of IgG for the groups of waste collectors exposed to "high" (n=33) and "low" (n=30) levels of endotoxins (***: p < 0.001).

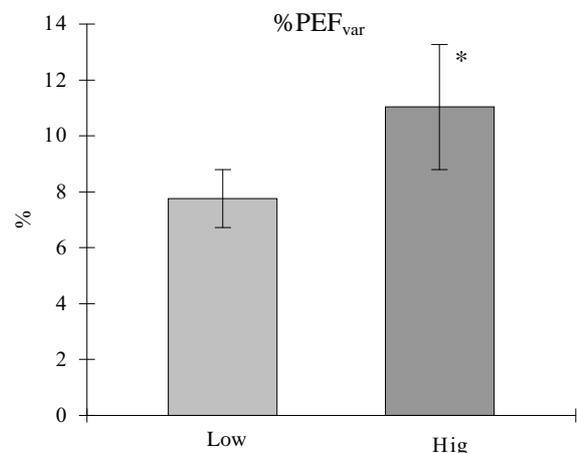


Figure 3. Percentage of peak expiratory flow variability for waste collectors exposed to "low" (n=50) or "high" (n=13) levels of *Aspergillus fumigatus* (*: p < 0.05).

Table 2. Percentage of waste collectors showing respiratory symptoms in relation to endotoxin and *Aspergillus fumigatus* exposure or "System 2000" compared to waste collectors in other systems.

	n	Cough	Productive cough	Chronic bronchitis	Wheeze	Chest tightness	Asthma	Smoker	
"System 2000"	15	20%	13%	7%	13%	7%	0%	67%	
Rest	48	35%	19%	17%	17%	8%	8%	67%	
<i>A. fumigatus</i> exposure	Low	50	28%	12%	8%	16%	8%	4%	70%
	High	13	46%	38% *	38% *	15%	8%	15%	54%
Endotoxin exposure	Low	30	47%	18%	18%	5%	5%	3%	55%
	High	33	18% *	12%	6%	24%	9%	9%	61%

* significantly different from low exposure ($p < 0.05$, Fisher-exact test).

concentrations between "low" or "high" level exposure to endotoxins. All 15 workers were in the "low" exposure group with respect to the other microbiological parameters. Apart from "System 2000" the different collection systems (Tab. 1) were not associated with differences in PEF variability and serum immunoglobulin concentrations.

Immunoglobulins. Waste collectors working under conditions involving "high" levels of exposure to endotoxins had significantly ($p < 0.001$) higher concentrations of IgG than those exposed to "low" levels of endotoxins (Fig. 2).

No significant differences were found in IgE concentrations between the different groups of waste collectors. However, 20.8% of the waste collectors had a higher IgE concentration than normal (> 100 kU/l) and 5.6% reported to have asthma.

Peak expiratory flow. Percentage of peak expiratory flow variability (% PEF_{var}) values were significantly affected by exposure to *Aspergillus fumigatus*. "High" exposure to *Aspergillus fumigatus* results in a higher % PEF_{var} ($p < 0.05$) (Fig. 3). However, there was no association between concentrations of immunoglobulins (IgG, IgA and IgE) and the PEF variability, and no association was found between exposure to endotoxin and PEF variability.

Exposure. The exposure levels recorded during collection of household waste showed the following ranges (minimum - maximum values): $< 2 \times 10^2 - 1.4 \times 10^6$ cells/m³ for total microorganisms, $< 4 \times 10^2 - 7 \times 10^5$ cfu/m³ for viable fungi, $< 10^2 - 5.0 \times 10^4$ cfu/m³ for *Aspergillus fumigatus*, $< 4 \times 10^2 - 2.8 \times 10^5$ cfu/m³ for viable bacteria, and 0.3 - 72 EU/m³ (0.02 - 5 ng/m³) for endotoxin.

Briefly, both the type of waste, frequency of collection, environmental temperature, storage and collection equipment, and job function - all appear to have influence on the waste collectors' exposure to dust and microorganisms. However, with some few exceptions, the exposure level of dust and microorganisms during handling of waste from private households is generally in the low range compared to that seen in other occupational settings where biological

materials are handled. Detailed analysis of the exposure assessment has been reported separately [1, 12, 13].

Questionnaire. Apart from the waste collectors collecting garden waste in plastic sacks ($n = 5$), who complained of eye and upper airway irritation, there were no work-related symptoms. An overview of the prevalence of self reported symptoms in the "high" and "low" exposure groups is given in Table 2. Prevalence of both productive cough ($p < 0.05$) and chronic bronchitis ($p < 0.05$) were highest for waste collectors exposed to "high" levels of *Aspergillus fumigatus*. On the other hand, "low" endotoxin exposure was associated with a high prevalence of cough ($p < 0.05$).

DISCUSSION

Compared to other occupations involving exposure to bioaerosols, the waste collectors in this study were exposed to relatively low concentrations of organic dust and microorganisms, and their serum concentrations of immunoglobulins IgA and IgG generally did not exceed the reference values. However, our findings indicate that waste collectors with "high" exposure to endotoxins had significantly elevated concentrations of IgG compared to those with "low" level exposure. Since the serum IgG concentrations do not exceed the reference values, an increase in serum IgG may be interpreted as a sub-clinical response. Increased serum IgG concentrations may be regarded as an indicator of exposure to immune activating agents. Several investigations concerning farmers with allergic alveolitis have shown that IgG antibodies to microorganisms also can be found in sera from healthy farmers. These antibodies are therefore not regarded to be related to the disease *per se* but to exposure to microorganisms [5]. This can also be the case in our study. No clinical symptoms, related to an immune response, were observed among the waste collectors in this study.

In a 1994 Danish national survey among adults concerning health and morbidity [9] it was reported that approximately 5% of the population suffered from

asthma. Our results agree with this survey. In the same survey it was reported, that 10% and 5.4% of the Danish males between 25-44 and 45-66 years of age respectively suffered from allergic disease. Zetterström & Johansson [18] found that a serum IgE value above 100 kU/l in a patient is a strong evidence for the presence of an atopic allergic disease. In a study concerning the correlation between atopic allergic disease and IgE level, it was found, that the risk to develop an atopic allergic disease was 78% if the IgE level was above 100 kU/l [19]. In our study we observed, that 20.8% of the waste collectors had IgE levels above 100 kU/l. Hence in 16.2% (78% of 20.8%) of the waste collectors with an increased IgE level there is estimated a risk of developing an atopic allergic disease. Compared to the prevalence of allergic disease among normal Danish males with an age between 25-66 years, waste collectors prevalence of allergic disease could hypothetically be approximately 50-300% higher.

Waste collectors' exposure to organic dust and microorganisms is dependent on the type of waste handled and the methods used in waste collection [2, 13]. We observed that the 15 waste collectors working with the waste collecting system "System 2000" had significantly higher concentrations of both serum IgG and IgA compared to all other waste collectors participating in this survey. Thirteen out of these 15 workers were also classified as being exposed to a "high" level of endotoxins, though there was no difference in mean immunoglobulin concentrations between these workers and those who were classified as having received a "low" exposure. All 15 workers were in the "low" exposure group considering the other microbiological parameters. Concerning these findings one could assume, that exposures other than endotoxins, may be responsible for the higher immunoglobulin concentrations found in the "System 2000" employees. One might suspect that pre-separated waste, as is the case in "System 2000", can create ideal growth conditions for certain microorganisms, which might activate the immune system. This could explain the high serum concentrations of IgG and IgA among the waste collectors working with "System 2000". However, it does not explain why this phenomenon is seen only in waste collectors using this kind of waste collection system and not in workers collecting source separated waste in other systems.

In system I (Tab. 1) the five waste collectors collecting garden waste in plastic sacks and bulky waste complained about mucous membrane irritation. These complaints were probably the result of short-term high-level exposure to bioaerosols and gases during the special collection procedure, which involved manual opening and emptying of the sacks into the scoop of the truck. While emptying the plastic bags, which is accompanied by agitation of the waste, the workers cannot avoid being exposed to relatively high concentrations of irritating gases and bioaerosols which may lead to airway sensitisation.

In our study, waste collectors who were exposed to "high" levels of *Aspergillus fumigatus* had a higher peak

flow variability compared to workers exposed to "low" levels of *Aspergillus fumigatus*. This is in accordance with the drop in FEV₁ over a workshift, which was significantly associated with exposure to organic dust [17]. Also Cernelc & Vozelj [3] found a change in peak flow among workers exposed to air conditioning systems contaminated with *Aspergillus fumigatus*.

We did not find any difference in PEF variability between the two groups ("low" and "high") of waste collectors with exposure to endotoxin. Several studies in humans on the effect of acute endotoxin inhalation have shown that a decrease in lung function usually occurs with endotoxin concentrations in the µg/m³ range, although sensitive subjects are likely to react at lower concentrations [8, 16]. In our study the maximum endotoxin exposure found was 5 ng/m³. Similarly Sigsgaard *et al.* [17] reported endotoxin exposure levels of ≤ 50 ng/m³ in waste recycling workers, but no effect on lung function resulting from endotoxin exposure was observed.

CONCLUSION

The changes in PEF variability and the elevated concentrations of immunoglobulins we found in this study can be used as indicators of sub-clinical effects of relatively low exposure levels to organic dust. While the predictive value of these indicators for effects is still unknown, one could assume that e.g. the increased PEF variability is an indication of increased risk for the development of pulmonary problems among susceptible individuals. Hence assessment of PEF variability (or serum IgG concentrations) could be used in surveillance programs to avoid of clinical symptoms caused by organic dust exposure.

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