Respiratory effects of fire smoke exposure in firefighters and the general population

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Respiratory effects of fire smoke exposure in firefighters and the general population

Blootstelling aan rook van branden en respiratoire gezondheidseffecten bij brandweerlieden en de algemene bevolking

(met een samenvatting in het Nederlands)

Proefschrift

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Contents

CHAPTER 1

General introduction

Environmental incidents

In acute environmental incidents, i.e. chemical spills, explosions, and fires, hazardous substances are released into the environment. Chemical spills and explosions are often thought to cause more relevant exposures than fires. But fires are much more frequent and can cause heavy air pollution. From 1990 to 2008 yearly 42.000 to 55.000 accidental fires occurred in the Netherlands[1]. Accidents with hazardous material take place far less frequently. In 2006 12 of the 25 safety regions of the Netherlands reported 536 accidents with hazardous materials[2]. Of these 215 involved accidental fires.

Fires emit many different chemical compounds. Their origin is of 4 sorts:

- Substances of the burning material.
- Products of incomplete combustion (*pyrolysis*).
- Products of complete combustion.
- Compounds that originate *de novo* in or nearby the fire.

Many compounds are composed in fires, as fires constitute in fact chemical reactors. These substances can be gaseous or particulate matter[3;4]. Fires can emit enormous fluxes of gases and particulate matter. These emissions depend partially on the burning material[5] (Table 1). Additionally, the composition of the combustion production is influenced by the stage of the fire, the availability of oxygen, the temperature of the fire, and other factors. A sizable part of the emissions is formed *de novo* in the fire and does not depend upon the burning material.
The dispersal of combustion products of fires into the environment vary in time and space, depending on plume rise, heat release, diffusion, chemical transformations and other characteristics of the release, such as weather conditions (precipitation, wind direction and speed). The dispersion determines to a large extent levels of pollutants in the surroundings and neighbourhoods and subsequently exposure of humans, which can lead to health effects.

Table 1: A few examples of burning materials and products released in the environment[5].

Material	*Particulate matter*	*Inorganic gases*	*Organic gases*
Plastics	+++	CO, NO_x, SO_2, HCN, HCl	Aromatic and aliphatic compounds, aldehydes, ketones, phenols, furans, nitriles, isocyanates
Paints, solvents, pesticides and other chemicals	++	CO, NO_x, SO_2, HCN, NH_3, PO_x	Aromatic and aliphatic compounds, aldehydes, ketones, phenols, furans, nitriles, isocyanates
Oil and related fuels	+++	CO, NO_x, SO_2	Aromatic and aliphatic compounds, aldehydes, ketones, phenols
Buildings	++	CO, NO_x, SO_2, HCN, HCl, HBr	Aromatic and aliphatic compounds, aldehydes, ketones, phenols, furans, nitriles, isocyanates
Wood	+	CO, NO_x, SO_2	Aromatic and aliphatic compounds, aldehydes, ketones, phenols, furans

Health effects of air pollution

Evidence of adverse health effects of general out door air pollution, mainly the result of traffic and industrial activity, has substantially increased in recent years[6]. Life expectancy[7], vascular function[8], and respiratory function[9] are affected, among others. Associations were found also between chronic exposure to air pollution and cardiopulmonary health[10], or lung cancer[11]. Importantly, in the context of this thesis, the severity of asthma was found to be associated with air pollution[12;13]. However, it should be noted that the chemistry of traffic and industry related air pollution is substantially different from the chemistry of fire smoke. There are some general population air pollution studies which involve fire smoke exposure[14-16]. These studies indicate that inhalation of particulate matter released by wildfires leads to increased respiratory morbidity of health effects.

Most evidence comes from studies among occupationally exposed individuals such as firemen. These studies show that occupational inhalator exposures are related with new occurrence of COPD and asthma[17] as well as exacerbations.

Occupational asthma (OA) has been defined as asthma induced by exposure in the working environment to airborne dusts vapours or fumes, with or without pre-existing asthma[18]. Two types of OA are distinguished based on their appearance after a latency period[19]. First, the category with an allergic mechanism and a latency period. Second, a non-immunological asthma without a latency period, following single (Reactive Airways Dysfunction Syndrome, RADS) or multiple (Irritant-Induced Asthma) exposures to non-specific irritants.

Although, numerous studies have investigated the relation between respiratory health and long-term and short-term exposure to air pollutants, far fewer studies have been directed at the relation between respiratory health and accidentally acute exposures. These acute exposures occur in environmental incidents like chemical releases and fires.

There are various reasons for the paucity of data regarding effects of accidental acute exposures.

- First, subject-specific information regarding the magnitude, the duration and the type of exposure during a fire or a chemical incident is seldom available. Additionally, fire smoke consists of a multitude of chemicals, depending on the materials and the circumstances. Contrary to general air pollution, which is a product from mostly complete combustion, fire smoke comprises far more incomplete combustion products, especially during non-flaming or under-ventilated flaming stages of a fire[20].
- Second, knowledge about relations between substances and respiratory health are mostly based on long-term exposures of relatively low-level smoke concentrations.
- Third, knowledge about respiratory health consequences of accidentally acute exposure is to a large extent based on research after the World Trade Centre disaster[21-25], which was not restricted to fire smoke. Although epidemiological studies on exposure to fire smoke exist, many studies focused on respiratory symptoms and lung function parameters[26-31]. Some studies have included bronchial challenge testing, but mostly in acute studies[32;33]. Some studies have focused on firefighters compared to other occupations[30;34] or the general public[35].
- Fourth, knowledge about the health status of subjects preceding acute exposure incidents is mostly absent.

Therefore, gaps in the knowledge about respiratory health consequences of accidentally acute exposures remain. During a fire or a chemical incident the exposure time is mostly in the order of minutes to hours and therefore much shorter than the long-term or short-term exposure in the epidemiology of air pollution.

Respiratory health hazards resulting from fire smoke

Hazardous substances produced by fires or otherwise fall roughly in three categories: 1) particulate matter, 2) respiratory irritants and 3) systemic toxins, including asphyxiants. Systemic toxins are substances that affect other parts of the body than the organ that has primarily been exposed. Carbon monoxide enters the body via the lung, and combines with haemoglobin to carboxyhemoglobin, which is ineffective for delivering oxygen to bodily tissues. Respiratory irritants include substances that cause non-specific inflammation of the lung after they are inhaled. Depending on water-solubility and concentration, damage may occur mainly in the upper or the lower airways. Particles act as vehicles of absorbed toxicants into the respiratory tract. Particles between 5 and 30 µm impact on the nasopharyngeal region, whereas particles of 1 to 5 µm penetrate the large airways of the trachea, bronchi, and bronchioles and particles smaller than 1 µm reach the alveoli[36-38]. Furthermore, particulate matter causes inflammation, and may be allergenic[4].

Respiratory health consequences after exposures to high concentrations of particulate matter and chemicals fall in 4 major categories: 1) upper respiratory disease, 2) lower respiratory disease, 3) parenchymal or interstitial lung diseases, and 4) cancers of the lung and pleura[39]. Epidemiology involves complex mixtures of respiratory health consequences.

Upper respiratory disease usually manifests as cough, and a chronic inflammation of nose and sinuses[39-43]. In some studies Reactive Upper Airways Dysfunction Syndrome (RUDS), defined as a chronic rhino sinusitis initiated by high-level exposure to inhaled irritants, with recurrence of symptoms after re-exposure to irritants has been mentioned[39-41]. Following the WTC disaster a high prevalence of upper respiratory symptoms, such as congestion, runny nose, headache, sinus pain, sore throat and hoarse voice has been described[24;42;43]. However, in this disaster residents, commuters, and rescue workers were exposed to an unprecedented complex mixture of debris from the collapsing towers and fire smoke[44].

Lower respiratory disease manifests in different ways. In subjects exposed to WTC dust an accelerated decline in FEV_1 and FVC has been described[42;43;45;46]. Aldrich et al. found that in the first year post-exposure the mean FEV_1 of rescue workers decreased in the first, but that little or no recovery in FEV_1 during the subsequent 6 years was found[47].

Accelerated decline in lung function parameters has also been found in firefighters in the 70's[28;48], which was associated with the number of fires fought[49] and might also be associated with high exposure[50]. Reactive airways dysfunction syndrome (RADS) has been defined as non-immunological asthma without latency produced by a single high exposure to airway irritants, characterized by an abrupt onset of symptoms (within 24 hours after exposure) and the presence of non-specific bronchial hyperresponsiveness and airflow obstruction[51;52]. In several studies it has been found that RADS can occur after exposure to any variety of chemicals generated as a gas or aerosol as well as of particles, such as acetic acid[53], chlorine[54] and fire smoke[51;55;56]. More generally defined is Irritant-Induced Asthma (IIA) as an asthmatic syndrome that results from single or multiple exposures to airway irritants[57].

The third group of sequels is identified as parenchymal or interstitial lung diseases such as sarcoidosis or sarcoid-like granulomatous lung disease in WTC rescue workers[23]. In the aftermath of the WTC disaster case reports have appeared on interstitial lung diseases such as eosinophilic pneumonia[58] and bronchiolitis obliterans[59].

Finally, a last group consists of neoplastic changes following long-standing exposure to fire smoke and combustion products[39]. Up to now no associations have been found in the WTC disaster between exposure to carcinogens and malignancies. However, the time that passed since this disaster is at the moment too short to examine these associations.

Aims and outline of this thesis

The main aim of this thesis is to investigate associations between airway irritant exposure of short duration and the occurrence of respiratory effects in adults. Specifically, the risk of developing respiratory symptoms due to exposure to fire smoke is investigated.
Chapter 2 describes a cross-sectional study of a population involved in a major fire in a hazardous waste depot. Identified by telephone interview six years later, subjects with persistent respiratory symptoms were identified as having suspected Reactive Airways Dysfunction Syndrome (RADS). Medical tests were performed. Suspected RADS cases were compared with healthy controls for exposure to combustion products, lung function, and bronchial responsiveness.
Chapter 3 describes a Dutch web-based version of the European Community Respiratory Health Survey questionnaire study among 1330 firefighters. Associations were examined between general respiratory symptoms and exposure variable.
Chapter 4 describes lung function, bronchial responsiveness and atopy in a subset of the study population of chapter 3 (n=402). Associations between exposure variables and medical tests were examined.
Chapter 5 describes the associations between exposure variables and serum pneumoproteins in the same population as chapter 4.
Chapter 6 describes the changes examined in a subset (n=51) of firefighters who had been accidentally exposed to fire smoke. Associations between exposure variables and lung function, bronchial hyperresponsiveness, sputum cell differential parameters, serum pneumoproteins and serum cytokines were examined.
Chapter 7 provides a general discussion on the topics covered in this thesis. In addition, the implications of the findings are described

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CHAPTER 2

Respiratory effects in the aftermath of a major fire in a chemical waste depot

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ABSTRACT

Objectives: To investigate respiratory effects among first responders and residents with exposure to combustion products in the aftermath of a fire in a chemical waste depot.
Methods: The study population comprised 138 subjects present in the area downwind of an accidental fire. Subjects with persistent respiratory symptoms were identified by telephone interview as suspected cases of Reactive Airways Dysfunction Syndrome (RADS). Medical tests were performed six years after the incident, including bronchial challenge testing. For bronchial responsiveness a cut-off point of PD_{20}<2.39 mg histamine was taken and a dose-response slope (DRS) was calculated. Suspected RADS cases were compared with healthy controls for exposure to combustion products, lung function, and bronchial responsiveness.
Results: 25 suspected cases were more frequently exposed than 99 controls; the crude odds ratio (OR) for high versus low exposure was 6.5 (95% CI 2.4-18.0). Suspected cases showed lower FEV_1/FVC (P=0.028). Overall, suspected cases had higher DRS than controls. The difference was significant for males only (P=0.006), and non-smoking males (P=0.014). Highly exposed subjects had higher DRS than low exposed subjects (P=0.056). These differences were significantly different when restricted to non-smokers (P=0.034) and to males (P=0.019). Differences between cases and controls were stronger when the population was restricted to current non-smokers.
Conclusions: Persistent respiratory symptoms and bronchial responsiveness were associated with exposure to combustion products of a chemical waste depot fire which occurred more than 6 years earlier. Authorities and first responders are recommended to take this into consideration when managing incidents with possible exposure to airway irritants.

INTRODUCTION

On May 12th 2000, 480 metric tons of chemical waste were burnt in an accidental fire at a hazardous waste disposal site where waste included batteries, acids, bases and paint. This resulted in major smoke development and combustion products were dispersed over a large area to the west of the provincial town of Drachten, Friesland, the Netherlands. Fire fighters, police officers and others were exposed to combustion products while performing their emergency tasks. Of those involved professionally only fire fighters used protective gear. Residents of the rural community downwind of the fire were exposed to some extent. During subsequent clean-up activities, over a period of a week, workers were exposed to dust and debris. No fatalities have been reported.

The Environmental Incident Service of the Dutch National Institute of Public Health and the Environment (RIVM) measured high levels of particulate matter, heavy metals, dioxins, volatile organic compounds and HCN starting 7 hours after the beginning of the fire. No structured sampling strategy was followed. Analyses of compounds did not focus on those potentially relevant for acute respiratory health effects. Irritant gases, apart from HCN, were not sampled by the RIVM. HCN exceeded the Dutch Threshold Limit value (Maximum Allowable Concentration). Air samples 20-30 m downwind of the fire demonstrated 5500 $\mu g/m^3$ Total Suspended Particulates. These measurements indicated that exposure levels were high at some point of time, but do not allow estimation of exposure of inhabitants of the area and first responders. Dispersion modelling indicated that at a distance of 1000m (the distance to the nearest dwellings) the maximum concentrations of compounds would be diminished by a factor of about 500. Therefore health risks other than temporal irritation by inhalation of combustion products were considered unlikely beyond that distance. Direct health effects due to inhalation of smoke at short distance of the fire were not assessed[1;2].

In the literature, respiratory effects as a consequence of exposure to smoke or combustion products of fires and accidents have been described. Studies were mainly focused on short-term effects[3-6] and on long-term respiratory effects restricted to fire victims[7] or to those occupationally exposed, such as fire fighters[7;8].

One of the few incidents in which persisting respiratory effects due to exposure to airway irritants has been studied among residents[9] and first responders[10-12], was the 2001 WTC disaster, New York.

Since the Drachten fire, inhabitants and professionals presented themselves with respiratory symptoms to health care providers over a period of several years. The symptoms seemed indicative of Irritant-Induced Asthma (IIA), a non-immunological asthma phenotype caused by exposure to airway irritants[13] or Reactive Airways Dysfunction Syndrome (RADS)[14]. RADS has been defined as non-immunological asthma produced by a single high exposure to airway irritants characterised by an abrupt onset of symptoms (within 24 hours after exposure) and presence and persistence of nonspecific bronchial hyperresponsiveness. To our knowledge, this is the first study to investigate whether lung function and bronchial challenge parameters

of residents and first responders are associated with accidental exposure more than 6 years previously.

MATERIALS AND METHODS

Population and study design

Combustion products of a fire in a chemical waste depot were dispersed over an area approximately 3x8 km (fig1) downwind. 5 years after the fire, health authorities ordered a follow-up study among subjects present in the affected area at the moment of the fire. Subjects were invited to participate through a letter to their home address, a letter to their employer's address and an announcement in the local media.

After enrolment, the study protocol consisted of two parts: a telephone interview with all eligible persons, to register symptom status, followed by a medical examination, including bronchial challenge testing of a selection of the interviewed population.

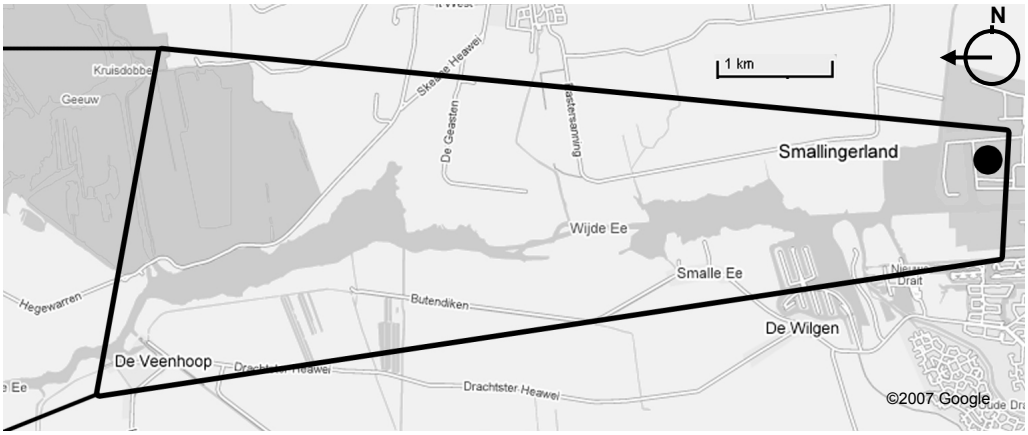

Figure 1 Area approximately 3x8 km downwind of the fire in a chemical waste depot over which combustion products were dispersed on May 12th 2000. Location of the fire is depicted by a black dot. Wind direction during the fire was east.

Telephone interview

Health questionnaire

In a telephone interview, 138 subjects were interviewed in March and April of 2006. A health questionnaire, based on a previously validated questionnaire (the Dutch version of the European Community Respiratory Health Survey questionnaire[15]), was used to obtain

standardized data. Questions were added to identify the onset of symptoms, the duration of symptoms and to determine possible exposure during the fire or the following clean-up.

Case definition

In this study, a subject was classified as a suspected RADS case if he/she met the following criteria in the telephone interview: no pre-existing respiratory symptoms or known pulmonary pathology, acute respiratory symptoms following the Drachten fire, such as cough, wheezing, and dyspnoea, recurrence of these symptoms during specified weather conditions or when inhaling irritants or environmental tobacco smoke, and persistence of these respiratory symptoms for at least three months. All subjects who met these criteria were classified as suspected cases and invited to participate in the medical examination.

A subject was classified as a potential RADS case, when the diagnosis of current or recovered RADS could not be ruled out by the examining lung physician. Subjects meeting the following criteria were defined as controls: no persisting 'asthma-like' symptoms for more than 3 months following the fire and no pre-existing lung-pathology. Controls were randomly selected for the medical examination in a control:case ratio of 3:1. If a control underwent the medical examinations he was classified as an examined control.

Exposure definition

Exposure was defined using different proxies; self-reported exposure to combustion products, date of exposure (date of fire or dates of clean-up) and distance to the source. The distance to the source was assessed using a map on which the dispersion of the combustion products was plotted, as well as the location of subjects, using information such as residential addresses, activities during the fire and the area isolated by police. Exposure was considered high if a subject reported exposure and had been within 100 m from the fire. Exposure was considered intermediate if the distance was from 100 m up to 1 km and exposure was defined as low if the distance was more than 1 km, or exposure occurred only during clean-up activities or there was no self-reported exposure.

Medical examination

The medical examination was performed from June 2006 to February 2007 in the Nij Smellinghe Hospital, Drachten, the Netherlands. Subjects visited a lung physician for an interview and physical examination. Venous blood samples were obtained for immunological testing for inhalation allergens (tree and grass pollen, house dust mite, cat and dog) by immunoCAP assay (ImmunoCAP®250, Phadia AB, Uppsala, Sweden). Total IgE was assessed by Immulite assay (Immulite® 2500, Diagnostic Products Corporation, Siemens AG, Germany).

Spirometry

Each subject was offered lung function testing (Masterscreen-PFT, Jaeger/Toennies, Hoechberg, Germany) using ERS guidelines[16], measuring FVC (forced vital capacity), FEV_1 (forced expiratory volume in one second). These parameters are presented as % of predicted.

In subjects with baseline FEV_1/FVC< 0,7 or baseline FEV_1< 85% predicted, the reversibility of the bronchial obstruction was measured after inhalation of 400 micrograms salbutamol by means of a Ventolin® Diskus. Significant spirometric bronchodilator response was defined by an absolute increase of 200 ml or an increase of at least 9% of predicted[17;18] of the FEV_1.

Histamine challenge testing

After written informed consent, histamine challenge testing (HCT) was performed using ERS guidelines. Histamine diluted in sterile 0.9% saline was inhaled in doubling doses from 0.027-1.493 mg. HCT was performed using the 2-min tidal breathing protocol with histamine delivered via a nebulizer up to a maximum dose of 32 mg/ml (Master Screen-PFT, analyzer unit, Jaeger) using the Cockroft-criteria. Administration of increasing histamine doses continued until FEV1 declined by 20% of baseline (PD_{20}) or the maximum cumulative dose of 2.968 mg histamine was administered.

Results of the HCT were presented using PD_{20}, the calculated dose of histamine that caused the FEV_1 to fall 20%. Bronchial hyperresponsiveness (BHR) was considered to be present if PD_{20}≤ 2.39 mg histamine. To make the best use of the HCT data, additionally the dose-response slope (DRS)[19] was calculated as the % fall in FEV_1 per mg inhaled histamine.

Statistical methods

SPSS v14.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) was used for the statistical analysis. Odds ratios between symptoms and exposure variables were calculated with confidence intervals using logistic regression. Lung function parameters (FVC, FEV_1 and FEV_1/FVC) were analysed using the independent-samples T-test. DRS was non-parametrically analysed using Mann-Whitney U-test.

For all tests, *p*-values < 0.05 were considered statistically significant.

RESULTS

Questionnaire

Population characteristics

The adult population (n=138) recruited for the study, consisted of 65 workers (47%) involved in the incident, such as police officers and fire fighters and 73 (53%) employees from neighbouring firms, bystanders and residents, living in the potentially exposed area in 2006. Data of 14 subjects (10%) were excluded from the analysis, because they suffered from pre-existing pulmonary pathology or were not present in the catchment area during the incident. Of the remaining 124 interviewed subjects, 25 subjects (18%) were classified as suspected cases and 99 (72%) were classified as interviewed controls. General characteristics of the baseline study population are shown in Table 1.

Table 1 Population characteristics of cases and controls on the basis of the interview.

		Case	Control
n		25	99
Sex	Male (%)	19 (76.0)	72(72.7)
Smoking status	Current smoker (%)	5 (20.0)	19 (19.2)
	Former smoker (%)	13 (52.0)	40 (40.4)
	Never smoked (%)	7 (28.0)	40 (40.4)
Exposure	Low (%)	7 (28.0)	63 (63.6)
	Intermediate (%)	2 (8.0)	14 (14.1)
	High (%)	16(64.0)	22 (22.2)
Age-yr	Mean (sd)	46.7 (9.9)	49.6 (11.0)
	Range	28-69	22-72

Symptoms

25 suspected cases reported having experienced respiratory symptoms shortly after the fire. Table 2 shows proportions of acute respiratory symptoms in suspected cases, interviewed and examined controls. Examined controls did not differ from non-examined controls with respect to acute respiratory symptoms.

Table 2 Acute respiratory symptoms[a] of suspected cases and controls.

Symptoms		Case	Interviewed control	Examined control
	n	25	99	50
Cough	Yes (%)	18 (72)	14 (14)	7 (14)
	No (%)	3 (12)	83 (84)	43 (86)
	Can't remember (%)	4 (16)	2 (2)	0 (0)
Wheezing	Yes (%)	9 (36)	3 (3)	1 (2)
	No (%)	7 (28)	90 (91)	46 (92)
	Can't remember (%)	9 (36)	6 (6)	3 (6)
Shortness of breath	Yes (%)	21 (84)	5 (5)	1 (2)
	No (%)	3 (12)	89 (90)	46 (92)
	Can't remember (%)	1 (4)	5 (5)	3 (6)
Dyspnoea	Yes (%)	13 (52)	4 (4)	3 (6)
	No (%)	7 (28)	90 (91)	45 (90)
	Can't remember (%)	5 (20)	5 (5)	2 (4)
Chest tightness	Yes (%)	8 (32)	1 (1)	0 (0)
	No (%)	8(32)	90 (91)	46 (92)
	Can't remember (%)	9 (36)	8 (8)	4 (8)
Runny nose	Yes (%)	7 (28)	4 (4)	1 (2)
	No (%)	11 (44)	89 (90)	46 (92)
	Can't remember (%)	7 (28)	6 (6)	3 (6)
Sore throat	Yes (%)	12 (48)	12 (12)	3 (6)
	No (%)	8 (32)	82 (83)	44 (88)
	Can't remember (%)	5 (20)	5 (5)	3 (6)
Other symptoms	Yes (%)	9 (36)	9 (9)	4 (8)
	No (%)	14 (56)	89 (90)	46 (92)
	Can't remember (%)	2 (8)	1 (1)	0 (0)

[a]Symptoms were considered acute when onset of symptoms was within a week after exposure to the combustion products of the Drachten fire.

Associations with exposure

Odds ratios between interview case-control status and exposure were all statistically significantly different from unity (Table 3). The crude odds ratio for high versus low exposure was 6.5 (95% CI 2.4-18.0). Associations were stronger when interviewed controls were restricted to those without acute respiratory symptoms and when subjects were restricted to

males only. Although associations weakened when subjects were restricted to current non-smokers, and to males the odds ratios remained significant.

Table 3 Odds ratios [OR (95% C.I.] between case-control status and exposure on the basis of the interview.

Odds Ratios	All	Men	Non-smokers	Non-smoking men
High (n=38) versus low (n=70) exposure	6.5 (2.4-18.0)	5.2 (1.6-16.4)	4.6 (1.6-13.7)	3.8 (1.1-12.8)
Intermediate (n=16) versus low (n=70) exposure	1.3 (0.2-6.9)	<0.1 (0.0-5.1)[a]	1.7 (0.3-9.4)	<0.1 (0.0-50.4)[a]

[a]one of the cells empty. 0.1 was imputed for the calculations.

Medical examination

75 interviewed controls (75%) were randomly selected to enrol in the medical examination. Because no pathological findings were observed in the first series of examined controls, it was considered unethical to continue evaluating controls. Therefore inviting controls was stopped after 58 were invited. The response rate among invited controls was 86% (58-8/58). The response rate among invited suspected cases was 96% (25-1/25). Responders did not differ from non-responders in terms of age, gender, smoking status and respiratory symptoms.

Bronchial challenge and spirometry

Suspected cases showed lower average values for FVC, FEV_1 and FEV_1/FVC in comparison with examined controls (table 4). Restriction to controls without acute respiratory symptoms following the fire increased the differences with cases.

A similar pattern was observed for potential cases.

Table 4 Lung function parameters and total IgE results in case-control classes.

	Examined Control	Examined control without acute respiratory symptoms	Suspected Case	Potential Case
N	50	41	24	10
FVC % of predicted (%)	108.6(13.9)	108.2(14.1)	107.6(11.9)	104.0 (6.6)
FEV_1 % of predicted (%)	104.0(18.9)	105.1(18.1)	101.5(14.3)	97.4 (10.8)***
FEV_1/FVC % of predicted (%)	95.3(11.6)	96.7(10.6)	94.5(8.3) **	93.6(8.3)
PD_{20} positive (%)	11 (23.9)	7 (17.1)	6 (28.6)	6 (60.0)
DRS (25^{th}, 75^{th} percentile)	3.6 (2.1, 8.3)	3.5 (2.0, 5.8)	5.9 (3.0,17.2)****	6.2 (3.9, 22.9)*
Total IgE (ln kU/l)	3.4 (1.3)	3.3 (1.3)	3.8(1.7)	5.1(1.9)

* Significantly different from controls without acute respiratory symptoms (P=0.004).

** Significantly different from controls without acute respiratory symptoms, when restricted to current non-smokers (P=0.028) and to currently non-smoking males (P=0.021).

*** Significantly different from controls without acute respiratory symptoms (P=0.042), when restricted to current non-smokers (P=0.015).

**** Significantly different from controls without acute respiratory symptoms, when restricted to males (P=0.006) and to currently non-smoking males (P=0.014).

DRS: dose-response slope calculated as the % fall in FEV_1 per mg inhaled histamine; FEV_1: forced expiratory volume in one second; PD_{20}: the calculated dose of histamine that caused the FEV_1 to fall 20%; FVC: forced vital capacity. Lung function parameters and total IgE results presented as geometric means with standard deviations, positive PD_{20} presented as absolute numbers with percentage and DRS presented as median with 25^{th} and 75^{th} percentiles. A positive PD_{20} (i.e. abnormal) was defined as a $PD_{20} \leq 2.39$ mg histamine.

Odds ratio between BHR and case-control status was 1.8 (95% CI 0.8-5.9). Although this association was stronger when controls were restricted to those without acute respiratory symptoms, this difference was not significant. Controls had lower DRS than suspected cases, which was significant when the population was restricted to males only (P=0.006), and to currently non-smoking males (P=0.014). A similar patter was seen for potential cases.

Associations with exposure

Highly exposed subjects had lower FVC, FEV_1 and FEV_1/FVC than subjects with low exposure (P>0.10) (table 5). Odd ratio between BHR and exposure was 1.7 (95% CI 0.5-5.9). Overall, high exposure showed higher DRS than low exposure (P=0.056). Associations grew stronger when the population was restricted to males only (P=0.016), current non-smokers (P=0.034) and currently non-smoking males (P=0.019).

Table 5 Lung function parameters and total IgE results in exposure classes.

	Exposure		
	High	Intermediate	Low
n	25	7	32
FVC % of predicted (%)	106.4 (10.6)	110.0 (9.4)	110.5 (13.2)
FEV_1 % of predicted (%)	100.7 (14.3)*	104.2 (14.3)	107.3 (15.0)
FEV_1/FVC % of predicted (%)	94.4 (8.5)	94.6 (8.8)	97.3 (8.7)
PD_{20} positive (%)	7 (28.0)	1 (14.3)	6 (18.7)
DRS (25^{th}, 75^{th} percentile)	4.6 (3.2,15.6)**	3.5 (2.0,8.0)	3.4 (1.6, 7.0)
Total IgE (ln kU/l)	3.8 (1.7)	3.5 (1.6)	3.2 (1.2)

* Borderline significantly different from low exposure (P=0.099).

** Borderline significantly different from low exposure (P=0.056). Differences become significant when restricted to current non-smokers (P=0.034), to males (P=0.016) and to currently non-smoking males (P=0.019).

DRS: dose-response slope calculated as the % fall in FEV_1 per mg inhaled histamine; FEV_1: forced expiratory volume in one second; PD_{20}: the calculated dose of histamine that caused the FEV_1 to fall 20%; FVC: forced vital capacity. Lung function parameters and total IgE results presented as geometric means with standard deviations, positive PD_{20} presented as absolute numbers with percentage and DRS presented as median with 25^{th} and 75^{th} percentiles. A positive PD_{20} (i.e. abnormal) was defined as a $PD_{20} \leq 2.39$ mg histamine.

Clinical Investigation

In 14 suspected cases (56%), RADS was ruled out by the lung physician. In 10 suspected cases (40%) RADS could not be ruled out.

From other sources bronchial challenge test data were obtained from the years following the fire for three potential cases. All three cases were smokers at the time of the Drachten fire without pre-existing respiratory complaints. They experienced acute respiratory symptoms which deteriorated during the summer. Independently they visited a lung physician who performed histamine or methacholine challenge test (ranging from PC_{20}=1.3 mg/ml histamine to PC_{20}=2.5 mg/ml methacholine). 2 cases had quit smoking after they had visited a physician because of new-onset respiratory symptoms following the fire. In the following years BHR decreased and respiratory symptoms ameliorated. In 2 cases symptoms were still present in 2006. In 2 cases BHR was absent in 2006 and in one case it was still increased (PD_{20}=0.5671 mg histamine).

DISCUSSION

To our knowledge, this is the first study to investigate whether lung function and bronchial challenge parameters of residents and first responders are associated with exposure more than 6 years previously. In this study, a strong positive association was found between exposure to combustion products of a fire in a chemical waste depot and self-reported persisting respiratory symptoms among workers involved in the incident and the general population in the affected area. These findings were not seriously affected by correction for gender, involvement as a first responder or smoking status, which mainly resulted in a loss of precision for some odds ratios.

Although no significant differences were found between exposure and PD_{20}, the dose-response slope (DRS) was significantly lower in low exposed compared to highly exposed subjects, when restricted to current non-smokers and to males. Suspected cases also showed lower FEV_1/FVC and higher DRS than controls. FEV_1 was lower in potential cases than in controls. In these cases, FEV_1/FVC showed borderline significant differences compared to controls.

The findings of this study are consistent with earlier findings from studies which involved exposure to airway irritants. These findings included persisting respiratory symptoms, spirometric changes and bronchial hyper-responsiveness in first responders[10] and in residents[9]. In some studies cases with persisting respiratory effects were diagnosed with Reactive Airways Dysfunction Syndrome[10;20]. The term RADS was introduced in 1985 by Brooks et al[14] to describe the development of respiratory symptoms in the minutes or hours after a single accidental inhalation of high concentrations of irritant gases, aerosols, or particles. Later observations have made it clear that RADS can occur after exposure to a great variety of chemicals generated as gases or aerosols, such as sulphuric acid[21], as well as to smoke[7;14] or particles with an irritant nature[10;22;23]. Inhalation accidents may occur at home[24], in the workplace[14;20;25;26] or in the general environment[27;28]. There is common agreement that RADS is best described as non-immunological occupational asthma without a latency period, occurring after a single exposure to airway irritants and should be distinguished from the more commonly observed occupational asthma which has in many cases an underlying immunologic mechanism, which is characterized by sensitization[29]. Most of the highly exposed subjects emphasized the irritant nature of the combustion products. Given the structure of the questionnaire, cases most likely include new-onset cases. None of the interviewed subjects experienced a clinically severe inhalation injury, neither did a considerable part of subjects with persisting respiratory effects in other cohort studies[10;30]. The medical evaluation started more than 6 years after the incident had taken place. To overcome selection bias we approached the whole population that was present in the affected area at the moment of the fire. To recruit both residents, occupationally involved persons and chance bystanders we approached the subjects through a letter to their home address, a letter to their employer's address and an announcement in the local media. In the

telephone interview subjects were asked standardized questions about their health and exposure. Cases were selected exclusively on the basis of health criteria. Therefore, selection probability was not related to exposure and health outcome combined[31]. The proportion of first responders and others was evenly distributed over cases and controls. We conclude that self-selection, considering the fact that self-selection always lies in wait in this kind of studies, will undoubtedly have played a role. A careful examination of our data indicated that if present, the effects seemed limited.

In addition, non-responding controls did not differ from the examined controls for age, gender, smoking status and exposure. Therefore, terminating the invitation of subjects appeared to be a random event, and probably did not affect associations found, with the exception of reducing the statistical power.

Another issue in case-control studies is the possibility of recall bias resulting in exposure misclassification. This could have played a role because suspected cases could have recalled the onset of their symptoms as very shortly after the fire, whilst in fact the onset occurred later, or they could have remembered higher than actual exposure. Perception of what happened during and immediately after the incident and attribution of symptoms to the incident may have played a role. Some of the subjects could not accurately recall which respiratory symptoms they had experienced, or for how long these symptoms had persisted. This could have resulted in an overestimation of affected associations. On the other hand, controls who were free of symptoms during the study could have forgotten that they had experienced persisting respiratory symptoms, or they could have become so accustomed to their symptoms that they had become insignificant and therefore not worth mentioning. It therefore remains difficult to establish how these potential biases affected the associations.

Although high exposure consistently showed more BHR, only significant associations with exposure were found for DRS. Using DRS as a continuous measure of non-specific airway responsiveness avoids information loss because HCT data of all subjects could be used, as opposed to BHR which only used data of subjects with a demonstrated PD_{20}.

Improvements over time usually reduce differences between cases and controls and between highly and less exposed subjects. The observation that some associations were still detectable after 6 years indicated that the respiratory effects were possibly more severe shortly following the Drachten fire. This indication was supported by the observed improvements of the three aforementioned subjects, consistent with other studies[32].

CONCLUSION

Despite uncertainties we think that we have found some intriguing associations, which have implications for disaster management. In the major disasters in the Netherlands, such as the Bijlmer disaster and the Enschede fireworks incident, major fires were involved and large and costly studies were undertaken. However, Irritant-Induced Asthma and RADS were not

considered as potentially relevant endpoints. RADS and related respiratory effects were considered and demonstrated in the aftermath of the 9/11 disaster. An increased awareness amongst emergency services of these respiratory effects may help to avoid unnecessary exposure of first responders and the general population. We therefore recommend considering respiratory effects in incidents in which exposure to airway irritants is possible[9-12].

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CHAPTER 3

Respiratory symptoms in firefighters

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ABSTRACT

Background: The aim of the present study was to determine the prevalence and risk factors associated with respiratory symptoms in common firefighters in the Netherlands.
Methods: A total of 1330 firefighters from the municipal fire brigades of 3 provinces of the Netherlands were included in the study. All subjects were administered a Dutch web-based version of the European Community Respiratory Health Survey questionnaire.
Results: General respiratory symptoms were associated with the number of fires fought in the last 12 months with odds ratios between 1.2 (CI 95% 1.0-1.4) and 1.4 (CI 95% 1.2-1.7) per 25 fires. A strong association was found between an inhalation incident and present respiratory symptoms with odds ratios between 1.7 (CI 95% 1.1-2.7) and 3.0 (CI 95% 1.9-4.7). Adjustments for smoking, sex, atopy and age did not change any of the associations. After stratification, atopics showed elevated odds ratios.
Conclusions: It is recommended that firefighters are aware of these elevated health care risks associated with exposure to fire smoke and that they increase as much as possible the use of self-contained breathing apparatus.

INTRODUCTION

Fire fighting is a strenuous activity during which personnel is exposed to risks such as smoke inhalation. Firefighters should carry self-contained breathing apparatus (SCBA) to prevent smoke inhalation. Nevertheless, exposure to toxic hazards is a concern because these devices are often not or not continuously used during firefighting, especially owing to the visual impression of low smoke concentration[1;2]. Smoke contains substances injurious to airways such as acid gases, aldehydes and respirable particulate matter[1;3]. Earlier studies have indicated that exposure of firefighters to smoke may result in acute lung function impairment[4-6], acute increase of airway responsiveness[7], and acute increase of respiratory symptoms[8]. Furthermore studies have suggested that firefighters are at risk of chronic respiratory symptoms and lung function impairment[9-11].
Information about possible respiratory effects of exposure during firefighting has been collected in different countries over several decades. The use of self contained breathing apparatus has increased over the years and many studies have focused specifically on high risk subcategories such as forest firefighters[8;12;13], or firefighters in the 9/11 disaster[14;15].
Only few studies with sufficient power have been conducted among common fire fighters, and little is known about potential respiratory health risks with modern breathing apparatus. In this study we investigated the respiratory health in common firefighters in the Netherlands in relation to exposure to combustion products of fires.

MATERIALS AND METHODS

Population and design

In the present cross-sectional survey, all active firefighters of the municipal fire brigades of 3 provinces in the Netherlands (Groningen, Friesland and Drenthe) were invited to fill in a web-based questionnaire. The management of the fire brigades was approached to give information on fires and hazmat incidents in their communities.
The institutional review board for human studies of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved the protocol and informed consent was obtained from all participants.

Questionnaires

The questionnaire was a web-based version of the European Community Respiratory Health Survey questionnaire translated into Dutch[16]. Questions were added to identify the type and number of incidents, the type, the onset and the duration of symptoms and to determine possible exposure during an incident.
General respiratory symptoms during the last 12 months, work-related respiratory symptoms and symptoms indicative of the presence of atopy and bronchial hyper-responsiveness (BHR)

were the health outcomes of interest. Atopy, asthma and BHR were defined on the basis of questionnaire items involving treatment of allergies (asthma, hay fever, eczema and other allergies), ('Have you ever had asthma') and BHR-like symptoms (respiratory symptoms in humid air, during acute temperature changes, and when exposed to second hand cigarette smoke, etc.). Exposure was defined on the basis of questionnaire items involving working years, inhalation incidents ('have you ever inhaled a large amount of smoke') and the number of fires fought during the last 12 months.

Comparison with ELON

Prevalence of common respiratory symptoms was compared with information from a random sample (2,711 people) of the Dutch population. These symptoms originated from the execution of the Dutch version of the European Community Respiratory Health Survey [European Respiratory Health Study the Netherlands: ELON][17].

Statistical analyses

The association between firefighter characteristics and reported health symptoms was calculated using a multiple logistic analysis, which also allowed for adjustment for potential confounders. Associations were adjusted for smoking, sex, atopy and age. Effect modification was examined by analyzing atopic and non-atopic individuals separately and never smoking, formerly smoking and currently smoking individuals separately. The level of statistical significance was set at $p<0.05$.

RESULTS

General information

In this study all municipal fire brigades (n=54) in the three northern provinces of the Netherlands were invited. Fire brigades ranged from 1 up to 10 fire stations (n=142) in a municipal fire brigade. Of all fire brigades contacted through surface mail and telephone, 98% responded; the average worker participation rate per fire brigade was 49%.The management of all fire brigades consisted of professional career firefighters, whereas most of the active firefighters were volunteers (83.9%). Of 197 professional firefighters (18.8%) 84 also worked as volunteers (6.9%).

In the Netherlands, firefighters, professionals and volunteer, complete the same education, are equally trained, train the same minimal amount of hours monthly and have equal access to SCBA. Furthermore, they undergo mandatory pre-employment as well as periodical (frequency depending on age) medical examinations, but no clear-cut exclusion criteria are being used.

Total number of fires in 2008 in the area was 4301 (no data available from 4 fire stations within 1 fire brigade) and ranged from 5 in a small rural community to 485 in Groningen

(185,000 inhabitants). Fire fighter characteristics are outlined in table 1. From the 2814 firefighters, 1330 (47.3%) responded, of which the completed questionnaires (n=1249) were used for analyses. Most of the firefighters were male (90.3%). Firefighters had a mean age of 39.8 years.

Table 1: Descriptive characteristics of the firefighters (n=1,249).

	Total	***Male***	***Female***
Sex	1249 (100)	1128 (90.3)	120 (9.6)
Current smoker (no., %)	377 (30.2)	340 (30.1)	37 (30.8)
Ex-smoker (no., %)	332 (26.6)	302 (26.8)	30 (25.0)
Age, years (mean, SD, range)	39.8 ± 8.5 (19-60)	40.1 ± 8.5 (20-60)	37.2 ± 7.4 (19-52)
Working as fire fighter, years (mean, SD, range)	10.8 ± 8.5 (0-39)	11.4 ± 8.6 (0-39)	5.4 ± 4.8 (0-18)

Questionnaire study

The frequency of symptoms occurring during or immediately following fire fighting in the last 12 months was 31.2% and ranged from 0.7% (nosebleeds) to 28.8% (coughing) (table 2). Eighteen employees (1.8%) visited a doctor with complaints related to exposure to fire smoke. No differences were found in gender and in smoking behavior among volunteer and other firefighters. Employment as a firefighter was shorter among subject who worked as volunteers (10.3 ± 8.0 years) than professional firefighters (13.5 ± 10.3 years; p=0.01) or those who combined functions (13.1 ± 10.0 years; p=0.03).

The prevalence of respiratory symptoms ranged from 0.5% (asthma attack during last 12 months) to 19.2% (BHR-like symptoms). The prevalence of general respiratory symptoms and atopy are shown in table 3.

Table 2 Frequency of acute symptoms following a fire in the past 12 months.

Acute symptoms	*Seldom (1-5x) no., (%)*	*Frequently (6-10x) no., (%)*	*Very frequently (>10x) no., (%)*	*Total no., (%)*
Wheezing	30 (3.0)	2 (0.2)	1 (0.1)	33 (3.3)
Coughing	271 (27.4)	12 (1.2)	2 (0.2)	285 (28.8)
Shortness of breath	74 (7.5)	4 (0.4)	2 (0.2)	80 (8.1)
Irritation of the lungs	77 (7.8)	2 (0.2)	2 (0.2)	81 (8.2)
Itchy eyes	151 (15.3)	5 (0.5)	0	156 (15.8)
Itchy nose	109 (11.0)	10 (1.0)	1 (0.1)	120 (12.2)
Sore throat	98 (9.9)	5 (0.5)	0	103 (10.4)
Headache	126 (12.8)	10 (1.0)	1 (0.1)	137 (13.9)
Nosebleeds	4 (0.4)	3 (0.3)	0	7 (0.7)
Dizziness	33 (3.3)	2 (0.2)	0	35 (3.5)
Nausea	13 (1.3)	1 (0.1)	0	14 (1.4)
Chest pain	18 (1.8)	1 (0.1)	0	19 (1.9)
Feeling of weakness	25 (2.5)	1 (0.1)	0	26 (2.6)

Table 3 General respiratory symptoms

General respiratory symptoms	***Number (%)***
Wheeze during last 12 months	95 (7.7)
Yes, with shortness of breath	59 (4.8)
Woken up by shortness of breath during last 12 months	31 (2.5)
Cough at wake up during winter	64 (5.2)
Cough at day/night time during winter	85 (6.9)
Phlegm at wake up during winter	56 (4.5)
Phlegm at day/night time during winter	41 (3.3)
Dyspnea when walking on a flat surface with people of the same age	14 (1.1)
Have you ever had asthma?	93 (7.5)
Was it doctor diagnosed	89 (7.2)
Asthma attack during last 12 months	6 (0.5)
Asthma medication at moment	30 (2.4)
Bronchial hyperresponsiveness-like symptoms	235 (19.2)
Allergy treatment	245 (19.8)

Table 4 Association between general respiratory symptoms and exposure.

General respiratory symptoms	***Exposure estimates***			
	Number of fires Crude odds ratio (95% CI)	**Number of fires Adjusted odds ratio (95% CI)**	**Inhalation incident Crude odds ratio (95% CI)**	**Inhalation incident Adjusted odds ratio (95% CI)**
Wheeze during last 12 months	1.2 (<1.0-1.4)	1.1 (0.9-1.3) [¶]	2.3 (1.5-3.5)	2.3 (1.5-3.6) [¶]
Woken up by shortness of breath during last 12 months	1.0 (0.7-1.5)	1.0 (0.6-1.5) [¶]	1.2 (0.6-2.6)	1.2 (0.5-2.5) [¶]
Cough at wake up during winter	1.1 (0.9-1.4)	1.1 (0.9-1.4) [¶]	2.3 (1.4-3.8)	2.3 (1.4-3.9) [¶]
Cough at day/night time during winter	1.3 (1.1-1.5)	1.3 (1.1-1.5) [¶]	3.0 (1.9-4.7)	3.0 (1.9-4.7) [¶]
Phlegm at wake up during winter	1.4 (1.2-1.7)	1.4 (1.1-1.7) [¶]	2.8 (1.6-4.8)	2.8 (1.6-4.8) [¶]
Phlegm at day/night time during winter	1.4 (1.1-1.6)	1.3 (1.1-1.6) [¶]	1.8 (<1.0-3.4)	1.8 (<1.0-3.4) [¶]
Have you ever had asthma?	1.3 (1.1-1.5)	1.2 (1.0-1.5) [¶]	1.7 (1.1-2.7)	1.8 (1.1-2.8) [¶]
Bronchial hyperresponsiveness-like symptoms	1.1 (<1.0-1.3)	1.1 (<1.0-1.3) [¶]	2.4 (1.8-3.2)	2.5 (1.8-3.4) [¶]
Allergy treatment	1.2 (1.0-1.4)	1.2 (1.0-1.4) [§]	1.1 (0.8-1.5)	1.1 (0.8-1.5) [§]

†¶: Odds ratio adjusted for age, gender, smoking and atopy. §: Odds ratio adjusted for age, gender and smoking.

In an exploratory logistic regression analysis between possible determinants and respiratory symptoms we found associations between the number of fires fought in the last 12 months and some respiratory symptoms with odds ratios between 1.2 (CI 95% 1.0-1.4) and 1.4 (CI 95% 1.2-1.7) per 25 fires (table 4). The average number of fires fought was 12.2 and the maximum was 302. 152 firefighters (12.5%) fought 25 fires or more last year. Exclusion of 3 subjects fighting more than 200 fires in the last 12 months did not change the associations. Adjustments for smoking, sex, allergy treatment and age did not change associations. A strong association was found between any inhalation incident and present respiratory symptoms with odds ratios between 1.7 (CI 95% 1.1-2.7) and 3.0 (CI 95% 1.9-4.7). Adjustments for smoking, sex, atopy and age did not change any of the associations. Being volunteer or professional firefighters was not a determinant of respiratory symptoms.
When we stratified the population into atopic and non-atopic individuals based on the item 'Have you ever been treated for an allergic disease' in the questionnaire we found, that odds ratios were slightly elevated for all respiratory symptoms for atopic individuals. The association between the determinant 'number of fires fought during the last 12 months' and respiratory symptoms was not significant for non-atopic individuals.
Stratified analyses for smoking behavior did not give any evidence of effect modification for respiratory symptoms.
Comparison with ELON showed a statistically significantly lower prevalence of several respiratory symptoms in firefighters compared with the Dutch general population (table 5). ORs ranged from 0.5 (95%CI 0.3-0.7) for woken up by shortness of breath to 0.3 (95%CI 0.2-0.4) for wheeze. There was a statistically significant elevated prevalence of firefighters who had ever had asthma compared to the ELON population an OR of 1.5 (CI95% 1.1-2.0).

Table 5: Comparison of general respiratory symptoms in firefighters and general respiratory symptoms in the general Dutch population, ELON (n=2,711), adjusted for age smoking and gender.

General respiratory symptoms	***Adjusted odds ratio (95% CI)***
Wheeze during last 12 months	0.3 (0.2-0.4)
Woken up by shortness of breath during last 12 months	0.5 (0.3-0.7)
Cough at wake up during winter	0.4 (0.3-0.6)
Cough at day/night time during winter	0.4 (0.3-0.5)
Phlegm at wake up during winter	0.4 (0.3-0.6)
Phlegm at day/night time during winter	0.3 (0.2-0.5)
Have you ever had asthma?	1.5 (1.1-2.0)
Dyspnoea when walking on a flat surface	0.3 (0.3-0.5)

DISCUSSION

We found a positive association between an increased prevalence of general respiratory symptoms and exposure in firefighters. Furthermore, a positive association was found between general respiratory symptoms and the number of fires fought in the last 12 months. Firefighters showed a higher prevalence of asthma than a Dutch general population sample. On the other hand, firefighters showed a lower prevalence of several respiratory symptoms than this population sample. It was found that the effect of exposure to fire smoke was higher in atopics.

The crude associations were adjusted for gender, age, smoking and atopy, which had no major effect.

In this study, the exposure was estimated using a questionnaire. The questionnaire items working years, inhalation incidents and number of fires fought in the last 12 months were used as proxies for exposure. Other studies have shown an association between exposure and reduction in pulmonary function[6;10;18]. However, since most of these associations were found in a time when self-contained breathing apparatus (SCBA) was not yet commonly used, the more remarkable it is that respiratory symptoms are associated with the number of fires fought in the last 12 months in a time when SCBA's are widely used. Others have suggested that, although the availability and effectiveness of protective devices such as SCBA's has increased, SCBA is insufficiently used by firefighters due to its weight and inconvenience, especially when smoke is not visible and during phases of overhaul or work in the second line (drivers, pump manipulators), when important exposure to combustion products may persist[2,9] Our results that respiratory symptoms are associated with the number of fires fought, suggests that the use of SCBA's should still be increased. Additionally, the development of a lighter SCBA would facilitate the use of it, and should therefore be encouraged. In our study, respiratory symptoms were strongly associated with an inhalation incident with a large amount of smoke considerable smoke during which a large amount of smoke may have been inhaled. These associations are consistent with studies among residents, firefighters and other workers involved in the WTC disaster[19-21]. Working years was not associated with symptoms which is also consistent with earlier studies[22;23]. By contrast, Mustajbegovic et al. demonstrated a positive relationship of respiratory symptoms with working years[10]. Austin et al. suggested that reliance on years of employment as a surrogate for exposure might lead to misclassification of exposure and therefore underestimation of the risk of disease[24]. The observation that working years is a poor proxy for exposure is supported by our finding that the median number of fires fought in the last working year was 6 with a minimum of 0 fires (13.9%) and a maximum of more than 100 (0.8%).

It is of interest that the risk of respiratory symptoms in atopics was elevated compared to non-atopics. This is a strong indication for effect modification by atopy that should be further investigated in a study with objective measures.

Brooks et al. suggested before that atopy may be a risk factor for (not-so-sudden onset) Irritant-Induced asthma[25]. In a recently performed study it was found that the prevalence of atopy based upon skin prick test was higher among urban firefighters compared with a Swiss population sample[9]. Associations of atopy based on IgE with exposure of non-allergenic compounds have been found in earlier studies[26]. A possible mechanism underlying these associations could be a disruption of the lung epithelial barrier facilitating the penetration of allergens in the lung[27]. As the prevalence of allergic rhinitis has risen in the world over the last decades[28], it is of importance to evaluate the role of atopy in the prevalence of respiratory symptoms in firefighters.

The presence of general respiratory symptoms in firefighters might partly be explained by temporary acute respiratory symptoms following exposure to fire smoke. However, we cannot rule out that some exposed firefighters might suffer from RADS (Reactive Airways Dysfunction Syndrome) or IIA (Irritant-Induced Asthma). The current study showed a lower prevalence of several respiratory symptoms in firefighters compared to a Dutch general population sample. In the Netherlands firefighters undergo mandatory pre-employment as well as periodical (frequency depending on age) medical examinations, including lung function testing, but no clear-cut exclusion criteria are being used. Depending on their age, they will be re-examined as frequently as every two or four years. This selection mechanism combined with the healthy worker effect might explain the differences in respiratory symptom prevalence between the two populations. However, the observation of a higher prevalence of asthma among firefighters is remarkable and cannot be explained this way. In this light, it should be emphasized that the finding that exposure is associated with respiratory symptoms was demonstrated in a relatively healthy population. The higher prevalence of asthma in this study therefore is unexpected. We do not at current have an explanation. Information bias is an unlikely explanation, because in a subset of 402 fire fighters, asthma and wheezing in the last 12 months were associated with BHR (OR [95% CI], 5.5 [2.6–11.6] respectively OR [95% CI], 2.7 [1.3–5.6]). The apparent increase in asthma occurrence seems real and we can thus not rule out an increased risk for developing Irritant-Induced Asthma in firefighters.

Since only 49% of the firefighters completed the questionnaire, selection bias might have occurred. However, reported symptom prevalence in fire brigades with a high response (>60%) were comparable with fire brigades with a lower response (<40%). Responder bias would probably be a more important limiting factor. A good contact with the study population was difficult, because they could only to be approached with an invitation letter with general encouragement by the management.

As in other questionnaire studies, recall bias may have occurred. Possibly low-level smoke concentration is not judged as a health risk. For instance, firefighters who have experienced a substantial inhalation incident will probably not refer to minor incidents. During the most recent fire 55.4% of the firefighters was exposed to visible smoke and/or noticed inhalation of

smoke. Furthermore an inhalation incident was associated with the number of fires (OR [CI 95%], 1.2 [1.0-1.4] per 25 fires), which argues against a major influence of recall bias.
In conclusion, we have found clear associations between number of fires and inhalation incidents with respiratory symptoms at present. Despite a potential healthy worker effect and the possible influence of pre-employment medical examinations, exposed individuals reported respiratory symptoms more often. It is recommended that firefighters are aware of these elevated health care risks associated with exposure to fire smoke and that they increase as much as possible the use of self-contained breathing apparatus.

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CHAPTER 4

Lung function, bronchial hyperresponsiveness and atopy among firefighters

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ABSTRACT

Objectives: The aim of the present study was to determine associations between lung function, bronchial hyperresponsiveness and atopy with exposure to fire smoke in firefighters.
Methods: The study comprised of 402 firefighters, a randomly chosen subset of a previous survey among firefighters in the Netherlands. Subjects underwent spirometry and methacholine provocation, and blood samples were taken to assess atopy. Exposure to fire smoke was registered by a questionnaire.
Results: Hyperresponsiveness expressed as dose-response slope was positively and significantly associated with the number of fires fought in the last 12 months with and without adjustments for smoking, sex, atopy, age and exposure in the main job held. Limiting the analysis to firefighters without exposure within 7 days of testing did not change any of the associations. The association between number of fires and the dose-response slope was stronger among atopics, and hyperresponsiveness expressed as PD_{20} was also significantly associated, indicating that atopics are at higher risk of developing hyperresponsiveness as a result of smoke exposure. Respiratory protection devices were not optimally used.
Conclusions: It is recommended that awareness is strengthened among firefighters to avoid exposure to all fire smoke and that the management is fully perceptive in the adequate use of self-contained breathing apparatus by their personnel.

INTRODUCTION

Although firefighters have access to self-contained breathing apparatuses to prevent smoke inhalation, exposure to toxic hazards is a concern, because the devices are not consistently used during firefighting, especially owing to the visual impression of low smoke concentration[1;2]. Previous studies have indicated that smoke exposure may result in acute lung function impairment[3;4] and acute increase of airway responsiveness[5]. Furthermore, studies have suggested that firefighters are at risk of chronic respiratory symptoms and lung function impairment[6-8]. On the other hand, some studies did not find associations between exposure and lung function impairment[9;10]. Bronchial hyperresponsiveness in firefighters was mostly assessed shortly following exposure to fire smoke in a small population[5;11] or assessed following the World Trade Center disaster in New York, USA[12;13]. Miedinger et al. found increased bronchial hyperresponsiveness (BHR) in a population of 101 firefighters as compared to the Swiss general population. However, an association with levels of exposure was not demonstrated[6].
Information about possible respiratory effects of firefighting has been collected in different countries over several decades. The use of self-contained breathing apparatuses has increased over the years though firefighter-procedures vary in different parts of the world. Only few studies with sufficient power have been conducted among common firefighters, and little is known about potential respiratory health risks using modern breathing apparatus. In this study we investigated the respiratory health of firefighters in the Netherlands in relation to exposure to combustion products from fires.

METHODS

Population and design

The current cross-sectional study was carried out in a subset of a population of firefighters of a previous study in the Netherlands[14]. All tests were carried out at the fire stations between December 2008 and June 2009.
The institutional review board for human studies of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved the protocol and written consent was obtained from all participants.

Questionnaire and exposure estimates

Questionnaire items have been previously described elsewhere[14]. Due to the time lapse between the previous questionnaire and the execution of the tests at the fire stations (between 6 and 11 months), questions were asked again to (i) identify the type and number of incidents, and the type, the onset and the duration of symptoms following the last fire and (ii) to determine possible exposure during an incident. Several personal exposure estimates

were obtained by questionnaire items on working years, inhalation incidents ('have you ever inhaled a large amount of smoke'), the use of self-contained breathing apparatus, the number of days since the last fire preceding the test, and the number of fires fought during the last 12 months. Additionally, job titles were reviewed for potential occupational exposure to airway irritants (dusts, gases or fumes). The exposure was graded in no, possible, and certain exposure. Exposure in the main job was considered as a potential confounding variable.

Serology

Blood samples were processed within 4 hours and serum aliquots were stored at -80 ºC until serologic assays were done. Specific immunoglobulin E (IgE) to common aeroallergens were analyzed in our laboratory based on adjusted previously published methods[15] as a measure of atopy. In short, the common panel of allergens consisted of house dust mite, cat, dog, grass pollen mixture (Phleum Pratense and Lolium Perenne, protein 1:1), and birch pollen (Allergon AB, Angelholm, Sweden). Coating concentrations were: House dust mite (HDM) 10, Cat 15, Dog 10, Grass mixture 15 and Birch 20 µg protein/ml. The final Optical Density (OD) signal was double corrected for the serum blank and the reagent blank. OD above 0.05 was considered positive. Total IgE was measured as described elsewhere[15], using a detecting system consisting of Polyclonal rabbit anti human IgE 1:3500 (DAKO A00094 , Dakopatts, Copenhagen, Denmark) and Swine anti- Rabbit/HrP (DAKO PO364) 1:3500 in physiological salt buffer (PSB) 0.05% v/v Tween 20 and 0.2 % w/v gelatine. Performance remained equal to the original system. Atopy was defined as positive reaction to the specific IgE panel or total IgE exceeding 100 kU/l[16;17]. We also explored specific atopy defined as positive reaction to the specific IgE panel[18].

Spirometry and methacholine challenge

Experienced technicians carried out the spirometry according to European Respiratory Society standards[19]; results are presented as % of predicted[20].

Bronchial hyperresponsiveness (BHR) was assessed by inhalation of increasing doses of methacholine delivered via a de Vilbiss 646 nebulizer chamber using a ZAN 200 breath triggered pump (Zan, Oberthulpa, Germany). Dosing was started with PBS followed by a 0.03 mg methacholine dose after three quadrupling doses up to a cumulative dose of 1.92 mg (short schedule). FEV_1 was measured 30 and 90 seconds after each challenge and the lowest FEV_1 from a technically acceptable manoeuvre was used. After a fall in FEV_1 of 5%, doubling doses were used (long schedule). The test was stopped when a fall of 20% in FEV_1 was observed (PD_{20}) or the maximum cumulative dose was reached. Bronchial hyperresponsiveness (BHR_{20}) was considered to be present if $PD_{20} \leq 1.92$ mg methacholine. To make optimal use of all available data, we also calculated the dose-response slope (DRS) as the % fall in FEV_1 per mg inhaled methacholine[21].

Statistical analyse

SAS version 9.1 statistical software was used (SAS Institute, Cary, NC). Associations between (log-transformed) exposure variables and continuous health outcomes were calculated using a linear regression analysis. Logistic regression was used to describe associations for binary health outcomes. The level of statistical significance was set at $p<0.05$. Associations were adjusted for smoking, sex, atopy and age. Effect modification was examined by analyzing atopic and non-atopic individuals separately and never smoking, formerly smoking and currently smoking individuals separately.

RESULTS

Population characteristics

In a previously conducted survey, 1249 active firefighters from 54 municipal fire brigades in 3 provinces of the Netherlands (Groningen, Friesland and Drenthe) filled in a web-based version of the European Community Respiratory Health Survey questionnaire[14]. For the present study 21 fire brigades were randomly chosen until the required number of 400 firefighters was examined. The invited study population comprised of 424 subjects, who had indicated in the previous survey that they were willing to participate. In total 402 of 424 firefighters (94.8%) were examined. Of 22 non-participating subjects, 13 could not fit in the planned schedule of measurements, 4 refused participation, 2 reported ill, 2 quitted working as firefighter and 1 was pregnant. One fire brigade consisted solely of professional firefighters, 2 fire brigades consisted of both professional and volunteer firefighters. Remaining fire brigades consisted for the majority of volunteers. All tests were carried out at fire stations between December 2008 and June 2009.

General characteristics of the study population and exposure estimates are shown in table 1. Of 402 firefighters 305 worked as volunteer, 60 as professional, and 37 as both. Compared to males, females worked less time as firefighters ($p<0.0001$), were younger ($p=0.002$), fought their last fire preceding the examination longer ago ($p=0.0036$), and used self-contained breathing apparatus more often during the last fire they fought preceding the examination ($p=0.006$).

Table 1 Descriptive characteristics of the firefighters.

	Total	*Male*	*Female*
Sex	402 (100)	356 (88.6)	46 (11.4)
Smoker	111 (27.7)	100 (28.1)	11 (24.4)
Ex-smoker	115 (28.7)	102 (28.7)	13 (28.9)
Age [year]	41.3±8.1 (20-60)	41.8±8.0 (20-60)	37.9±7.8 (22-53)*
Length of employment as firefighter [year]	12.5 ± 8.5 (0-40)	13.3 ± 8.6 (0-40)	6.1 ± 4.8 (0-19)*
Number of fires fought in the last 12 months	16.7 ± 18.9 (0-200)	17.1 ± 19.6 (0-200)	13.0 ± 11.8 (0-51)
Inhalation incident cases ever	139 (34.8)	122 (34.6)	17 (37.0)
Time passed since last fire [day]	14.0 [9.0, 100.0]	14.0 [7.5, 100.0]	35.5 [14.0, 100.0]*

Data are presented as n, n (%), mean ± SD (min-max), or median [25th, 75th percentile]; * p<0.05.

Occupational exposure to airway irritants in the main job was certain for 9 firefighters (2.2%). For 325 firefighters (80.8%) additional exposure seemed unlikely, and for 13 firefighters (3.2%) we had no information about job titles. For 55 firefighters (13.7%) main job exposure seemed uncertain and therefore we conducted a sensitivity analysis considering this category once being exposed and once being not exposed.
Table 2 shows that, during the last fire fought before the examination, 344 subjects (86.6%) did not use SCBA at all/all the time. Exposure to fire smoke was considered intolerable after a few minutes by 1 subject (0.4%), discomforting by 48 (20.4%), or perceptible by 162 (68.9%).

Table 2 Use of self-contained breathing apparatus during the last time firefighting preceding the examination.

	Not all the time	*Not at all*
	N (%)	N (%)
Use of self-contained breathing apparatus	136 (34.3)	208 (52.4)
Unprotected exposure to visible smoke or inhalation of smoke	110 (27.7)	125 (31.5)

Symptoms
Prevalence of respiratory symptoms ranged from 0.7% (asthma attack during last 12 months) to 10.0% (Wheeze during last 12 months) (table 3). Prevalence of respiratory symptoms among medically tested subjects (n=402) did not differ from the prevalence of symptoms among the initial sample of firefighters who responded to the questionnaire (n=1249).

Table 3 General respiratory symptoms.

General respiratory symptoms	***Total (n=402)***
Wheeze during last 12 months	40 (10.0)
Wheeze and shortness of breath during last 12 months	30 (7.5)
Woken up by shortness of breath during last 12 months	14 (3.5)
Cough upon waking up during winter	26 (6.5)
Cough at day/night time during winter	38 (9.5)
Phlegm at wake up during winter	22 (5.5)
Phlegm at day/night time during winter	20 (5.0)
Dyspnoea when walking on a flat surface with people of the same age	4 (1.0)
Have you ever had asthma?	34 (8.5)
Was the asthma doctor diagnosed?	34 (8.5)
Asthma attack during last 12 months	3 (0.7)
Current asthma medication	10 (2.5)

Data are presented as n, n (%).

Bronchial hyperresponsiveness, spirometry and atopy

BHR could not be determined for 10 subjects because (i) the spirometric maneuver was technically unacceptable (n=5), (ii) discomfort was experienced following the baseline lung function test (n=3), (iii) FEV_1 was <50% reference (n=1) or (iv) the individual was temporarily using oral corticosteroids (n=1). Serology was not available for 4 subjects, because insufficient blood was obtained (n=3) or giving a blood sample was refused (n=1).

In table 4, BHR, baseline spirometry and atopy are presented. FEV_1 was significantly higher in females (p=0.046), as were FVC (p=0.001) and FEV_1/FVC (p<0.001). A total of 19 firefighters (4.7%, all male) showed a FEV_1 below the Lower Limit of Normal (LLN is defined as 1.645 times the standard deviation of predicted value), and 35 (8.7%, all male) firefighters showed a FEV_1/FVC below the LLN.

Table 4 Lung function, bronchial hyperresponsiveness and atopy.

	All (n=402)	*Male (n=355)*	*Female (n=46)*
$FEV_1\%_{predicted}$	101.6 ± 12.8 (50.7-138.7)	101.1 ± 12.7 (50.7-138.7)	105.1 ± 12.9 (82.3-131.1)*
$FVC\%_{predicted}$	107.9 ± 12.3 (70.6-149.2)	107.2 ± 11.8 (70.6-149.2)	113.4 ± 14.2 (92.8-144.1)*
FEV_1/FVC%	77.3 ± 6.3 (52.1-97.1)	76.9 ± 6.4 (52.1-90.5)	80.5 ± 4.7 (71.2-97.1)*
MMEF%	82.2 ± 22.1 (18.0-145.0)	81.8 ± 22.4 (18.0-145.0)	86.1 ± 19.0 (53.5-128.8)
BHR_{20}, n (%)	63 (16.1)	52 (15.0)	11 (23.9)
DRS	4.2 [2.4,7.3]	4.2 [2.4,7.2]	4.7 [2.7,10.8]
Atopy, n (%)	126 (31.4)	115 (32.4)	11 (23.9)

Data are presented as n (%), mean ± SD (min-max), or median [25th, 75th percentile].

BHR_{20}: bronchial hyperresponsiveness, PD_{20}≤1.92 mg methacholine causing a fall in forced expiratory volume in one second (FEV_1); FVC: forced vital capacity; MMEF: maximal mid-expiratory flow; DRS: dose-response slope; * p<0.05.

Associations with exposure

Hyperresponsiveness expressed as log-transformed DRS was associated with the log-transformed number of fires fought in the last 12 months (table 5). BHR_{20} was not associated with number of fires fought [OR=1.5 (CI 95% 0.8-3.1)].

Stratification for atopy showed a positive association of BHR_{20} with the number of fires fought with an odds ratio of 4.9 (CI95% 1.4-16.6) per 10 fires in atopic subjects, whereas in non-atopic individuals no association was found [OR=0.8 (CI 95% 0.3-1.9)]. This association became stronger after adjustment for smoking [OR=6.0 (CI 95% 1.6-22.0)]. Stratification for specific atopy showed also an association of BHR_{20} with the number of fires fought with an odds ratio of 3.7 (CI95% 1.1-12.9) per 10 fires in atopic subjects, whereas in non-atopic individuals no association was found [OR=0.9 (CI 95% 0.4-2.1)]. Again, this association became stronger after adjustment for smoking [OR=4.4 (CI 95% 1.2-16.5)]. Equally, the DRS was positively associated with number of fires fought in atopic subjects (β= 0.309, P=0.03) after adjustment for smoking, whereas in non-atopic subjects no association was found (β= 0.091, P=0.19). The DRS was borderline significantly associated with the number of fires fought in (specific) atopic subjects (β= 0.349, P=0.06) after adjustment for smoking. In non-atopic subjects no association was found (β= 0.084, P=0.18).

The aforementioned associations between hyperresponsiveness and the number of fires fought were hardly affected when we excluded subjects who had fought a fire within 7 days of testing. The association between exposure and BHR_{20} was hardly affected when cases with FEV_1/FVC below Lower Limit of Normal were excluded from the analysis [OR=4.6 (CI95% 1.3–16.7)] per 10 fires. No signs of effect modification were found for smoking.

The associations were also adjusted for exposure to airway irritants during the main job. A positive association was found between exposure to airway irritants and DRS (β = 0.734,

P<0.001) or BHR_{20} [OR=4.6 (CI95% 1.2–17.7)], when uncertain job exposure was defined as no additional exposure. When uncertain main job exposure was also designated as positive exposure the association with DRS became weaker (β= 0.132, P=0.062) and the association with BHR_{20} disappeared [OR=1.6 (CI95% 0.8–3.1)]. Adjustment for the strict definition of main job exposure strengthened the relation between DRS and the number of fires (β= 0.159, P=0.013). Adjustment for the lenient definition of exposure did not affect the association between DRS and number of fires (β= 0.144, P=0.029).

No associations were found between the effect parameters, DRS and BHR_{20}, and the exposure estimates, working years, inhalation incidents and the time passed since the last fire. Furthermore, DRS and BHR_{20} were not associated with any exposure, nor with the level of exposure during the last fire fought (data not shown).

Table 5 Factors affecting lung function and methacholine provocation parameters.

	Exposure parameter	***Coefficient***	***p-value***
FEV_1*	Number of fires	0.019	0.78
	Inhalation incident	-0.035	0.55
	Working years	0.008	0.94
	Time passed since last fire	0.0004	0.36
FVC*	Number of fires	-0.010	0.90
	Inhalation incident	-0.088	0.21
	Working years	0.004	0.97
	Time passed since last fire	-0.001	0.31
FEV_1/FVC*	Number of fires	0.485	0.55
	Inhalation incident	0.437	0.52
	Working years	0.174	0.88
	Time passed since last fire	0.001	0.77
MMEF*	Number of fires	0.163	0.96
	Inhalation incident	1.533	0.53
	Working years	-1.763	0.68
	Time passed since last fire	0.004	0.81
DRS[§]	Number of fires	0.157	0.02
	Inhalation incident	-0.016	0.79
	Working years	0.060	0.41
	Time passed since last fire	0.0002	0.67

DRS, working years and number of fires are log-transformed; * adjusted for smoking, height and age;
[§] adjusted for smoking.

No associations were found between exposure and baseline lung function (table 5). We adjusted for age-dependency of lung function parameters comparing subjects younger than 25 years with the reference values for 25 years[20;22]. Also PEF (Peak Expiratory Flow) and

Maximum expiratory flow (MEF_{25}, MEF_{50}, and MEF_{75}) were not associated with exposure. No significant associations were found between any exposure estimate and atopy.

DISCUSSION

We found a positive association between bronchial hyperresponsiveness and the number of fires fought in the last 12 months. Adjustment of the crude associations for gender, age, smoking, atopy and exposure to airway irritants during the main job had no major effect. In addition, we found indications that in case of exposure to fire smoke, atopics seem more at risk for bronchial hyperresponsiveness as a result of fire smoke exposure.

The questionnaire items working years, inhalation incidents, number of days since the last fire, and number of fires fought in the last 12 months served as exposure estimates. Prior studies on respiratory health of firefighters mainly focused on respiratory symptoms and baseline lung function. Studies which assessed bronchial hyperresponsiveness in firefighters are mostly confined to acute response[5;11] or the respiratory sequelae of the World Trade Center disaster[12;13]. Although Miedinger et al. found a higher prevalence of bronchial hyperresponsiveness in firefighters compared to a general Swiss population, a relation to length of employment as exposure estimate could not be demonstrated[6]. However, in our study we found a positive relation between number of fires fought in the last 12 months and bronchial hyperresponsiveness (β=0.146, p=0.03) adjusted for smoking. When we additionally adjusted for atopy and asthma, this association was slightly stronger (β=0.156, p=0.018). In our analyses we used two definitions of atopy; a positive reaction to specific IgE or total IgE exceeding 100 kU/L, and a positive reaction to specific IgE only. The results for both analyses were very similar. The association between DRS and the number of fires fought became slightly weaker adjusted for specific IgE only, whereas this association was not affected when it was adjusted for smoking, job exposure and specific IgE simultaneously.

Acute effects of exposure to fire smoke can be ruled out as an explanation for our findings, because associations were hardly affected when we limited the analysis to firefighters without exposure 7 days prior to testing (β=0.148, p=0.047). Additionally, this association was not affected, when we excluded 9 individuals for a second analysis, to eliminate the possible effect of a subpopulation that had not yet fought a fire and worked less than 6 months as a firefighter (β=0.162, p=0.023). As the majority of firefighters were volunteers, we further explored the influence of occupational exposure to airway irritants other than during firefighter tasks. Although a positive association was found between main job exposure and bronchial hyperresponsiveness, the association between hyperresponsiveness and the number of fires fought in the last 12 months was not negatively affected after adjustment for this exposure. Furthermore, hyperresponsiveness was not related to any exposure during the last fire fought. Therefore, this result seems to be indicative of a persisting respiratory effect of smoke inhalation.

Associations of atopy and bronchial hyperresponsiveness have been described before[23;24]. In our study, association of exposure to fire smoke with bronchial responsiveness was more pronounced in atopics. This is in line with other studies. Leuenberger et al demonstrated that occupational exposure, particularly to dust and fumes, was associated with increased bronchial reactivity in never smokers. The magnitude of the effect was larger among atopics[25]. To our knowledge, effect modification of bronchial hyperresponsiveness for atopy has not been demonstrated before in case of exposure to fire smoke.

We found no associations between exposure estimates and baseline lung function parameters [FEV_1, FVC, PEF, maximal mid-expiratory flow (MMEF), MEF_{25}, MEF_{50} and MEF_{75}]. We performed several sensitivity analyses, in which firefighters with possible exposure within 7 days of testing were excluded. The shown data were adjusted for age-dependency of lung function parameters comparing subjects younger than 25 years with the reference values for 25 years[20;22]. Additional sensitivity analyses were performed without adjustments for age-dependency and adjustment for age-dependency up to 30 years. Again, no associations were demonstrated between any exposure estimate and baseline lung function parameters. We stratified the population for atopy and smoking behaviour both separately, and combined. No indications for effect modification were found. In addition, adjusting by smoking behaviour by categories (never smoking, formerly smoking and currently smoking) as well as by pack-years did not provide any evidence of effect modification.

This study was executed as a subset of an earlier study[14]. The response rate of the participants of 21 randomly chosen fire brigades, who were invited for the testing at the fire stations, was 94.8%, and therefore selection bias was deemed improbable. General respiratory symptoms did not differ between both populations. Comparison with the Dutch version of the European Community Respiratory Health Survey [European Respiratory Health Study the Netherlands: ELON][26], showed a statistically lower prevalence of several respiratory symptoms in firefighters compared with a Dutch general population. Odds ratios ranged from 0.3 (95%CI 0.2-0.5) for phlegm at day/night time during winter to 0.5 (95%CI 0.3-0.7) for for woken up by shortness of breath during the last 12 months. There was a statistically significant elevated prevalence of fire fighters who ever had asthma compared to the ELON population with an OR of 1.5 (CI95% 1.1-2.0). These findings are also in line with the comparison between the questionnaire study and ELON[14]. Therefore, selection bias seems unlikely. A healthy worker effect, in combination with selection of both professional and volunteer firefighters by mandatory pre-employment and periodical medical examinations, might explain the relatively low prevalence of general respiratory symptoms among firefighters. In this light, the relatively high prevalence of asthma based on the questionnaire study among firefighters, supported by the methacholine provocation data, is remarkable.

It is notable that an association was found between bronchial hyperresponsiveness and the number of fires fought, whereas no association existed with the number of working years. A likely explanation is that the number of fires fought can vary widely per working year and the number of fires is therefore a better estimate of exposure. The fact that bronchial

hyperresponsiveness was associated with the number of fires fought in the last 12 months, particularly in atopic firefighters, might be due to the poor compliance in using self-contained breathing apparatus. As mentioned in other studies, the devices are often not used or not all the time during fighting a fire, especially owing to the visual impression of low smoke concentration. Our results suggest that the use of self-contained breathing apparatuses should even be encouraged at low-level smoke concentrations.

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CHAPTER 5

Serum pneumoproteins in firefighters

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ABSTRACT

Serum Clara cell protein (CC16) and surfactant-associated protein A (SP-A) were measured in a cross sectional study in 402 firefighters. For the population as a whole, no associations were detected between serum pneumoproteins and smoke exposure. SP-A levels were increased in symptomatic subjects exposed to fire smoke within 2 days prior to testing. SP-A levels were higher following an inhalation incident ever. CC16 was negatively associated with the number of fires fought in the last 12 months in current non-smokers. These associations between pneumoprotein levels reiterate the importance of adequate use of self-contained breathing apparatus by firefighters.

INTRODUCTION

Fires produce a complex mixture of airway irritants. Occupational exposure to fire smoke amongst firefighters should be avoided by the use of self-contained breathing apparatuses. Still, exposure to toxic hazards remains a concern, because devices are often not used in overhaul situations, and not permanently during firefighting itself, especially owing to the visual impression of low smoke concentration[1;2].

The evidence on respiratory effects of smoke inhalation relies mostly on evaluation of symptoms and lung function testing[3-5]. Previous studies have indicated that smoke exposure may result in acute respiratory obstruction[6;7] sometimes accompanied by an acute increase of airway responsiveness[8]. Furthermore, studies have suggested that firefighters are at risk of developing chronic respiratory symptoms and obstructive airway changes[9-11]. Miedinger et al. found increased bronchial hyperresponsiveness (BHR) in a population of 101 firefighters as compared to the Swiss general population[9]. We previously reported a positive association between the number of fires fought in the last 12 months and bronchial hyperresponsiveness, which we interpreted as an indication of an elevated risk for Irritant-Induced Asthma (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health*. Accepted for publication*).

Recently several lung-specific proteins, pneumoproteins, have been proposed as new biomarkers for lung epithelial injury following exposure to airway irritants[12]. Clara cell protein (CC16), produced in Clara cells along the tracheal-bronchial tree, and surfactant protein A (SP-A), predominantly produced in alveolar type II cells, are normally found in small amounts in the blood circulation[13;14]. Their presence is explained by leakage through the air-blood barrier[12]. Therefore, toxicants that affect the integrity of this barrier have an effect on the concentration of these pneumoproteins in serum. Serum pneumoproteins have been used as biomarkers in studies of exposure to respiratory irritants including tobacco-smoke[14-16], asbestos[17], silica[18], bioaerosols[19], general air pollution[16], bitumen fume[20], trichloramine exposure in swimming pools[21] and fire smoke[2;22-24].

To investigate the nature of respiratory health effects caused by fire smoke, serum pneumoproteins were assessed in firefighters. The present study was performed in a sample form a source population in which we previously examined the effect of smoke inhalation on spirometry and bronchial responsiveness in relation to atopy (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health*. Accepted for publication*). Furthermore, studies found associations between chronic exposures to agents such as foundry [25], crystalline silica[18] and sulphur dioxide[26]. Therefore, we hypothesized that exposure to fire smoke could be assessed by serum CC16 and SP-A as markers of lung-blood leakage and cytotoxicity.

Several studies have investigated associations between CC16 and SP-A, and determinants, such as sex[14;24], age[14;15;27], BMI[28] and exercise[29]. Up till now, adjustments in studies on associations between serum pneumoproteins and exposure to environmental factors have

mostly been limited to a few potential confounders with seemingly arbitrary selection procedures. As an additional goal of our study we therefore analyzed associations between exposure to fire smoke and serum pneumoproteins and systematically considered all potential confounders mentioned earlier in the literature, i.e. sex, age, atopy, body mass index and smoking behaviour as well as lung function. Furthermore, we analyzed associations between serum pneumoproteins and respiratory endpoints (asthma symptoms, atopy, BHR). To assess variability in serum pneumoproteins over time, we compared results from blood samples obtained on two different occasions from a subset of the present study for CC16 and for SP-A.

METHODS

Population and design

The current cross-sectional study was carried out in a randomly chosen subset of 402 firefighters of 23 fire brigades of a previous survey in the Netherlands[30]. All tests were carried out at the fire stations between December 2008 and June 2009.
The institutional review board for human studies of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved the protocol and written consent was obtained from all participants.

Questionnaire and exposure variables

Personal exposure variables were obtained by questionnaire items involving questions on working years, inhalation incidents ('have you ever inhaled a large amount of smoke'), the use of self-contained breathing apparatus, the number of days since the last fire preceding the test, the perception of exposure to fire smoke during the last fire preceding the test, the presence of respiratory symptoms following the last fire preceding the test, and the number of fires fought during the last 12 months. Details of the questionnaire can be found elsewhere[30].

Spirometry and methacholine challenge

Spirometry was obtained by experienced technicians according to European Respiratory Society standards[31], and presented as % of predicted[32] as described (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health. *Accepted for publication*). Bronchial hyperresponsiveness to methacholine was measured using a previously described protocol (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health. *Accepted for publication*). Bronchial hyperresponsiveness was considered to be present if $PD_{20} \leq 1.92$ mg methacholine (BHR_{20}). To make optimal use of all available data, we also calculated the dose-response slope (DRS) as the % fall in FEV_1 per mg inhaled methacholine[33].

Serology

Blood was obtained from each subject by venipuncture. Each sample was processed within 4 hours and serum aliquots were stored at -80 ºC until analysis. Specific IgE (the common panel of allergens consisted of house dust mite, cat, dog, grass pollen mixture and birch pollen [Allergon AB, Angelholm, Sweden]) and total IgE (DAKO A00094 and DAKO PO364, Dakopatts, Copenhagen, Denmark) were assessed in our laboratory based on previously published methods[34]. We defined atopy as a positive reaction to the specific IgE panel or total IgE exceeding 100 kU/l.

Serum pneumoproteins

CC16 was measured with a BioVendor Human Clara Cell Protein ELISA (Enzyme-Linked Immuno Sorbent Assay) kit (Brno, Czech Republic) as described in the manufacturer's protocol. SP-A was measured using a sandwich ELISA technique. Therefore, immunoassay plates (immune plates medisorp 96 well NUNC, Roskilde, Denmark) were coated and incubated for 2h at 37°C with a polyclonal goat antibody for human SP-A (AB3422, Millipore, Billerica, MA, USA) diluted 1:1100 in phosphate buffered saline (PBS). The plates were washed three times with PBS/0.05% Tween-20 (PBST), and blocked with 5% gelatine in PBST. After washing, standards and samples were added. As standard, we used pooled serum samples with high SP-A. Samples were tested undiluted and, if necessary, diluted in PBST. The plates were incubated at 4°C overnight. The following day the plates were washed and detection was performed with 1:1100 diluted biotin labeled monoclonal SP-A antibody (HYB 238-04B, BioPorto, Gentofte, Denmark). After washing, avidin-HRP conjugate (P0364, 1:2000, Dako, Glostrup, Denmark) was added for 2h at 37°C. For colour development, OPD/H_2O_2 (Dako, Glostrup, Denmark) was added and the reaction was stopped with 2 M HCL. The optical density was read at 492 nm with a spectrophotometer (Molecular Devices, Versamax, tunable microplate reader Softmax Pro 4.1). SP-A results were expressed in Units/ml with the highest point in our serum pool standard set at 100 U/ml. The detection limit was 5 U/ml.

Statistical analyses

Serum pneumoprotein concentrations, the dose-response slope and the exposure variables, working years, the number of days since the last fire preceding the test and the number of fires fought in the last 12 months were log-transformed for analysis. SP-A levels below the limit of detection were given a value of 5 U/ml. Associations between (log-transformed) exposure variables and serum pneumoprotein data were calculated using a linear regression analysis using SAS version 9.1 statistical software was used (SAS Institute, Cary, NC). We investigated the influence of the potential confounders sex, age, atopy, body mass index, smoking behaviour, and lung function variables FEV_1 and FVC on associations between pneumoproteins and exposure. If the regression coefficient of this association changed more than 10% when a potential confounder was included in the model, associations were adjusted for the confounder involved. Furthermore, associations were analyzed between serum

pneumoproteins and respiratory endpoints (asthma symptoms, atopy, BHR). An analysis of variance was applied on results from blood samples obtained on two different occasions from a subset of the population study for CC16 and for SP-A. The level of statistical significance was set at $p<0.05$.

RESULTS

Population characteristics

The randomly chosen subset comprised 402 firefighters. One fire brigade consisted solely of professional firefighters, 2 fire brigades consisted of both professional and volunteer firefighters. The remaining fire brigades consisted for the majority of volunteers. All tests were carried out at fire stations between December 2008 and June 2009.

General characteristics of the study population and exposure variables are shown in table 1. Of the 402 firefighters, 305 worked as volunteer, 60 as professional, and 37 as both. Females worked significantly shorter as a firefighter ($p<0.0001$), were younger ($p=0.002$), fought their last fire preceding the examination longer ago ($p=0.0036$), and used self-contained breathing apparatus more often during the last fire they fought preceding the examination compared to males ($p=0.006$).

Table 1 Descriptive characteristics of the firefighters.

	Total	***Male***	***Female***
Sex (*n,%*)	402 (100)	356 (88.6)	46 (11.4)
Smoker (*n,%*)	111 (27.7)	100 (28.1)	11 (24.4)
Ex-smoker (*n,%*)	115 (28.7)	102 (28.7)	13 (28.9)
Age (*year*, mean ± SD (min-max),	41.3 ± 8.1 (20-60)	41.8 ± 8.0 (20-60)	37.9 ± 7.8 (22-53)*
Working years as firefighter (*year*, mean ± SD (min-max)	12.5 ± 8.5 (0-40)	13.3 ± 8.6 (0-40)	6.1 ± 4.8 (0-19)*
Fires fought in last 12 months (*n*, mean ± SD (min-max)	16.7 ± 18.9 (0-200)	17.1 ± 19.6 (0-200)	13.0 ± 11.8 (0-51)
Time since last fire (*day*, median 25^{th}, 75^{th} percentile])	14.0 [9.0, 100.0]	14.0 [7.5, 100.0]	35.5 [14.0, 100.0]*
Inhalation incident cases ever (*n,%*)	139 (34.8)	122 (34.6)	17 (37.0)

* $p<0.05$.

Serum pneumoproteins

In table 2, serum concentrations of CC16 and SP-A are presented. Serum SP-A concentrations were below the detection limit in 168 subjects (39.9%). Serum SP-A was significantly lower in current smokers as compared to currently non-smokers (p=0.018) and to never smokers (p=0.036). No differences were found between male and female firefighters.

Table 2 Serum pneumoproteins.

Serum pneumoproteins				
	All (n=402)	**Current smokers (n=110)**	**Current non-smokers (n=277)**	**Never smokers (n=170)**
CC16 [ng/ml]	5.80 ± 2.40 (0.99-18.62)	5.76 ± 2.53 (0.99-12.83)	5.85 ± 2.35 (1.11-18.62)	5.97 ± 2.41 (2.00-18.62)
SP-A [U/ml]	80.64 ± 265.79 (5.0-3217.41)	29.98 ± 108.47 (5.0-1015.63)*	95.03 ± 294.38 (5.0-3217.41)	120.73 ± 349.06 (5.0-3217.41)

Data are presented as mean ± SD (min-max), U= Units. *: p<0.05.

In table 3, associations are presented between potential confounders and log-transformed serum pneumoproteins. Serum CC16 were higher in male firefighters, positively associated with FEV_1 and FVC (β=0.034, p=0.04 and β=0.023, p=0.03, respectively), and negatively associated with BMI (β=-0.007, p=0.01). Serum CC16 levels tended to be lower in ever smokers as compared to never smokers (β=-0.033, p=0.09) and CC16 did not differ between current smokers versus ex-smokers (p>0.10). The number of cigarettes per day was not significantly associated with serum CC16 levels (p>0.10) among smokers. Surfactant protein A was negatively associated with age, smoking behaviour and FEV_1. The association with FVC was statistically borderline significant (table 3).

Table 3 Factors associated with serum pneumoprotein levels.

	CC16		*SP-A*	
Variable	**Coefficient**	**p-value**	**Coefficient**	**p-value**
Sex	-0.065	0.03*	-0.019	0.85
Age	0.002	0.08	-0.011	0.01*
BMI	-0.007	0.01*	-0.011	0.26
Smoking [0/1]	-0.033	0.09	-0.270	<0.0001*
Pack-years[¶]	-0.001	0.22	-0.012	<0.01*
FEV_1 [L]	0.034	0.01*	-0.133	<0.01*
FVC [L]	0.023	0.03*	0.069	0.06
Atopy [0/1]	0.011	0.60	0.086	0.22

BMI= body mass index; DRS= dose-response slope; Smoking= never as compared to ever smoking; Serum pneumoprotein levels and DRS are log-transformed; [¶] analyses restricted to ever smokers; * p<0.05.

Additionally, we analyzed associations of serum pneumoproteins with respiratory endpoints (asthma symptoms, atopy, BHR). Firefighters with diagnosed asthma as defined by the questionnaire tended to have lower CC16 levels (β=-0.055, p=0.10). Firefighters who were bronchial hyperresponsive or had a higher dose-response slope had lower CC levels (table 4). These associations were hardly affected when adjusted for smoking and atopy. No associations were found between SP-A and bronchial hyperresponsiveness.

When the analysis was stratified for atopy, a weak association was found between CC16 and DRS (β= -0.507, p=0.07) in atopics, which grew stronger when the association was adjusted for smoking (β= -0.582, p=0.04). These associations were weaker in non-atopic subjects (p>0.10).

Stratification for smoking showed the following associations between CC16 and the DRS adjusted for atopy: current non-smokers (β= -0.336, p=0.054) and never smokers (β= -0.433, p=0.11). In never smoking atopic subjects a strong association was found with both DRS (β= -1.379, p=0.008) and BHR_{20} [OR=0.02 (CI 95% <0.001-0.88)].

Table 4 Associations between serum pneumoprotein levels and bronchial hyperresponsiveness.

Serum pneumoprotein	BHR_{20}	$BHR_{20,\ adjusted}$ ‡	*DRS*		$DRS_{adjusted}$ ‡	
	OR (95% CI)	**OR (95% CI)**	**B**	**p-value**	**β**	**p-value**
CC16	0.23 (0.06, 0.92)	0.22 (0.06, 0.88)	-0.314	0.023*	-0.323	0.018*
SP-A	0.82 (0.52, 1.28)	0.79 (0.50, 1.25)	0.007	0.87	-0.002	0.96

BHR_{20} = bronchial hyperresponsiveness; DRS= dose-response slope; Serum pneumoprotein levels and DRS are log-transformed; ‡ Adjusted for atopy and smoking; * p<0.05.

Associations between exposure and pneumoproteins

No crude associations existed between (log-transformed) serum pneumoprotein levels and any of the exposure variables. SP-A was positively associated with exposure to fire smoke within 2 days preceding testing among those who also had respiratory symptoms (β=1.118, p=0.003), although the group involved was small (table 5). This association became clearly stronger (β=1.241, p=0.0007) after adjustment for smoking. When exposure took place within the last 24 hours prior to testing the association became stronger (β=1.910, p<0.0001), and when it took place within the last 3 days it became weaker (β=0.412, p=0.120). This trend continued when the period was extended (data not shown). The association between exposure within 2 days preceding testing and SP-A grew stronger when we excluded SP-A levels below the detection limit (β=1.571, p=0.0008). Serum SP-A levels tended to be higher when subjects had ever inhaled a large amount of fire smoke, which became significant when adjusted for age (β=0.138, p=0.04), smoking (β=0.139, p=0.04), FEV_1 (β=0.135, p=0.047) and FVC (β=0.131, p=0.056). No associations were found between other exposure variables and SP-A.

No associations were found between any exposure variable and CC16 in the total population. A negative association between the (log-transformed) number of fires fought in the last 12 months and serum CC16 levels (β=-0.054, p=0.04) was found in current non-smokers. This association grew stronger when adjusted for FEV_1 (β=-0.061, p=0.02). Additionally, no associations were found between CC16 and SP-A (β=-0.244, p=0.15).

Table 5 Associations between exposure and serum pneumoprotein levels.

Exposure variables	*logCC16*		*logCC16$_{adjusted}$*‡		*logSP-A*		*logSP-A$_{adjusted}$*¶	
	B	**p-value**	**B**	**p-value**	**B**	**p-value**	**β**	**p-value**
Fires fought in the last 12 months [n]	-0.024	0.31	-0.029	0.22	0.078	0.34	0.061	0.44
Inhalation incident ever [0/1]	0.016	0.44	0.024	0.21	0.115	0.09	0.151	0.02*
Working years as firefighter [year]	0.028	0.24	-0.033	0.20	-0.091	0.27	0.065	0.57
Time since last fire [day]	-0.008	0.63	-0.004	0.81	-0.011	0.83	0.000	0.996
Exposure during recent fire [0/1]	0.018	0.68	0.014	0.75	0.002	0.99	0.032	0.83
Exposure during recent fire accompanied with respiratory symptoms [0/1]	0.093	0.40	0.116	0.29	1.118	0.003*	1.275	0.0004*

Serum pneumoprotein levels, fires fought in the last 12 months, working years and days since last fire are log-transformed; ‡ Adjusted for sex, body mass index, FEV_1 and smoking; ¶ Adjusted for age, FEV_1 and smoking;
* $p<0.05$.

Variability within and between individuals

In a subset of 45 subjects blood samples were obtained with a 4 months interval. Analysis of variance revealed that for CC16 about 56% of the total variability was variability between individuals and 44% was variability over time. For SP-A about 99% of the total variability was inter-individual variability and only the remaining 1% was variability over time. The levels of pneumoproteins in serum appeared to be relatively stable in each subject, but differences between individuals were relatively high.

DISCUSSION

We hypothesized that exposure to fire smoke is associated with serum pneumoproteins as markers of lung-blood leakage and cytotoxicity. Although this was not the case in the whole population, we did observe associations between serum pneumoprotein levels and several exposure variables. Serum SP-A levels were higher after exposure to fire smoke within 2 days before blood sampling among a small group of subjects who experienced respiratory symptoms following this exposure, after adjustment for smoking. Furthermore, serum SP-A levels tended to be higher in subjects who reported an inhalation incident ever. This

association grew stronger adjusted for the confouders age, smoking and lung function. CC16 levels in serum were lower for current non-smokers who fought more fires in the last 12 months. This association also grew stronger when adjusted for FEV_1. Furthermore, lower CC16 levels were associated with bronchial hyperresponsiveness, as expected.

Our finding of higher SP-A levels in recently exposed firefighters are supported by other studies, which described acute increases in serum SP-A levels following exposures to trichloramines[21] and fire smoke[2;23]. To investigate whether this association was influenced by the chosen time frame, we executed sensitivity analysis by shifting the days prior to testing. When exposure took place within the last 24 hours prior to testing the association became stronger, and when it took place within the last 3 days it became weaker. This trend continued when the period was extended. This pattern of associations is indicative of an transient respiratory effect of smoke inhalation[2;22]. However, no associations were found between this exposure variable and CC16 levels. A possible explanation is that the half-life of serum CC16 levels is shorter than the half-life for serum SP-A levels. Serum CC16 levels are determined by three main mechanisms: (i) production of CC16 by the Clara cells in the airways; (2) intravascular leakage of CC16 from the lung; (3) elimination by glomerular filtration[12;35;36]. The serum half-life of CC16 is estimated to be 2-3 hours due to glomerular filtration, whereas the clearance of SP-A is not associated with clearance through the kidney[27]. An alternative explanation is that serum CC16 levels are less sensitive to fire smoke exposure than serum SP-A levels. Researchers have observed higher serum SP-A levels following short-term exposures to chlorination products in swimming pools without changes in serum CC16 levels[21;37]. Additionally, when exposure was not accompanied by symptoms, no associations between serum SP-A and acute exposure was found.

Higher serum SP-A levels in subjects who inhaled a large amount of fire smoke ever, cannot be explained as the result of a transient response. At the moment, it is unclear if this association might be caused by permanent damage to the pulmonary epithelial barrier. Burgess et al. described lower serum SP-A levels in firefighters compared with police officers[24]. However, in this study inhalation incidents were not described. As far as we know associations between a fire smoke inhalation incident ever and serum SP-A levels have not been described before.

We found lower CC16 levels when subjects had fought more fires in the last 12 months. Chronic exposure to foundry[25], crystalline silica[18] and low levels of sulphur dioxide[26] are associated with lower serum CC16 levels. Burgess et al. found that serum pneumoproteins were lower in firefighters as compared to police officers[24]. Additionally, in a number of studies lower serum CC16 levels are associated with smoking[14-16]. By contrast, acute or repeated exposures to airway irritants such as bitumen fume[20], bioaerosols[19] and fire smoke[2;22;23] have been associated with higher serum CC16 levels. It has been suggested that increases of serum CC16 following exposure to irritants are caused by leakage through a disrupted epithelial barrier[12], while a mechanism of reduced CC16 production by Clara cells are caused by cytotoxic effects of inhaled substances explains decreased serum CC16

levels[14;26]. A tentative explanation of our results might be that multiple exposures to fire smoke caused decreased CC16 production, which dominated increased lung-blood leakage. To our knowledge, associations between frequency of exposure to fire smoke and lower CC16 levels have not been described before.

Interestingly, bronchial hyperresponsiveness was associated with lower levels of serum CC16. This is supported by our earlier result that bronchial hyperresponsiveness was associated with more frequent exposures to fire smoke (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health*. Accepted for publication*). Other investigators observed lower levels of CC16 levels in asthmatics compared with healthy controls as well for bronchoalveolar lavage fluid[38] and serum[39;40]. A possible explanation for the lower serum CC16 levels in asthmatics is the markedly decreased number of CC16-positive cells in small airways of asthmatics[36;39].

Several other potential determinants of CC16 levels have been investigated, such as gender, age, BMI and exercise[36]. We found that serum CC16 levels were higher in males as observed before[24], whereas other studies found no effects[15;19], or found the same pattern in a subset of smokers[14]. The adjustment for gender had a marginal effect on the relationship between smoke exposure and CC16 levels. In accordance with other studies, we found that CC16 levels tended to be higher in non smokers[14-16] and in older age[14;15]. Our finding of lower serum CC16 levels with higher body mass index corresponds with results of a subset in a study by Steiner et al.[19], whereas others found no association[41;42], or, in contrast a positive association between serum CC16 and BMI[43] or body weight[27]. BMI did not confound the relationship between exposure and CC16. We consider that our population was healthy and well trained, which may influence CC16 levels[29]. Additionally, none of the subjects in this study was involved in firefighting tasks less than 12 hours preceding the tests. Adjustment for FEV_1 changed the associations found between both serum SP-A and inhalation incidents, and serum CC16 and the number of fires fought. FVC influenced these associations in the same way, but for serum CC16 the change was smaller (9.6%). . We decided to include adjustments for FEV_1 or FVC although these variables have not been described earlier as determinants of SP-A or CC-16 and thus as potential confounders. Nevertheless, adjustment for lung function strengthened associations and there were no indications that FEV_1 and FVC were intermediate effects. We have no clear explanation for this association. It is unlikely that this association occurred because of correlations between FEV_1 or FVC with BHR. Adjustment for BHR has weaker effects than adjustment for lung function, while BHR is considered a hallmark of asthma and BHR has been mentioned to be associated with pneumoprotein levels. On the other hand, if we did not adjust for lung function, generally similar associations were observed, indicating that our findings are not dependent on the adjustments made.

In our study serum SP-A levels were lower in smokers, whereas others found higher levels in smokers[14;16]. The direction of associations remained unchanged when we analysed smoking as pack-years, as ever smoking compared to never smoking, and as currently smoking compared to currently non-smoking. We have considered variability in our measurements of

pneumoproteins as contributing to lack of associations. Therefore, in a subset we re-analysed sera of firefighters on average 4 months later and found extremely stable levels within individuals (and much higher differences between individuals). This observation lends support to the robustness of our findings.
In this study exposure variables were obtained by questionnaire. An inhalation incident ever was defined by the item 'have you ever inhaled a large amount of smoke'. The possibility exists that this could lead to exposure misclassification. Other items such as the number of fires fought in the last 12 months seem less prone to misclassification. A second limitation was that we had no information about the number of cigarettes smoked per day in our study, nor when the last cigarette was smoked before blood sampling.
In conclusion, we have found effects in serum pneumoprotein levels of both acute and repeated exposures. However, the influence of many factors on these protein levels complicates the possibility to use these markers as an easy tool to assess effects of exposure to fire smoke. Nevertheless, the short-lived increases in SP-A in blood, and the long-lived changes in CC16 point to systemic consequences of exposure and reiterate the importance of adequate use of self-contained breathing apparatus by firefighters.

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CHAPTER 6

Acute respiratory effects in firefighters

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ABSTRACT

Associations between exposure to fire smoke, respiratory inflammatory responses, and changes in bronchial hyperresponsiveness, serum pneumoproteins have not been studied in an integral way.

We studied 51 firefighters. Blood samples were taken within 24 hours following exposure to fire smoke, and after a week and 3 months. Sputum was induced within 5 days post-exposure and subjects underwent spirometry and methacholine provocation one week post-exposure. Exposure was registered by a questionnaire.

No changes were observed following smoke exposure in bronchial hyperresponsiveness and serum pneumoprotein levels. Nevertheless, in a sizable proportion of the firefighters (44%) elevated sputum neutrophil levels (≥60%) were found. Serum IL-8 was higher 24 hours post-exposure compared to pre-exposure. Sputum neutrophils were positively associated with serum IL-8 (β=0.010, p=0.004) and TNFα (β=0.005, p=0.034) within 24 hours post-exposure; IL-8 elevation lasted up to 3 months. Acute symptoms were associated with the change in IL-8 concentration after a week (β=2.476, p=0.03).

Acute exposure to fire smoke induces neutrophilic airway inflammation in healthy firefighters in the absence of bronchial hyperresponsiveness. Given the fact that the inflammatory response could still be observed after three months, we strongly reinforce the recommendation to avoid as much as possible exposure to fire smoke.

INTRODUCTION

Fires produce a complex mixture of particulate matter, asphyxiant and irritant gases. Occupational exposure to fire smoke amongst firefighters should be avoided by the use of self-contained breathing apparatuses but exposure remains a concern, because the devices are often not used in overhaul situations, or not during firefighting itself, especially owing to the visual impression of low smoke concentration[1;2].

The evidence of respiratory effects of airway irritants, such as fire smoke, relies mostly on reported symptoms and lung function testing[3-5]. Previous studies have indicated that smoke exposure may result in acute respiratory obstruction[6;7] sometimes accompanied by an acute increase of airway responsiveness[4], or even reactive airways dysfunction syndrome (RADS)[8]. We previously reported a positive association between the numbers of fires fought in the last 12 months and bronchial hyperresponsiveness, which we interpreted as an indication of an elevated Irritant-Induced Asthma risk (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health*. Accepted for publication*). Furthermore, studies have suggested that fire smoke inhalation leads to acute airway inflammation[9;10]. Fire smoke exposure has also been associated with a systemic inflammatory response[10;11].

Recently, several lung-specific proteins, such as Clara cell protein (CC16) and surfactant protein A (SP-A), have been proposed as new biomarkers for lung epithelial injury following exposure to airway irritants[12]. Serum pneumoprotein levels have been used as biomarkers in studies of exposure to general air pollution[13], respiratory irritants like trichloramines[14] and fire smoke[2;15-17].

Many studies have focused specifically on high risk subcategories such as forest firefighters[10;18;19], or firefighters in the 9/11 disaster[20;21]. Only a few studies with sufficient power have been conducted among common firefighters.

Few studies have explored pre- and post-exposure health status data in firefighters, nor did any study link inflammation in sputum to changes in hyperresponsiveness. We hypothesized that changes in hyperresponsiveness after exposure would be associated with respiratory inflammation markers. Additionally we explored pneumoprotein levels in blood as early markers of exposure and predictors of susceptibility to smoke exposure.

METHODS

Population and design

In a previously conducted survey 1249 active firefighters of 54 municipal fire brigades of 3 provinces of the Netherlands (Groningen, Friesland and Drenthe) filled in a web-based version of the European Community Respiratory Health Survey questionnaire[22]. Firefighters (n=402) who participated in a subsequently conducted cross-sectional study (Greven et al. *Lung*

function, bronchial hyperresponsiveness and atopy among firefighters; Scand J Work Environ Health*. Accepted for publication*) were invited to contact the researchers within hours following accidental fire smoke exposure. In a telephone interview perceived exposure to fire smoke was assessed as being positive if a firefighter noticed inhalation of fire smoke or unprotected exposure to visible smoke. Exposed subjects (n=51) were enrolled in the current study if a blood sample could be obtained within 24 hours following the exposure. Sputum was induced within 5 days following exposure, and after at least 24 hours more spirometry and bronchial responsiveness testing was carried out and a second blood sample was taken. After three months a third blood sample was taken. Sputum induction was carried out in the University Medical Center Groningen, and spirometry and bronchial responsiveness testing was carried out at the Municipal Health Services Groningen. All tests were executed between February 2009 and October 2009. Enrolment of subjects was stopped when the number of 51 firefighters was reached from whom a blood sample was taken within 24 hours. The institutional review board for human studies of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved the protocol and written consent was obtained from all participants.

Questionnaire and exposure estimates

Questionnaire items involved the type and number of incidents, and the type, the onset and the duration of symptoms following the last fire. Several questionnaire items reflecting exposure were included involving questions on job history, working years, the use of self-contained breathing apparatus, perceived exposure to fire smoke (discomforting as opposed to exclusively perceivable) during the last fire preceding the test, and the presence of acute symptoms following reported smoke inhalation.

Spirometry and methacholine challenge

Spirometry was obtained by experienced technicians according to European Respiratory Society standards[23] and as described previously (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health*. Accepted for publication*).

Bronchial hyperresponsiveness (BHR_{20}) was considered to be present if the provocative dose of methacholine causing a 20% fall in FEV_1 (PD_{20}) ≤1.92 mg as described previously (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health*. Accepted for publication*). To make optimal use of all available data, we also calculated the dose-response slope (DRS) as the % fall in FEV_1 per mg inhaled methacholine[24].

Serology

Blood samples were processed within 4 hours and serum aliquots were stored at -80 ºC until analysis. Atopy was defined as positive reaction to the specific IgE panel of house dust mite, cat, dog, grass pollen mixture (Phleum pratense and Lolium perenne, protein 1:1), and birch

pollen (Allergon AB, Angelholm, Sweden), or total IgE exceeding 100 kU/l as described previously (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health*. Accepted for publication*).

Induced Sputum

Sputum was induced according to European Respiratory Society guidelines[25]. Whole sputum samples were processed for cell counts within 120 min, as described before[26]. A total cell count was performed on sputum samples after addition of 0.1% dithiothreitol equal to the sample's volume and filtration. Viability was checked by means of trypan blue exclusion. Two slides for differential cell counts were stained with May–Grunwald–Giemsa. Differential cell counts were performed by two technicians counting 300 nonsquamous cells in a blinded fashion, and the mean was used for analysis. Significant eosinophil associated airway inflammation was defined if sputum eosinophil levels were ≥ 2%[27]. The percentage of neutrophils was also assessed and compared to normal values[28;29], and values above 60% were judged to be elevated. Samples with contamination of >80% squamous cells were excluded from analyses.

Sputum for analysis of smoke particles in sputum

Sputum was homogenized and put in a 100 µm deep Bürker-Türk counting chamber (BT, Brand, Wertheim, Germany) for the counting procedure. A 0.40 mm cover slip (VWR International, Tåstrup, Denmark) was put in position. After loading, it was assessed whether sputum was evenly distributed. In the Bürker-Turk counting chamber, the counted area in each side of the chamber was 0.4 mm^2 giving a total counted volume for each side of 4.0 × 10^{-5} ml in the first step. Counts were performed using 100 × magnifications. Particle count was expressed as numbers/ml. Log-transformed particle count was used as an exposure variable.

Serum pneumoproteins

Blood was obtained from each subject by venipuncture. Each sample was processed within 4 hours and serum aliquots were stored at -80 ºC until analysis.Clara cell protein (CC16) was measured with a BioVendor Human Clara Cell Protein ELISA (Enzyme-Linked Immuno Sorbent Assay) kit (Brno, Czech Republic) as described in the manufacturer's protocol. Surfactant protein A (SP-A) was measured using a homemade sandwich ELISA technique as previously described (data submitted). The optical density was read at 492 nm with a spectrophotometer (BioTek Instruments, Inc. Winooski, USA). SP-A was expressed in Units/ml (U/ml) with the highest point in our serum pool standard set at 100 U/ml. The detection limit was set at 5 U/ml.

Cytokines

Blood was obtained, processed and stored as described above.

IL-1β, IL-6, IL-8, IL-10, INFγ and TNFα were simultaneously quantitatively determinated in serum with a High Sensitivity Human Cytokine Lincoplex kit (Millipore, Billerica, MA, USA) as described in the manufacturer's protocol. Values below the detection limit were allotted the value of the detection limit. Prevalence of values below detection limit were 76.1% for IL-1β, 6.5% for IL-6, 0.0% for IL-8, 1.5% for IL-10, 51.7% for INFγ and 0.0% for TNFα.

Statistical analyses

SAS statistical software version 9.1 was used (SAS Institute, Cary, NC). Associations between (log-transformed) exposure variables and (log-transformed) continuous health outcome variables were calculated using a linear regression analysis. Associations with binary health outcome data were calculated using a logistic regression analysis. The level of statistical significance was set at $p<0.05$.

RESULTS

Population characteristics

In a previously conducted cross-sectional study in 21 fire brigades in the Netherlands, lung function, bronchial responsiveness, atopy, CC16 and SP-A were determined in 402 active firefighters (Greven et al. *Lung function, bronchial hyperresponsiveness and atopy among firefighters;* Scand J Work Environ Health. *Accepted for publication*). Afterwards 54 firefighters contacted us following acute exposure, of which 51 (94.4%) entered into this study. The 3 non-participating subjects were not able to complete testing within 24 hours following exposure. The exposure took place 1 day to 6 months after the cross-sectional tests were carried out.

General characteristics of the subgroup are shown in table 1. Of 51 firefighters 37 worked as volunteer, 8 as professional, and 6 as both. The subgroup was not different from the original cross-sectional population in sex distribution, smoking, working years, FEV_1, FVC, atopy, bronchial responsiveness, and age though the subgroup was slightly younger than the source population (41.3 ± 8.1 years; p=0.06). For some parameters we did not obtain a complete dataset from all participants since not all subjects could fit in the time window allowed for the tests. From all 51 subjects (100%) post-exposure blood was obtained. In 4 subjects (7.8%) only post-exposure blood was obtained, in 13 subjects (25.5%) additionally only lung function tests and bronchial provocation tests were performed, in 4 subjects (7.8%) additionally only sputum induction was performed, and in 30 firefighters (58.8%) all tests were executed.

Table 1 Descriptive characteristics of the firefighters.

	Total (n=402)	***Subgroup post-exposure (n=51)***
Age (year)	41.3 ± 8.1 (20-60)	39.1 ± 7.5 (20-55)
Working years (year)	12.5 ± 8.5 (<1-40)	11.1 ± 6.9 (1-27)
Male *n* (%)	356 (88.6)	43 (84.3)
Current smoker *n* (%)	111 (27.7)	10 (19.6)
Former smoker *n* (%)	115 (28.7)	20 (39.2)
FEV_1 [% predicted]	101.6 ± 12.8 (50.7, 138.7)	100.9 ± 12.0 (78.1, 130.1)
FVC [% predicted]	107.9 ± 12.3 (70.6, 149.2)	107.8 ± 12.4 (82.5, 139.4)
FEV_1/FVC (%)	77.3 ± 6.3 (52.1, 97.1)	77.4 ± 5.0 (62.1, 86.9)
BHR_{20} *n* (%)	63 (16.1)	7 (13.7)
Atopy *n* (%)	126 (31.4)	14 (28.0)

Data are presented as *n* (%) or mean ± SD (min-max); BHR_{20}: bronchial hyperresponsiveness, PD_{20}≤1.92 mg methacholine causing a fall in forced expiratory volume in one second (FEV_1); FVC: forced vital capacity.

Symptoms

Exposure was reported as discomforting by 31 subjects (60.8%), and as just perceivable by 20 subjects (39.2%). Symptoms immediately following accidental exposure to fire smoke were reported by 34 firefighters (68%) and ranged from 2.0% (nausea) to 31.4% (coughing) (table 2). No wheezing, chest pain, dizziness, nose bleed and weakness were reported. The presence of symptoms was clearly more frequent in the current study than in the cross-sectional analysis of the source population. Symptoms lasted less than an hour in 22 subjects (43.1%) while in 9 subjects (17.6%) symptoms were still present when the first blood sample was taken. No information was available about the persistence of symptoms beyond this moment.

Table 2 Acute symptoms.

Acute symptoms	*Total with reported exposure following the last fire preceding the tests (n=241)*	*Subgroup (n=50)*
Acute symptoms	38 (15.8)	34 (68.0)
Coughing	14 (5.8)	16 (31.4)
Itchy eyes	13 (5.4)	15 (29.4)
Sore throat	7 (2.9)	11 (2.2)
Headache	2 (0.8)	5 (9.8)
Itchy nose	6 (2.5)	2 (3.9)
Lung irritation	1 (0.4)	2 (3.9)
Shortness of breath	1 (0.4)	2 (3.9)
Nausea	0 (0.0)	1 (2.0)
Wheeze	0 (0.0)	0 (0.0)
Chest pain	0 (0.0)	0 (0.0)

Data are presented as n (%).

Bronchial hyperresponsiveness, spirometry

Baseline lung function and bronchial responsiveness parameters of the source population (n=402) and the current population (n=51) are provided in table 1. Spirometry and methacholine challenge testing were carried out 6.7 ± 1.7 (min 2, max 12) days following exposure. No significant changes were found in FEV_1 (-24.9 ± 290.0 ml; p=0.87), FVC (-27.6 ± 345.9 ml; p=0.90) and bronchial responsiveness (table 3).

Induced sputum

Induced sputum was assessed in 31 subjects between 1 to 5 days following exposure. Sputum contained > 80% squamous cells in six subjects while cell differential parameters were interpretable in the other 25 (table 4). Two subjects (8%) had eosinophils ≥ 2%, and 11 subjects (44%) had neutrophils ≥ 60%.

Table 3 Lung function and bronchial hyperresponsiveness (n=43)

	Pre-exposure	***Post-exposure (2-12 days)***
FEV_1 [% predicted]	102.5 ± 11.6 (79.8, 130.1)	101.6 ± 11.1 (79.3, 128.2)
FVC [% predicted]	109.1 ± 12.4 (83.3, 139.4)	108.1 ± 12.3 (87.0, 142.5)
FEV_1/FVC (%)	77.7 ± 4.6 (62.1, 86.9)	77.8 ± 4.7 (58.8, 85.3)
DRS	5.1 [2.9, 7.0]	3.9 [2.5, 6.8]
BHR20 *n* (%)	6 (15.0)	6 (15.0)

Data are presented as n (%), mean ± SD (min-max) or median [25^{th}, 75^{th} percentile]; DRS: dose response slope of the methacholine challenge test: a higher number signifies more hyperresponsiveness; BHR_{20}: bronchial hyperresponsiveness, PD_{20}≤1.92 mg methacholine causing a fall in forced expiratory volume in one second (FEV_1); FVC: forced vital capacity.

Table 4 Sputum inflammatory markers (n=25) and particle count (n=27)

Total cell count [10^6 *cells/ml]*	*3.0 ± 2.5 [0.1, 11.2]*
Eosinophils (%)	0.7 ± 1.3 (0.0, 5.7)
Lymphocytes (%)	2.5 ± 1.2 (0.8, 5.3)
Macrophages (%)	35.7 ± 17.2 (8.0, 72.8)
Neutrophils (%)	53.5 ± 20.1 (15.0, 87.7)
Basophils (%)	0.0 ± 0.0 (0.0, 0.2)
Bronchial epithelial cells (%)	7.5 ± 8.1 (1.8, 41.0)
Particle count [*10^3 particles/ml]	81.8 ± 83.1 [2.7, 385.5]

Data are presented as mean ± SD (min-max).

We found a relation between neutrophils and particle count assessed in sputum (figure 1). For each individual with particle count above 100,000 per ml, the percentage of neutrophils was greater than 58.8%. Below the threshold of 100,000 particles /ml, the distribution of neutrophils was unrelated to the particle count. There was a borderline positive association between neutrophils (expressed as quartiles) and particle count (expressed as quartiles) (β=0.430, p=0.051). When the population was restricted to current non-smokers the association became significant (β=0.449, p=0.036). Symptomatic non-smokers had higher neutrophil levels than non-smokers without acute symptoms(β=32.5, p=0.067). This association was not found when all subjects were included (β=10.1, p=0.298).

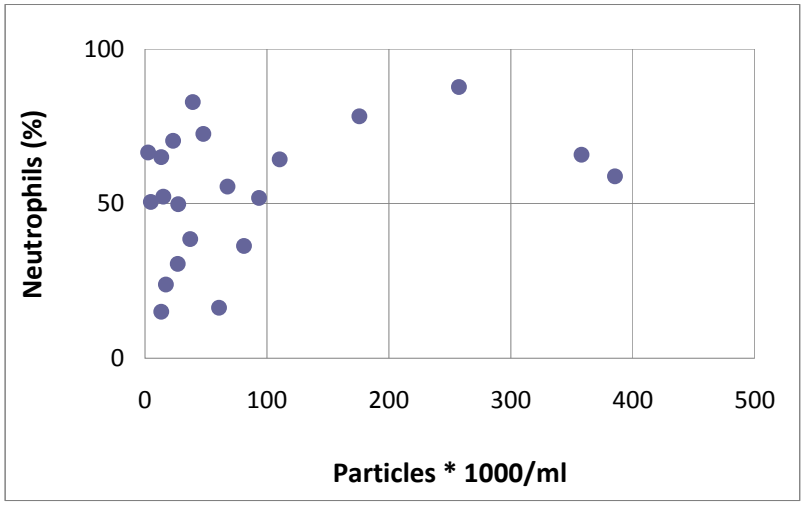

Figure 1: Association between post exposure neutrophil levels (%) and particle counts in induced sputum. Neutrophils (expressed as quartiles) were weakly associated with particle count (expressed as quartiles) (β=0.430, p=0.05).

Serum pneumoproteins and cytokines

Pneumoprotein levels in serum of the source population did not differ from the pre-exposure levels of the current study population (table 5). No changes were found within 24 hours post-exposure in log-transformed serum pneumoproteins (table 5). No associations were found between serum pneumoproteins and acute symptoms, perceived exposure, or particle cell count.

Log-transformed serum IL-8 concentrations were significantly higher 24 hours post-exposure (β=0.127, p=0.031), one week post-exposure (β=0.085, p=0.0007), and 3 months post-exposure (β=0.112, p<0.0001) compared to pre-exposure.

The presence of elevated neutrophils in sputum was positively associated with IL-8 (β=0.431, p=0.0023), IL-10 (β=0.383, p=0.023) and TNFα (β=0.227, p=0.011) in serum within 24 hours following exposure. Associations were also found between the percentage of neutrophils in sputum, and IL-8 (β=0.010, p=0.0044) and TNFα (β=0.005, p=0.034) in serum within 24 hours following exposure. No associations were found between sputum cell differentials parameters and serum pneumoproteins, IL-1β, IL-6 and INFγ, lung function parameters, and DRS.

Perceived exposure (just perceivable versus discomforting) was positively associated with a change in concentration in IL-8 after a week (β=3.437, p=0.001). The same pattern was seen when having acute symptoms was used as exposure variable (β=2.476, p=0.03).

Table 5 *Mean serum pneumoprotein and cytokine levels.*

	Source population (n=396)	Pre-exposure (n=50)	Post-exposure (24 hours)	Post-exposure (1 week)	Post-exposure (3 months)
Serum pneumoproteins					
CC16 [ng/ml]	5.80 ± 2.40 (0.99, 18.62)	5.87 ± 2.74 (2.00, 18.62)	5.98 ± 2.29 (2.24, 12.15)	5.89 ± 2.40 (2.14, 13.40)	4.72 ± 1.75 (1.16, 8.85)*
SP-A [U/ml]	80.64 ± 265.79 (5.0, 3217.41)	85.92 ± 213.55 (5.0, 872.53)	84.12 ± 204.74 (5.0, 826.31)	96.99 ± 245.90 (5.0, 1109.75)	90.02 ± 206.45 (5.0, 851.63)
Serum cytokines					
IL-1β [pg/ml]	n.a.	0.37 ± 0.83 (0.16, 5.45)	0.29 ± 0.26 (0.12, 1.31)	0.48 ± 1.24 (0.17, 8.00)	0.87 ± 2.46 (0.14, 11.58)
IL-6 [pg/ml]	n.a.	25.64 ± 58.41 (0.21, 250.15)	27.97 ± 67.22 (0.21, 302.87)	27.46 ± 70.04 (0.21, 364.16)	29.53 ± 58.63 (0.80, 259.43)*
IL-8 [pg/ml]	n.a.	4.98 ± 2.68 (1.28, 13.89)	8.16 ± 7.86 (1.81, 35.82)*	7.68 ± 4.88 (1.14, 27.24)*	16.18 ± 21.51 (2.56, 91.43)*
IL-10 [pg/ml]	n.a.	14.23 ± 17.41 (1.32, 95.97)	13.40 ± 11.89 (0.61, 66.40)	14.64 ± 17.80 (1.43, 88.66)	20.20 ± 27.97 (0.61, 138.78)
IFNγ [pg/ml]	n.a.	9.10 ± 26.81 (0.29, 168.98)	5.79 ± 9.38 (1.44, 47.67)	12.57 ± 43.88 (1.16, 274.51)	23.41 ± 78.95 (0.60, 400.89)
TNFα [pg/ml]	n.a.	7.00 ± 3.26 (1.89, 15.27)	7.61 ± 3.68 (0.92, 20.67)	7.68 ± 4.49 (0.94, 19.30)	9.52 ± 10.07 (1.40, 63.88)

Data are presented as mean ± SD (min-max); U= Units; n.a.= not assessed; * $p<0.05$ compared to pre-exposure.

DISCUSSION

In a sizable proportion of the firefighters elevated sputum neutrophils were found following fire smoke exposure. Additionally, sputum neutrophils and serum IL-8 levels were positively associated with exposure. Remarkably, we found (neutrophilic) inflammation without bronchial hyperresponsiveness following smoke exposure.

We found high neutrophil percentages in sputum (53.5 ± 20.1%) when compared to earlier studies in healthy volunteers by Spanevello et al[29]. (27.3 ± 13.0%) and Belda et al[28] (37.5 ± 20.1%). The high percentages could not be explained by smoking habits, because when we restricted the population to current non-smokers, the percentage of individuals with high neutrophil levels did not diminish, but slightly increased (56.9 ± 18.4%). Further exclusion of asthmatics hardly changed the percentage of individuals with high neutrophil levels. High neutrophil levels in sputum or broncho-alveolar lavage have also been found after exposure to several other irritants such as particulate matter[9;30-32], isocyanates[33;34], high molecular and low molecular weight agents[35]. Recently it has been described that in a high percentage of subjects with a diagnosed Irritant-Induced Asthma elevated neutrophils were found in induced sputum[27] and in broncho-alveolar lavage fluid[36] even more than 10 years following the onset of the Irritant-Induced Asthma. Additionally, neutrophils in sputum were positively associated with particle count in current non-smokers following exposure to fire smoke; this relation was slightly weaker in the whole population. We found a remarkable distribution between particles and neutrophils in sputum: above 100,000 particles/ml sputum all neutrophil percentages were 60% or higher. Subjects with these high particle counts comprised 3 never-smokers and 2 ex-smokers, who quitted smoking 14 respectively 16 years before. Additionally, neutrophil levels tended to be higher when acute symptoms were present. This relation is interesting since sputum was induced several days after exposure, while the mentioned symptoms generally lasted less than an hour (and were rather mild). Furthermore, in this study neutrophil levels were associated with the rise in IL-8 and TNFα. This is not unexpected, because these are pro-inflammatory cytokines which play a role in neutrophil recruitment. Moreover, the significant rise in IL-8 lasted for at least 3 months, and was accompanied by a rise in TNFα that was even higher at 3 months, but no longer statistically significant. We can not rule out that additional exposures might have occurred between these measurements. Nevertheless, recently, increases in IL-8 following exposure to wood fire smoke[10] and diesel exhaust[37] have been described. These results support the observation that exposure to fire smoke triggers neutrophil recruitment in the airways. Furthermore, these results are in line with toxicological studies in which IL-8 increased after exposure of human macrophages[38] or a co-culture of monocytes and pneumocytes with wood combustion particles[39]. Blood samples were stored between 6 and 16 months at -80 ºC until analysis. Although IL-8 post-exposure levels were associated with blood storage time, associations between exposure variables and changes in IL-8 levels were hardly affected when

adjusted for blood storage time, making it unlikely that blood storage time confounded results.

Unexpectedly we found no post-exposure associations between sputum inflammatory cells and bronchial responsiveness. Within our study, this was probably explained by the lack of change in airway responsiveness following exposure. Although the number of subjects with bronchial hyperresponsiveness rose from 4 preceding current exposure to 6 post-exposure when we excluded asthmatics (data not shown), and the maximum DRS was found post-exposure, no significant changes in bronchial hyperresponsiveness were found for our study group as a whole. This is in contrast to studies in which acute increases in hyperresponsiveness following exposure to fire smoke were described[4;40]. Park et al. found that victims of smoke inhalation with persisting inflammatory responses were still hyperresponsive at least 6 months post-exposure[41]. In the majority of subjects with a diagnosed Irritant-Induced Asthma, BHR remained present for at least 10 years and a third also had neutrophils ≥ 60%[27;36]. As we expected an association of inflammation with bronchial hyperresponsiveness, we performed several sensitivity analyses in which asthmatics and firefighters who happened to have been exposed to fire smoke within 7 days preceding the baseline tests and asthmatics were excluded, but this made no difference.

The higher neutrophil levels in sputum in the absence of bronchial hyperresponsiveness in this study might be explained by a possible higher exposure in Irritant-Induced Asthma cases and victims of smoke inhalation in former studies [27;36;41] compared to our study. In general, it is difficult to compare studies, as the spectre of causative agents is wide, and exposure levels are mostly not quantified. Our findings are comparable to the findings described by Swiston et al. who also described associations between wood smoke exposure, neutrophils and IL-8 levels, whereas the majority of acute symptoms were relatively mild and were not associated with exposure and lung function and bronchial hyperresponsiveness[10]. Changes in cytokine levels and neutrophil recruitment could be more sensitive to smoke exposure, than lung function and bronchial hyperresponsiveness.

Contrary to expectations, no clear associations were found between exposure and serum pneumoprotein levels. Serum pneumoproteins have been used as biomarkers in studies on exposure to respiratory irritants such as tobacco-smoke[42;43], tri-chloramines[14] and fire smoke[2;15;17]. Previously we found that CC16 and SP-A levels in serum are very stable within persons, whereas differences between individuals can be large (data submitted). This might imply that serum pneumoproteins are less applicable as an easy assessment of monitoring smoke exposure within individuals. Furthermore, the first post-exposure blood samples were obtained after a mean of 16 ± 6.5 (3, 23) hours. Both serum CC16 and SP-A levels were lower when the first blood sample was taken later within 24 hours. These associations were not found for the second and third post-exposure blood samples. This might be a further explanation why no associations were found between exposure and the within 24 hours post-exposure serum protein levels.

In conclusion, we have found acute pulmonary inflammatory responses to inhalation of fire smoke, in the absence of bronchial hyperresponsiveness. These health effects do not seem fully or quickly transitory in these healthy individuals, since the elevation in blood IL-8 lasted for at least 3 months. Given these adverse health effects we recommend more strictly reducing or avoiding exposure to fire smoke even in the absence of visible smoke in order to attenuate possible health consequences.

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CHAPTER 7

General discussion

The main objective of this thesis was to investigate associations between airway irritant exposure resulting from acute environmental accidents and respiratory health effects in first responders and the general population. In this thesis, environmental exposure incidents were restricted exclusively to fires to focus on a large category of potentially relevant exposure incidents. For this purpose, surveys were conducted consisting of questionnaire evaluations of symptoms and a more detailed medical evaluation consisting of spirometry, BHR testing, immunological evaluation, and evaluation of serum pneumoproteins among residents exposed to accidental fires and first responders. In addition, acute changes and sputum cell differentials were considered in a population recently involved in fighting a fire as well.

The sequelae of a major chemical fire were explored in a survey among residents, bystanders and first responders. In this population study, persisting respiratory symptoms and a steeper dose response slope of a histamine challenge test were significantly associated with exposure 6 years previously. Some individuals fulfilled the clinical criteria for diagnosis of RADS[1]. Individuals in the general population might be prone to higher exposure than firefighters, because they lack personal protective equipment. Furthermore, knowledge about the hazards and risks of exposure to fire smoke and the skills to minimize exposure may be less available to the general population. These results show that respiratory effects should be considered a public health risk. This is an important finding since respiratory effects have not been considered extensively in recent large studies involving fires and disasters in the Netherlands such as the Bijlmer airplane disaster and the Enschede fireworks explosion[2-5]. The results triggered additional studies focussed on first responders, which are a clear risk group for health effects resulting from fires.

A survey in firefighters demonstrated an increased prevalence of general respiratory symptoms, if they had ever experienced an inhalation incident or if they fought more fires in the last year. Furthermore, symptoms of bronchial hyperresponsiveness were positively associated with the number of fires fought in the last 12 months. It was expected that firefighters would show a lower prevalence of respiratory symptoms than a general Dutch population sample because of the so-called *healthy worker effect*. Although this was true for most respiratory symptoms, it was striking that the opposite was found for asthma: the asthma prevalence was clearly higher in firefighters in comparison to the general population controls. Additionally, the effect of exposure to fire smoke was higher in atopics.

Clear associations were observed between both respiratory symptoms and bronchial hyperresponsiveness, and exposure to fire smoke. Additionally, effects on serum pneumoprotein levels were found after both one acute and repeated exposures. The majority of reported respiratory symptoms following acute exposure were relatively mild complaints related to irritation of the respiratory tract. Interestingly, a sizable fraction of the acutely exposed firefighters appeared to have neutrophilic bronchial inflammation, accompanied by a post-exposure rise in IL-8 and TNFα; cytokines which are known to play a role in neutrophil recruitment. The significant rise in IL-8 lasted for at least 3 months. The neutrophilic inflammation was not accompanied by a reduction in FEV_1 and FVC, nor was an increase in

bronchial hyperresponsiveness found. This could indicate that, although airway inflammation seems to occur after acute exposure, more severe Irritant-Induced Asthma-like or asthmatic changes might only be seen after more frequent exposure to relatively low concentrations of fire smoke, which induce airway inflammation, might result in an elevated asthma risk in the end.

Aetiology

Exposure to fire smoke has several dimensions, those considered the most relevant are the exposure level and the frequency of exposures. Different combinations can occur and the simplest combination of accidental exposure levels and frequencies is depicted in figure 1.

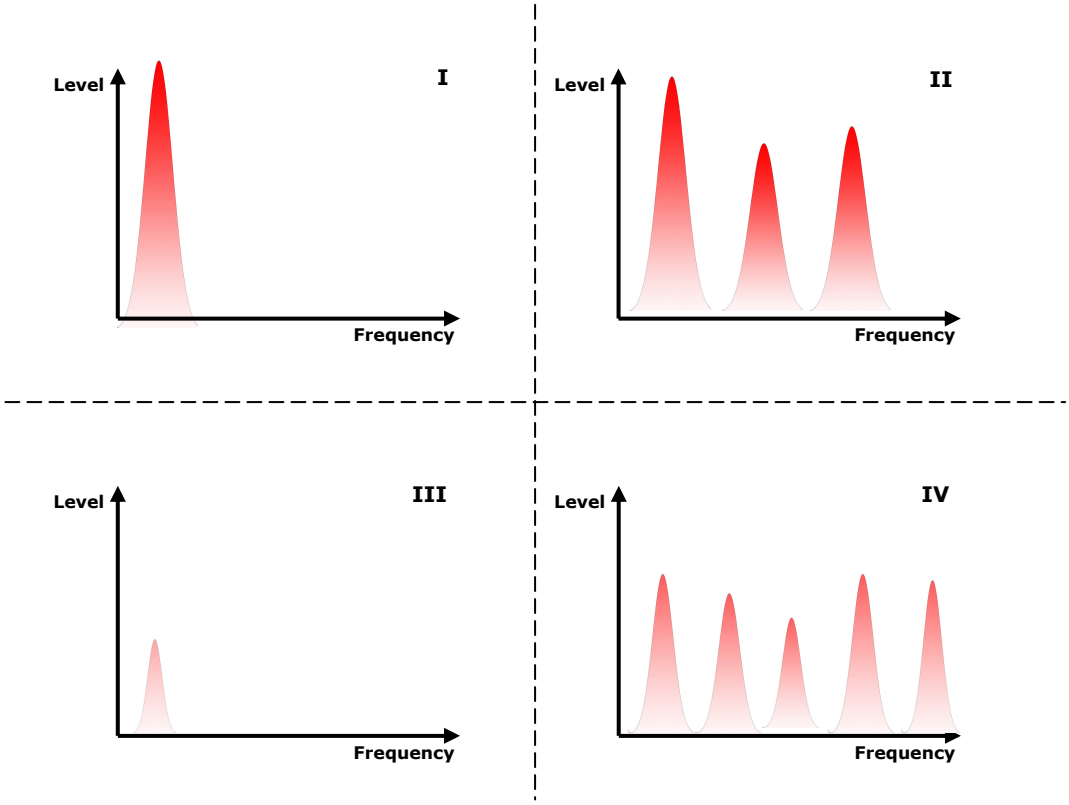

Figure 1: Combination of airway irritant exposure levels and frequencies

Single high exposures

The first cell refers to a single high exposure to airway irritants which appeared to potentially result to Reactive Airways Dysfunction Syndrome. The term RADS was first introduced in 1985 by Brooks et al.[1], and is used to describe the development of respiratory symptoms in the minutes or hours after a ***single*** accidental inhalation of high concentrations of irritant gases, aerosols, or particles accompanied by bronchial hyperresponsiveness[6]. RADS, which is also labelled the more consistent term Acute Irritant-Induced Asthma[7] (AIIA), is a type of occupational asthma without immunological sensitisation and without latency period following exposure to a single high dose of airway irritants as compared to many forms of asthma[7-9]. Later observations have shown that RADS can occur after a single exposure to a large spectre of agents generated as gases or aerosols, such as sulphuric acid[10;11], chlorine[11-13], hydrochloric acid[10;11;14], fire smoke or pyrolysis products[1;15], or particles with an irritant nature[6;16;17].

The criterion for RADS that the exposure was to a gas, smoke, fume or vapour which was present in very high concentrations and had irritant qualities to its nature[1], has been expanded to include less massive levels of exposure[16;18-20]. Exposures of short duration may have effects on bronchial processes leading to respiratory symptoms and these effects may occur after lower exposure levels than reported before. In several cases bronchial constriction and hyperresponsiveness is demonstrated[16;18;19]. Although the term RADS is already applicable when criteria return to normal from at least 3 months post-exposure, in many cases the health consequences persist for several years following the inhalation incident[21-23].

Multiple (high) exposures

The second cell involves RADS for which the exposure criteria have over the years been expanded to include multiple high-level exposures[11;24] as well as less massive levels of exposure[16;18-20;24]. Here the term RADS is abandoned in favour of the more comprehensive term Irritant-Induced Asthma (IIA)[7;9]. Many investigators consider RADS as a special phenotype of IIA[9;11], which therefore is often called Acute Irritant-Induced Asthma (AIIA))[7;21-23;25].

Single low exposures

In the third cell the effects of a single low-level inhalation of an airway irritant is described. When low-level exposure accidental exposures are considered these accidental exposures mostly occur in the normal work environment, are not disaster related, and usually exceed the concentrations observed in the pollution at large. Transient respiratory symptoms have been described following single exposure of an urban community to low levels of fire smoke[26]. Single accidental low-level exposures have been considered in several studies[27-29]. However, if subjects with low-level exposure are included in a study, the lower exposed subjects are mostly referred to as the control group as compared to the study population, or

highly exposed subjects[16;30]. Low-level exposure mostly refers to less exposed subjects of a study population and is treated as the normal situation. It should be noted that the definition of what has to be considered a single low exposure is not made explicit.
Up to date respiratory health effects of airway irritant inhalation (single or multiple high exposures) are broadly accepted to exist. The long-term health effects of this exposure pattern, i.e. single low exposures, are poorly described and are difficult to examine.

Multiple intermediate exposures
The fourth cell comprises more frequent intermediate or low exposures of airway irritants. New-onset asthma has been suggested to result from recurrent of chronic occupational exposures to low or moderate levels of airway irritants, such as exposures to fumes from waste acid drums, spray paint, sulphur dioxide, or a fire inspector who developed increasing symptoms after each of a series of 3 visits at a site[31-33]. Some authors conceive this subtype of Irritant-Induced Asthma as a low-dose Reactive Airways Dysfunction Syndrome[31;32;34]. Although low-dose Reactive Airways Dysfunction Syndrome as a clinical definition for a subtype of Irritant-Induced Asthma is not (yet?) applicable, associations have been found between asthma symptoms, airway hyperresponsiveness and frequent exposures to airway irritants, such as cleaning agents[35-38], trichloramines[39-42], and metalworking fluids[43]. It has been hypothesised that the onset or aggravation of asthma in domestic cleaners could be related to an irritant induced mechanism or to specific sensitisation[36]. Hypochlorous acid and chloramines cause an increase of epithelial permeability. Similar changes occur during inflammation, when inflammatory cells possessing a myeloperoxidase activity release hypochlorous acid and chloramines, as part of their bactericidal function or to facilitate cell recruitment. These observations suggest that the increased epithelial permeability caused by chlorination products might predispose children to the development of asthma[39].
A specific issue in the last two cells is the definition of low exposure. Historically, RADS has been associated with high exposures, first recognized in individual cases after a single exposure to comparably high concentrations of airway irritants such as uranium hexafluoride and hydrazine in the workplace, or spray paints, flour sealant and fire smoke in confined spaces[1]. Over the years RADS has also been described in relation to disasters and major accidents[16;18;19;44-46]. It cannot be ruled out that some situations, which are characterized as low exposure environments for instance owing to the visual impression of low smoke concentration are sometimes associated with high exposures[47;48].

Position of study populations in the exposure matrix
Chapter 2 of this thesis included clinical evaluation, and gave 3 overt RADS-cases with respiratory symptoms and bronchial hyperresponsiveness following a single high-level exposure to fire smoke. More than 6 years later in one case hyperresponsiveness persisted and in all cases respiratory symptoms persisted. These individuals fit most likely the first cell, i.e. a classical example of RADS following a single high exposure to fire smoke.

The second study population described in chapters 3-6 in this thesis was not clinically evaluated. Respiratory symptoms, bronchial hyperresponsiveness and serum pneumoprotein levels were related to repeated exposure to fire smoke. Acute exposure to fire smoke induced neutrophilic airway inflammation in healthy firefighters in the absence of bronchial hyperresponsiveness. The inflammatory response could still be observed after three months. Consequently, a single low-level exposure appears to have a small, but negative health effect. The inflammatory responses are probably transitory in healthy subjects, but might trigger airway hyperresponsiveness if repeatedly invoked by multiple low or intermediate exposure levels to fire smoke. It is hypothesised that predominantly subjects were found who fit in the fourth cell, i.e. acute neutrophilic airway inflammation following acute exposure to fire smoke, which when invoked repeatedly might result in an elevated asthma risk when this exposure is experienced repeatedly.

Impact on public and occupational health consequences

The three RADS-cases found in the study population exposed to smoke of a major fire in a chemical waste depot give an insight in the magnitude of the health risk. The data indicate that the magnitude of the risk varies between 1 per 50 (RADS with respiratory symptoms which last more than 6 years), and 1 per 150 (long-term RADS-case with proven bronchial hyperresponsiveness). If the non-responding inhabitants of the smoke exposed area and all deployed first responders are included in the denominator, risk would still be higher than 1 out of 1000 exposed. The maximum permissible risk level (MPR_{human}) in the Netherlands is defined as the highest levels of a potentially harmful pollutant concentration of a contaminant in an environment compartment below which no adverse effect for human health is to be expected. The Minister of Social Affairs and Employment recently described that for occupational allergy the excess likelihood from occupational exposures to become sensitised to airway allergens should be below 1 to 100[49]. The risk estimates encountered in this study are generally considered unacceptable in public health. The prevalence of asthma ('have you ever had asthma') in the Netherlands was 3-5% (ELON). Both studies investigated adults of the general populationIn this thesis the prevalence of asthma among firefighters in 2008 was higher than among the general population (OR 1.5 (1.1, 2.0). Therefore, this indicates that the risk of developing asthma is considerably higher in firefighters as compared to the general population. It should be noted that the ELON-data and the data in this thesis were collected 16 years apart, and it is unclear to what extent this influenced the risk difference; however, it seems unlikely that this had a major impact. However, more recent data on asthma prevalence in the Netherlands are not available. Thus, estimates of the risk are such that some consideration should be given to further prevention.

In this thesis a subgroup of previously examined firefighters was retested. Therefore, documented respiratory health data were available. However, in public health these data usually are not available. Furthermore, in occupational health it is neither common, especially when inhalation of hazardous substances is not considered to be a normal risk for

employees[14;36;50]. First responders, such as police officers without specific knowledge of hazardous materials may be the first to arrive on a scene with exposure risk to specific chemicals[14;50], or non-specific agents such as fire smoke or dusts[51-53]. A large number of studies have shown that high exposures to airway irritants, including those which exceed short term exposure limits even during overhaul [47;54], are a significant risk for firefighters[16;55-57]. Because firefighters often start using their protective equipment, when they perceive exposure as being discomforting, lower exposures to fire smoke occur more frequently. More asthma is found among firefighters compared to control populations such as police officers[57;58]. It is questionable if this is solely explicable by the higher incidence of high exposures. In this thesis it is hypothesised that repeated acute exposures induce multiple neutrophilic inflammations of the lung, which might lead to a non-immunological asthma in the end.

The general population might not encounter fires as often as first responders, but recently more studies appear that investigated the relation between forest fire smoke exposures and respiratory health. Investigators found an increase in care seeking or hospital admissions for asthma among a general population exposed to particulate matter of a large bushfire[59-62]. Wegesser et al. found that wildfire particulate matter contains chemical components that are more toxic to the lung than PM collected from normal ambient air[63]. Toxicity was manifested as neutrophils were found in lung lavage in a mouse bioassay[63]. This is in line with finding in this thesis of increased neutrophils after a single exposure to fire smoke. The general population might be prone to higher accidental exposures than firefighters, because they lack personal protective equipment and are not trained in handling accidental exposure situations. Knowledge about the hazards and risks of exposure to fire smoke and the skills to minimize exposure may be less available to the general population. These results triggered additional studies focussed on first responders, which are a clear risk group for health effects resulting from fires and that respiratory effects should be considered a public health risk.

Implications

Obviously, more frequent exposures predominantly occur in the occupational population, such as firefighters and police officers working on and around incident sites. In this thesis more frequent exposure to fire smoke is associated with increase in symptoms and airway hyperresponsiveness, despite the training of these first responders and their personal protective equipment. Procedures to avoid or minimise exposure should be stringently implemented and reconsidered. Occupational exposure to fire smoke amongst firefighters should be avoided by the optimal use of self-contained breathing apparatuses. In practice, these devices are often not used or not all the time during firefighting, especially owing to the visual impression of low smoke concentrations[48;54]. In this thesis the exposure variable number of fires fought in the last 12 months preceding the tests was associated with respiratory symptoms, bronchial hyperresponsiveness and serum pneumoproteins. It was found that during the last fire fought preceding the test a minority of the subjects (13.4%)

used their SCBA's all the time, whereas the majority (52.4%) did not use it at all. Of the not fully protected firefighters 68.9% perceived exposure and 20.4% more characterised the exposure as being discomforting. This suggests that the risk of health consequences of exposure to fire smoke is underestimated by firefighters. Furthermore, police officers, less informed about health risks of fire smoke than firefighters, should be aware of health risks of entering an area with smoke to alert inhabitants to close their windows etc. The police officers should weigh this potential damage to their own health against the cons of other methods to caution a population. From a practical point of view, reduction of exposure to fire smoke could be achieved relatively easy when first responders are instructed about these health consequences and how to reduce fire smoke exposure.

In this thesis, there were no signs that atopic status could be a potential effect modifier for association between acute exposure to fire smoke and neutrophilic bronchial inflammation, accompanied by a post-exposure rise in IL-8, which lasted for at least 3 months. However, the risk of respiratory symptoms and bronchial hyperresponsiveness in subjects with a higher number of fires fought in the last 12 months was elevated in atopics compared to non-atopics. This is a strong indication for effect modification by atopy. Further research is needed to elucidate the relations between neutrophilic inflammation, serum pneumoproteins, atopy and bronchial hyperresponsiveness.

The general population on the other hand is more vulnerable for a given exposure because it includes sensitive groups such as children and elderly. Elevated neutrophils and changes in biomarkers up to 3 months following a single exposure to fire smoke in firefighters were found. In vulnerable groups this effect might occur following exposure to lower doses. The general population should be made aware of small adverse effects and possible detrimental consequences of smoke inhalation.

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SUMMARY

Emergency services, such as fire brigades, the police and medical emergency services, should respond quickly and adequately in case of chemical accidents, like chemical spills, explosions, and industrial fires. Chemical spills and explosions are often thought to cause more relevant exposure to hazardous materials than fires. Therefore, first responders use personal protective equipment or stay at safe distances. On the other hand, fires occur more frequently and cause heavy air pollution. In case of a fire it is not unusual that first responders and bystanders inhale fire smoke. Moreover, the media often report in case of a fire that there was ´no threat of public health´ or ´no noxious substances have been emitted´. Apparently, the risk of exposure to fire smoke is estimated low as compared to exposure to other hazardous materials.

In this thesis, environmental exposure incidents were restricted exclusively to fires to focus on a large category of potentially relevant exposure incidents. The main aim of this thesis was to investigate associations between fire smoke exposure and the occurrence of respiratory effects in first responders and the general population. For this purpose, surveys were conducted consisting of questionnaire evaluations of symptoms and a more detailed medical evaluation consisting of spirometry, BHR testing, immunological evaluation, and evaluation of serum pneumoproteins among residents exposed to accidental fires and first responders. In addition, acute changes and sputum cell differentials were considered in a population recently involved in fighting a fire as well. One of the specific respiratory endpoints is Reactive Airways Dysfunction Syndrome (RADS) or Irritant-Induced Asthma (IIA), a type of occupational asthma without immunological sensitisation and without latency period following exposure to a single or multiple high doses of airway irritants respectively.

In the first survey respiratory effects among first responders and residents exposed to combustion products in the aftermath of a chemical waste depot fire were explored. The study population comprised 138 individuals who were present in the area downwind of an accidental fire. Identified by telephone interview six years later, subjects with persistent respiratory symptoms were suspected as having Reactive Airways Dysfunction Syndrome (RADS). Medical tests were performed. For bronchial responsiveness, a cut-off point of PD_{20}<2.39 mg histamine was taken and a dose-response slope (DRS) was calculated. Suspected RADS cases were compared with healthy controls for exposure to combustion products, lung function, and bronchial responsiveness. The 25 suspected RADS cases were more frequently exposed than the 99 controls; the crude odds ratio for high versus low

exposure was 6.5 [95% confidence interval (95% CI) 2.4–18.0]. Suspected cases showed a lower ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC; p=0.028). Overall, suspected cases had a higher DRS than controls. The difference was significant for males only (p=0.006), and non-smoking males (p=0.014). Highly exposed subjects had a higher DRS than low exposed subjects (p=0.056). These differences were significant when restricted to non-smokers (p=0.034) and males (p=0.019). Differences between cases and controls were stronger when the population was restricted to current non-smokers. For three potential cases, bronchial challenge test data were obtained for the years following the fire from other sources. All three cases were smokers at the time of the fire without pre-existing respiratory complaints. They experienced acute respiratory symptoms which deteriorated during the summer. Independently, they visited a lung physician who performed a histamine or methacholine challenge test (ranging from PC_{20}=1.3 mg/ml histamine to PC_{20}=2.5 mg/ml methacholine). Complaining of new-onset respiratory symptoms following the fire, two of the cases visited a physician and quit smoking thereafter. For these individuals, bronchial hyper-responsiveness decreased and respiratory symptoms ameliorated in the following years. In one of these two cases, symptoms were still present in 2006 and bronchial responsiveness was increased (PD_{20}=0.5671 mg histamine). In the second of the two cases, there were no symptoms in 2006. In the currently smoking case, bronchial hyper-responsiveness was absent in 2006, but respiratory symptoms were still being experienced.

These results triggered additional studies focussed on first responders, which are a clear risk group for health effects resulting from fires. The rest of the thesis describes several studies among firefighters. The study comprised 3 subsequent stages. First, all active firefighters of the municipal fire brigades of 3 provinces in the Netherlands (Groningen, Friesland and Drenthe) were invited to fill in a web-based questionnaire. Second, a randomly chosen subset of 402 firefighters underwent spirometry and methacholine provocation, and blood samples were taken to assess atopy and serum pneumoproteins. Of this subset 51 exposed subjects were enrolled in the third stage of the study when they were exposed to fire smoke and a blood sample could be obtained within 24 hours post-exposure. Sputum was induced within 5 days following exposure, and after at least 24 hours more spirometry and bronchial responsiveness testing was carried out and a second blood sample was taken. After three months a third blood sample was taken. In serum pneumoproteins and cytokines were assessed.

A first study describes the prevalence and risk factors associated with respiratory symptoms in common firefighters. A total of 1330 firefighters from the municipal fire brigades of 3 provinces of the Netherlands were administered a Dutch web-based version of the European Community Respiratory Health Survey questionnaire. General respiratory symptoms were associated with the number of fires fought in the last 12 months with odds ratios between 1.2 (CI 95% 1.0-1.4) and 1.4 (CI 95% 1.2-1.7) per 25 fires. A strong association was found between

an inhalation incident and present respiratory symptoms with odds ratios between 1.7 (CI 95% 1.1-2.7) and 3.0 (CI 95% 1.9-4.7). Adjustments for potential confounders did not change any of the associations. Atopics showed elevated odds ratios. Comparison with a survey among the Dutch general population (ELON) showed a statistically significantly lower prevalence of several respiratory symptoms in firefighters compared with the Dutch general population. Odds ratios ranged from 0.5 (95%CI 0.3-0.7) for woken up by shortness of breath to 0.3 (95%CI 0.2-0.4) for wheeze. There was a statistically significant elevated prevalence of firefighters who had ever had asthma compared to the ELON population an OR of 1.5 (CI95% 1.1-2.0).

In a subset of the source population associations between lung function, bronchial hyperresponsiveness and atopy were investigated with exposure to fire smoke in 402 firefighters, a randomly chosen subset of the source population of chapter 3. Hyperresponsiveness expressed as dose-response slope was positively and significantly associated with the number of fires fought in the last 12 months (β=0.157, p=0.02) with and without adjustments for smoking, sex, atopy, age. Limiting the analysis to firefighters without exposure within 7 days of testing did not change any of the associations. The association between number of fires and the dose-response slope was stronger among atopics (β=0.311, p=0.04), and hyperresponsiveness expressed as PD_{20} was also significantly associated (OR 4.9 (1.4, 16.6), indicating that atopics are at higher risk of developing hyperresponsiveness as a result of smoke exposure. Respiratory protection devices were not optimally used. Self-contained breathing apparatus (SCBA) was not used at all or all the time by 344 subjects (86.6%) during the last fire fought preceding the examination. The majority of these not fully protected firefighters perceived exposure (68.3%). Exposure was considered intolerable after a few minutes by 1 subject (0.4%), discomforting by 48 (20.4%), or perceptible by 162 (68.9%). Therefore, the number of fires fought in the last 12 months was a good proxy for repeated exposure to fire smoke.

In a following study associations between exposure to fire smoke and serum pneumoproteins were evaluated. For the population as a whole, no associations were detected between serum pneumoproteins and variables of smoke exposure. Nevertheless, surfactant protein A (SP-A) levels were higher in symptomatic subjects exposed to fire smoke within 2 days prior to blood sampling with and without adjustments for age, smoking and FEV_1. Serum SP-A levels tended to be higher when subjects had ever experienced a fire smoke inhalation incident. Adjusted for age, smoking and FEV_1 this association became significant. Clara cell protein (CC16) levels were negatively associated with the number of fires fought in the last 12 months in current non-smokers. The latter association grew stronger when adjusted for FEV_1. CC16 levels were lower in subjects with bronchial hyperresponsiveness.

A final study describes associations between acute respiratory inflammatory responses, changes in bronchial hyperresponsiveness, serum pneumoprotein levels and exposure to fire smoke. Blood samples of 51 firefighters from the study population described in chapter 4 and chapter 5 were taken within 24 hours following exposure to fire smoke, and after a week and

3 months. Sputum was induced within 5 days post-exposure and subjects underwent spirometry and methacholine provocation one week post-exposure. No changes were observed following smoke exposure in bronchial hyperresponsiveness and serum pneumoprotein levels. Nevertheless, in a sizable proportion of the firefighters (44%) elevated sputum neutrophil levels (≥60%) were found. Serum IL-8 concentrations were higher 24 hours post-exposure compared to pre-exposure. Elevated neutrophil levels in sputum were associated with elevated serum IL-8 (β=0.010, p=0.004) and TNFα (β=0.005, p=0.034) levels within 24 hours post-exposure and IL-8 elevation lasted up to 3 months. Acute symptoms were associated with the change in IL-8 concentration over a week (β=2.476, p=0.03).

To summarize, in firefighters and the general population significant positive associations were found between exposure to fire smoke and asthma-like outcomes. Although, Irritant-Induced Asthma following single and multiple exposures to very high concentrations of a gas, smoke, fume or vapour with irritant qualities are known, in this thesis it was found that multiple exposures to relatively low concentrations of fire smoke can invoke similar health effects. This suggests that the risk of health consequences of exposure to fire smoke is underestimated in public and occupational health. Further research is needed to elucidate the relations between neutrophilic inflammation, serum pneumoproteins, atopy and bronchial hyperresponsiveness. From a practical point of view, reduction of exposure to fire smoke could be achieved relatively easy when first responders are instructed about these health consequences and how to reduce fire smoke exposure.

SAMENVATTING

Hulpdiensten, zoals de brandweer, de politie en de ambulancediensten, behoren snel en adequaat te reageren in geval van ongevallen met gevaarlijke stoffen, zoals lekkages van chemicaliën, explosies en industriële branden. Daarbij kunnen zij worden blootgesteld aan gevaarlijke stoffen. Dit is de reden dat hulpverleners persoonlijke beschermingsmiddelen gebruiken of op een veilige afstand blijven.
Veelal wordt gedacht dat lekkages van chemicaliën en explosies een voor de gezondheid meer relevante blootstelling veroorzaken dan branden. Aan de andere kant komen branden veel meer voor en veroorzaken bovendien ernstige luchtverontreiniging. Het is niet ongewoon dat hulpverleners, omstanders en omwonenden in geval van een brand rook inademen. Daarnaast vermelden de media vaak dat in geval van een brand er 'geen sprake is van gevaar voor de volksgezondheid' of dat er 'geen schadelijke stoffen zijn vrijgekomen'. Schijnbaar wordt het risico van blootstelling bij branden lager geschat dat bij andere ongevallen met gevaarlijke stoffen.
In dit proefschrift zijn de milieu-incidenten waarbij blootstelling kan optreden aan gevaarlijke stoffen ingeperkt tot branden om gefocust te blijven op een grote categorie van mogelijk relevante blootstellingincidenten. Het belangrijkste doel van dit proefschrift was het onderzoeken van relaties tussen blootstelling aan rook van branden en het voorkomen van effecten op de luchtwegen bij hulpverleners en de algemene bevolking. Voor dit doel zijn enquêtes uitgevoerd op basis van telefonische en schriftelijke vragenlijsten en er zijn specifieke medische onderzoeken verricht. Het medische onderzoek bestond uit longfunctieonderzoek, een (histamine of methacholine) provocatietest, immunologisch onderzoek en de bepaling van serum pneumoproteïnen bij bewoners en hulpverleners die zijn blootgesteld bij branden. Aanvullend zijn differentiële tellingen gedaan van cellen in sputum uit luchtwegen en er zijn acute veranderingen van die sputumcellen onderzocht bij brandweerpersoneel dat kort tevoren was blootgesteld aan rook tijdens het bestrijden van een brand. Een van de mogelijke specifieke respiratoire gevolgen is *Reactive Airways Dysfunction Syndrome* (RADS) of *Irritant-Induced Asthma* (IIA). Dit is een type astma zonder immunologische sensibilisering en zonder latentie periode volgend op het inademen van een enkele hoge dosis respectievelijk meerdere hoge doses van stoffen die de luchtwegen kunnen irriteren.
In het eerste onderzoek zijn effecten aan de luchtwegen bij hulpverleners en bewoners onderzocht gedurende de nasleep van een brand in een opslagdepot voor gevaarlijk afval. De onderzoekspopulatie bestond uit 138 personen die aanwezig waren in het benedenwindse gebied van de brand. In deze groep zijn personen met bepaalde aanhoudende

luchtwegsymptomen na een telefonisch interview geclassificeerd als personen die mogelijk Reactive Airways Dysfunction Syndrome (RADS) hadden. Bij hen (en bij controlepersonen) zijn medische tests uitgevoerd. Voor bronchiale hyperreactiviteit is een grenswaarde van PD_{20}<2.39 mg histamine gehanteerd en bovendien is een dosis-respons curve (DRS) berekend. De mogelijke RADS-casussen zijn vergeleken met gezonde controles wat betreft blootstelling aan verbrandingsproducten, longfunctie en bronchiale reactiviteit.

De 25 mogelijke RADS-casussen bleken meer blootgesteld dan de 99 controles; de ruwe odds ratio voor hoge versus lage blootstelling was 6.5 [95% confidentie interval (95% CI) 2.4–18.0]. Mogelijke casussen vertoonden tevens een lagere ratio van geforceerd uitademingvolume in een seconde en geforceerde vitale capaciteit (FEV_1/FVC; p=0.028). Mogelijke casussen hadden bovendien een hogere DRS dan controles. Het verschil was significant voor uitsluitend mannen (p=0.006) en voor niet-rokende mannen (p=0.014).
Hoogblootgestelde personen hadden een hogere DRS dan laagblootgestelde personen (p=0.056). Deze verschillen zijn significant wanneer uitsluitend niet-rokers (p=0.034) of mannen (p=0.019) werden beschouwd.
Verschillen tussen casussen en controles waren groter wanneer de populatie was ingeperkt tot huidige niet-rokers. Voor drie mogelijke casussen zijn uit andere bronnen resultaten verkregen van bronchiale provocatietesten die al waren uitgevoerd in de jaren volgend op de brand. Alle drie casussen waren rokers ten tijde van de brand en hadden voorafgaand aan de brand geen luchtwegklachten. Zij ondervonden aansluitend aan de brand acute luchtwegsymptomen die verergerden gedurende de volgende maanden. Onafhankelijk van elkaar bezochten ze en longarts die een met histamine of methacholine een provocatietest liet verrichten (variërend van PC_{20}=1.3 mg/ml histamine tot PC_{20}=2.5 mg/ml methacholine). Dit bevestigt een aandoening van de luchtwegen volgend op de brand. Na een bezoek aan een arts stopten twee casussen met roken. Bij deze 2 casussen nam in de volgende jaren de bronchiale hyperreactiviteit af en werden de luchtwegklachten minder ernstig. In één van beide casus waren symptomen in 2006 nog aanwezig en was de bronchiale reactiviteit nog verhoogd (PD_{20}=0.5671 mg histamine). De tweede casus had geen luchtwegklachten meer in 2006. In de in 2006 nog rokende derde casus was de bronchiale hyperreactiviteit verdwenen, maar waren de luchtwegklachten symptomen nog aanwezig.

Deze uitkomsten leidden tot aanvullende onderzoeken bij hulpverleners, omdat zij een duidelijke risicogroep vormen voor gezondheidseffecten ten gevolge van branden. In de rest van het proefschrift worden onderzoeken beschreven die bij brandweerlieden zijn verricht.
Het onderzoek bestond uit 3 opeenvolgende fasen. In de eerste fase zijn alle repressieve brandweerlieden van de gemeentelijke brandweren van 3 provincies in Nederland (Groningen, Friesland and Drenthe) gevraagd om een web-based vragenlijst in te vullen. In de tweede fase zijn 402 in een steekproef gekozen brandweerlieden onderzocht met een longfunctietest en een bronchiale provocatietest met methacholine. Ook is bij hen een bloedmonster genomen om atopie te bepalen en serum pneumoproteïnen te meten. Uit deze subgroep zijn 51 personen in de derde fase van het onderzoek opgenomen, meteen nadat ze waren blootgesteld aan rook en als het mogelijk was binnen 24 uur na de blootstelling een

bloedmonster te nemen. Vervolgens is bij hen binnen 5 dagen een sputum inductie verricht, en na minimaal 24 uur extra zijn de longfunctietest en de bronchiale provocatietest opnieuw uitgevoerd en is bovendien een tweede bloedmonster afgenomen. Na 3 maanden is een derde bloedmonster genomen. In het bloed zijn pneumoproteïnen en cytokinen bepaald.

Een eerste deelstudie beschrijft de relatie tussen risicofactoren en de prevalentie van luchtwegklachten van brandweerpersoneel. In totaal hebben 1330 brandweerlieden van de gemeentelijke brandweren van 3 provincies in Nederland een Nederlandse web-based versie van de European Community Respiratory Health Survey vragenlijst ingevuld. Algemene luchtwegsymptomen toonden een statistisch verband met het aantal branden dat men in de voorafgaande 12 maanden had bestreden met odds ratios tussen 1.2 (CI 95% 1.0-1.4) en 1.4 (CI 95% 1.2-1.7) per 25 branden. Een sterke relatie is gevonden tussen een inhalatie-incident en huidige luchtwegsymptomen met odds ratios tussen 1.7 (CI 95% 1.1-2.7) en 3.0 (CI 95% 1.9-4.7). Correctie voor mogelijke confounders veranderde deze relaties niet. Personen met een atopie hadden verhoogde odds ratios. Een vergelijking met gegevens uit een enquête onder de algemene Nederlandse bevolking (ELON) vertoonde een statistisch significante lagere prevalentie van verscheidene luchtwegsymptomen bij de brandweer vergeleken met de algemene Nederlandse bevolking. Odds ratios varieerden van 0.5 (95%CI 0.3-0.7) voor wakker worden door kortademigheid tot 0.3 (95%CI 0.2-0.4) voor piepende ademhaling. Er was echter een statistisch significante verhoogde prevalentie van brandweerlieden die ooit astma hebben gehad vergeleken met de ELON populatie [OR=1.5 (CI95% 1.1-2.0)].
Uit deze groep van 1330 brandweerlieden is een random steekproef getrokken van 402 personen. In deze subgroep van de bronpopulatie is onderzoek gedaan naar verbanden tussen longfunctie, bronchiale hyperreactiviteit, atopie en blootstelling aan rook bij branden.
Hyperreactiviteit uitgedrukt als dosis-respons curve (DRS) was positief en significant geassocieerd met het aantal branden dat men had bestreden in de afgelopen 12 maanden (β=0.157, p=0.02) met en zonder correcties voor roken, geslacht, atopie en leeftijd. Geen van de associaties veranderde door de analyse te beperken tot brandweerlieden die geen blootstelling hadden gehad in de 7 dagen voorafgaand aan het onderzoek. De relatie tussen aantal branden en de DRS was sterker bij atopici (β=0.311, p=0.04), en hyperreactiviteit uitgedrukt als PD_{20} was dan ook significant geassocieerd (OR 4.9 (1.4, 16.6). Dit is een aanwijzing dat atopici een verhoogd risico hebben op het ontwikkelen van bronchiale hyperreactiviteit ten gevolge van blootstelling aan rook.
Persoonlijke beschermingsmiddelen waren niet optimaal gebruikt. Ademlucht (SCBA) was helemaal niet gebruikt door 344 personen (86.6%) gedurende de laatste brand die men had bestreden voorafgaand aan het onderzoek. De meerderheid van deze niet volledig beschermde brandweerlieden namen waar dat zijzelf waren blootgesteld aan rook (68.9%). De blootstelling werd na enkele minuten als ondraaglijk ervaren door 1 persoon (0.4%), als hinderlijk door 48 (20.4%), en als louter waarneembaar door 162 (68.9%). Dit vormt een argument waarom het aantal branden dat men in de afgelopen 12 maanden had bestreden een goede benadering was van blootstelling aan rook bij branden.
In een volgende deelstudie zijn de relaties tussen blootstelling aan brandrook en serum pneumoproteïnen onderzocht. In de populatie in zijn geheel zijn geen associaties gevonden

tussen serum pneumoproteïnen en variabalen voor subjectieve rookblootstelling. Desondanks waren de concentraties van surfactant protein A (SP-A) in serum hoger in personen met symptomen die blootgesteld waren aan rook minder dan 2 dagen voorafgaand aan de bloedafname. Dit gold met en zonder correcties voor leeftijd, roken en FEV_1. Het verband tussen verhoogde concentraties van serum SP-A en een inhalatie-incident was net niet significant. Correctie voor leeftijd, roken en FEV_1 leidde ertoe dat de relatie wel significant werd. Clara cell proteïne (CC16) concentraties waren negatief geassocieerd met het aantal branden dat men had bestreden in de afgelopen 12 maanden bij huidige niet-rokers. Deze associatie werd sterker wanneer ze werd gecorrigeerd voor FEV_1. Concentraties van CC 16 waren lager bij personen met bronchiale hyperreactiviteit

Een laatste deelstudie beschrijft de associaties tussen acute inflammatoire reacties, veranderingen in bronchiale hyperreactiviteit, concentraties van pneumoproteïnen in serum en blootsteling aan brandrook. Bloedmonsters zijn genomen binnen 24 uur na het inademen van rook en opnieuw na een week en na 3 maanden. Dit is gedaan bij 51 brandweerlieden die afkomstig waren uit de hiervoor beschreven onderzoekspopulatie van 402 personen. Binnen 5 dagen na de blootstelling is bij hen tevens een sputum inductie uitgevoerd, gevolgd door een longfunctietest, een bronchiale provocatietest met methacholine en een tweede bloedmonster na een dag of later.

Er zijn geen veranderingen in bronchiale hyperreactiviteit en serum pneumoproteïnen gevonden na de rookblootstelling. Wel werd bij een substantieel deel van de brandweerlieden (44%) verhoogde percentages neutrofiele cellen in het sputum (≥60%) gevonden. Serum IL-8 concentraties waren 24 uur na de blootstelling hoger dan de concentraties voorafgaand aan de blootstelling. Verhoogde concentraties neutrofielen in sputum waren gerelateerd aan verhoogde concentraties serum IL-8 (β=0.010, p=0.004) en TNFα (β=0.005, p=0.034) binnen 24 uur na de blootstelling. De verhoogde IL-8 concentraties bleven 3 maanden aanhouden. Acute symptomen waren geassocieerd met de verandering van de IL-8 concentraties na een week (β=2.476, p=0.03).

Samenvattend zijn er relaties gevonden tussen blootstelling aan rook en astma-achtige effecten in de bevolking en bij brandweerpersoneel. Hoewel het bekend is dat *Irritant-Induced Asthma* kan voortkomen uit een enkelvoudige of meerdere blootstellingen aan zeer hoge concentraties van een gas, rook, of damp met irriterende eigenschappen, is in dit proefschrift voor zover bekend voor het eerst beschreven dat herhaalde blootstelling aan relatief lage concentraties rook bij branden soortgelijke gezondheidseffecten kan uitlokken. Dit verschijnsel is opgetreden bij brand in chemisch afval maar ook bij branden in allerlei andere soorten materiaal. Dit doet vermoeden dat de kans op gezondheidseffecten bij “gewone” branden onderschat wordt in publieke en arbeidsgerelateerde gezondheidszorg. Aanvullend onderzoek is noodzakelijk om de relaties tussen neutrofiele inflammatie, serum pneumoproteïnen, atopie en bronchiale hyperreactiviteit op te helderen. Praktisch gezien is een reductie van blootstelling aan rook bij branden relatief gemakkelijk te realiseren wanneer de bevolking en de hulpverleners worden ingelicht over deze gezondheidseffecten en wanneer de mogelijkheden worden verruimd om de blootstelling aan rook te verminderen.

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Curriculum Vitae

Frans Greven werd op 26 juni 1961 te Winschoten geboren.
In 1980 behaalde hij zijn VWO diploma aan de Winschoter Scholengemeenschap te Winschoten.
Vervolgens heeft hij een jaar psychologie gestudeerd aan de Rijksuniversiteit Groningen. Daarna heeft hij verschillende functies vervuld in het Wilhelmina Ziekenhuis te Assen waaronder radiodiagnostisch laborant en milieukundig adviseur. Van 1987 tot 1995 heeft hij milieukunde gestudeerd aan de Open Universiteit Nederland. In deze periode was hij bovendien docent aan verschillende opleidingen. Van 1996 tot 2001 werkte hij als docent en als verantwoordelijk stralingsdeskundige aan de Hanzehogeschool te Groningen. Sinds 2001 werkt hij als gezondheidskundig adviseur gevaarlijke stoffen (GAGS) voor de Hulpverleningsdienst Groningen. In 2004 heeft hij de postdoctorale opleiding toxicologie afgerond. Bij de GGD Groningen was hij samen met zijn collega Frans Duijm betrokken bij de nasleep van de 'ATF-brand', een brand in een opslagplaats voor gevaarlijk afval die in 2000 in Drachten heeft gewoed. Het idee om een wetenschappelijk onderzoek op te zetten rondom het tot dan toe weinig beschreven Reactive Airways Dysfunction Syndrome ten gevolge van het inademen van irriterende stoffen bij milieu-incidenten werd uitgewerkt en vervolgens besproken met Prof. dr. H.A.M. Kerstjens van het Universitair Medisch Centrum Groningen en Prof. dr. B. Brunekreef en Prof. dr. D. J. J. Heederik, beiden van het Institute for Risk Assessment Sciences van de Universiteit Utrecht. Dit heeft geleid tot het onderzoeksvoorstel dat gehonoreerd werd met een subsidie van ZonMw.

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