Respiratory effects in workers of a diacetyl production plant with a special focus on bronchiolitis obliterans

An evaluation among currently working and retired workers

Confidential

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1 Summary

Recently, several reports have been published in the peer review literature in which a severe lung disease, consistent with the diagnosis bronchiolitis obliterans syndrome (BOS), is associated with exposure to flavorings. Within the spectrum of butter flavoring vapors, diacetyl is suspected to play a prominent role.
The present study was conducted in a diacetyl production plant among all workers who were potentially exposed to diacetyl in the period from 1960-2003. The aim of this study was to investigate respiratory health effects, including bronchiolitis obliterans syndrome and whether these could be attributed to exposure of diacetyl.
The cohort was defined as all workers ever involved in diacetyl production with no limit to a minimal employment time. The cohort was reconstructed on the basis of personnel files. Those eligible were invited at the company site.
Exposure profiles, occupational and medical history were collected through questionnaires. The lung function was evaluated by performing forced expiration lung function measurements.
Among individuals with irreversible airway obstruction a more detailed clinical evaluation was performed to identify cases of bronchiolitis obliterans syndrome and to investigate the potential of diacetyl to cause bronchiolitis obliterans syndrome and induce lung function abnormalities. These individuals underwent a medical history taking and a physical examination. Additional information on pulmonary function parameters including static lung volumes and diffusing capacity for carbon monoxide was collected. A high-resolution computed tomography (HRCT) scan of the chest during in- and expiration was performed.
Three cases of bronchiolitis obliterans syndrome were observed, all process operators, in the group of workers who were potentially exposed to diacetyl in the period 1960-2003. This is considerably higher than would be expected based on the background prevalence in the general population.
The prevalence of respiratory symptoms among workers was compared to a sample of the general Dutch population by logistic regression analysis, adjusting for smoking habits and age. This population sample is the Dutch contribution to the European Community Respiratory Health Survey (ECRHS). Compared with the ECRHS population, workers who were potentially exposed to diacetyl reported significantly more continuously trouble with breathing, daily cough and asthma.
There were no apparent differences in lung function.

A relation between lung function abnormalities and exposure to diacetyl could not be established.
Our study suggests an excess rate of bronchiolitis obliterans syndrome cases in a diacetyl production plant, which is consistent with findings in the literature of bronchiolitis obliterans (BO) cases associated with butter flavoring exposure and in particular diacetyl in popcorn workers. This supports the conclusion that an agent in the diacetyl production process has caused bronchiolitis obliterans syndrome in exposed process operators at this plant. The exact causal agent responsible for the health effect and which has been present in the production plant remains unclear and cannot be established from this study.

2 Background

Recently, several reports have been published in the peer review literature indicating a potential new severe occupational lung disease in workers exposed to flavorings. These studies include clinical case studies in former employees, cross-sectional epidemiological studies among current employees, explorative exposure studies and health hazard evaluation studies and a few animal exposure studies [Parmet & von Essen, 2002; King, 1998; Kreiss et al, 2002; NIOSH 2002, 2003, 2004a&b; Akpinar-Elci et al, 2004; Hubbs et al, 2002; Akpinar-Elci et al 2005; Kullman et al., 2005].
Taken into account the limited data, most studies were carefully performed, showing that clinical case series of workers exposed to occupational inhaled flavoring vapors conferred a high risk of a rare, severe lung disease, consistent with the diagnosis bronchiolitis obliterans syndrome. Epidemiologic studies showed that the prevalence of respiratory symptoms, fixed airway obstruction and neutrophilic airway inflammation as observed in popcorn production plant workers in the United States can most likely be attributed to worker’s occupational exposure to inhaled flavoring vapors. Clinically defined airway obstruction increased with increasing cumulative exposure [Kreiss et al., 2002]. The proportion of workers with abnormal spirometric lung function results also increased with increasing cumulative exposure. The meaning of these findings with regard to other respiratory health endpoints such as occupational asthma, more specifically irritant induced asthma (including RADS) related to irritant exposure or COPD, is not yet clear, but it is not unlikely that these respiratory effects occur more frequently in exposed workers as well. Some workers with severe disease have been put on national transplantation lists because of the severity of their illness and poor prognosis, and will require lung transplantation in the near future [Akpinar-Elci et al, 2004]. Within the spectrum of butter flavoring vapors, diacetyl plays a prominent role, but direct evidence for a causal relationship between diacetyl and brochiolitis obliterans has yet to be established [Kreiss et al, 2002].
In October 2004, the Netherlands Expertise Centre for Occupational Respiratory Disorders and the Institute for Risk Assessment Sciences were consulted by the management of a diacetyl production plant with the question whether employees of that specific plant had been at risk for developing bronchiolitis obliterans syndrome during the time diacetyl was produced. The management expressed their need for information about the possible effects of inhaled diacetyl, because of their intention and obligation to take care of their employees and their public responsibility.

Based on a survey of the available information in the international literature it was concluded that former workers of the diacetyl production plant may have been at risk for developing bronchiolitis obliterans syndrome during the time diacetyl was produced. [Houba et al. 2005].

On the basis of the literature survey the following research questions were addressed:

I. Are there cases of bronchiolitis obliterans syndrome in the group of workers exposed to diacetyl?

II. What is the prevalence of respiratory symptoms and lung function abnormalities in exposed workers compared to a general population sample

III. Does a relationship between bronchiolitis obliterans syndrome cases and exposure to diacetyl exist?

The present study was conducted in the diacetyl production plant among all workers of the plant who were potentially exposed to diacetyl in the period 1960-2003. Among these workers cases of bronchiolitis obliterans syndrome were not previously detected or suspected by the occupational health service or otherwise.

The aim of this study was to investigate respiratory health effects and whether these could be attributed to exposure to diacetyl. Therefore, we evaluated exposure profiles, occupational and medical history, and lung function in these workers. A more detailed clinical investigation among individuals with fixed irreversible airway obstruction was performed to identify cases of bronchiolitis obliterans syndrome and to investigate the potential of diacetyl to cause bronchiolitis obliterans syndrome and induce lung function abnormalities. Diacetyl was produced as a primary product for butter flavorings until 2003. The exposure to diacetyl differs from the use of butter flavoring agents in the production process of popcorn with regard to the qualitative exposure profile and process temperature, which may result in a different spectrum of exposure [Houba et al, 2005].

3 Methods

3.1 Study population

Each worker of the plant who potentially had been exposed to diacetyl in the period 1960-2003 was traced, based on the basis of information from employment records of the Human Resource department. A total of 206 present and former workers could be identified by the company. In this cohort 10 workers eligible for inclusion have died before the onset of this study. Their cause of death was not investigated. All 196 living workers were invited, and 174 (89%) responded and participated in the study. Written informed consent was obtained from all participating employees.

3.2 Exposure Assessment

Since the production of diacetyl at the plant stopped in 2003, it was not possible to measure the exposure at the time of this investigation. Instead, a qualitative and semi-quantitative exposure assessment was performed by a series of interviews with key representatives of the company and by evaluating all available retrieved exposure information which had been collected prior to 2003.

Production process, exposure potentials and sampling strategy

In the plant, diacetyl was produced by oxidation of 2,3-butylene glycol (Cas Number 513-85-9) into acetyl-methyl-carbinol (AMC; acetoin; Cas Number 513-86-0) which is partly further oxidized into diacetyl (2,3-butanedione; Cas Number 431-03-8). The required temperature for this catalyzed reaction is around 360 °C. In addition to the two primary reaction products (AMC and diacetyl), there is also side formation of acetaldehyde (Cas Number 75-07-0) and acetic acid (Cas Number 64-19-7). After the reactor vessel, the mixture of substances was separated in a series of steps (condensator, washer, distillation) into the primary product (diacetyl), co-product (AMC) and waste products (acetaldehyde and acetic acid). Waste streams were being washed and discharged into the regular waste water streams and via exhaust air. The production of diacetyl took place at an elevated process temperature in the reactor vessel, but this elevated temperature was not relevant for the process flows handled by the operators in the plant. All process flows were at room temperature before exposure to diacetyl became possibly relevant for the workers.
In the production plant, sampling results could be traced by the company for diacetyl and acetaldehyde between 1995 and 2003. According to the plant, the main goal of the

sampling scheme was compliance testing for exposure levels of acetaldehyde. However, available samples were also tested for some other chemicals. All samples were taken with cartridges containing silica gel coated with dinitrophenylhydrazine (DNPH-cartridges; firm Waters, Milford, MA, USA) at a flow rate varying from 33-122 ml/min and analyzed by a external commercial laboratory (Biochem) for diacetyl, acetaldehyde and acetone. Three types of samples were taken. First, environmental spot samples were taken at the work floor with sampling duration varying from 83-219 minutes. These spot samples were all taken in close range with the actual working sites of the operators and can therefore be used as a crude exposure estimate for the operators of the diacetyl plant. Second, personal task based sampling was performed during tapping containers of diacetyl by operators with sampling duration varying from 33-90 minutes. There is a general consensus that this activity had the highest actual exposure to diacetyl. Third, samples were taken nearby the scrubber and in the vents of the building to measure the emission of diacetyl and acetaldehyde to the environment. However, these types of samples can not be used for exposure assessment for the workers and were left aside for this particular study. In general, the exposure assessment strategies applied aimed at characterizing exposure of highest exposed workers during performance of high exposed tasks and are of limited value in an epidemiological context.

3.3 Questionnaire

A self administered standardized questionnaire supplemented with questions about respiratory, mucous membrane, atopic symptoms and work history was used. The questionnaire is based on NIOSH Health Hazard Evaluation Report No.2002-0408-2915 and Health Hazard Evaluation Report No.2001-0474-2943 [NIOSH 2003; NIOSH 2004b], and some of the questionnaire items were taken from the European Community Respiratory Health Survey (ECRHS) to allow a direct comparison with the Dutch contribution to this European study. After each worker completed the questionnaire, it was evaluated together with a trained professional to check whether all items were completed.

3.4 Spirometry

Spirometry was performed according to European Respiratory Society (ERS) standards [Quanjer, 1993]. by experienced technicians using a spirometer with specific software (Pneumotachograph and 4.66 software, Jaeger; Wurzburg, Germany). Spirometric age

and standing height adjusted reference values of the European Community for Steel and Coal (ECSC) were used [Quanjer, 1993]
Airway obstruction was defined as a FEV_1 to FVC ratio (FEV_1/FVC) $\leq$ 70% in combination with a FEV_1 of < 80% of predicted. Reversibility was measured after bronchodilation with 400 micrograms salbutamol MDI (metered dose inhaler) and defined as $\geq$ 9 percent increase in FEV_1 [Siafakas 1995, NVALT 1994].
Fixed airway obstruction was defined as irreversible airway obstruction. All participants with a post bronchodilator FEV_1/FVC of < 70% and a FEV_1 < 100% of predicted, were selected and referred to a hospital for further clinical investigation in order to identify cases of bronchiolitis obliterans syndrome. In addition, two workers who did not fulfill the criteria of fixed airway obstruction but in whom flow volume curve showed small airways obstruction, were also referred.
All spirometric lung function information was also used in an epidemiological analysis in which relationships between lung function, job category and exposure were analyzed. For this analysis, exposure information was retrieved from the company records and used in combination with job histories to estimate cumulative exposure.

3.5 Clinical evaluation

Referred workers underwent a medical history taking and a physical examination. Medical files of the Occupational Health Service were available. Additional information on pulmonary function parameters including static lung volumes and diffusing capacity for carbon monoxide was collected. A high-resolution computed tomography (HRCT) scan of the chest during in- and expiration was performed in workers with a fixed airway obstruction. HRCT scans were performed at the University Medical Centre (UMC) in Utrecht, The Netherlands, and read by a thoracic radiologist (prof dr M Prokop, Dept. of Radiology, UMC Utrecht, the Netherlands).

<u>Case definition</u>

The diagnosis bronchiolitis obliterans was accepted in cases with a fixed airway obstruction and a HRCT scan with features of air-trapping with hypo attenuation in segmental or lobular areas and mosaic pattern of perfusion [Hartman et al,1994; Padley et al., 1993; Stern et al,1994; Leung et al, 1998; Worthy et al.,1997; Ikonen et al.,1996].

3.6 Epidemiological and Statistical analysis

All statistical analyses were performed using SAS software (SAS System for Windows version 8.2, SAS Institute, Cary, NC). The prevalence of respiratory symptoms among workers was compared to a sample of the general Dutch population by logistic regression analysis (PROC LOGISTIC), adjusting for smoking habits and age. This population sample is the Dutch contribution to the European Community Respiratory Health Survey (ECRHS) [Rijcken et al., 1996]. To obtain comparable samples, only males between 30 and 70 years of age were included for this particular analysis. Personal characteristics and spirometric test results of workers (Caucasian males, between 30 and 70 years of age) were compared with the same general population sample.

For further analyses, data of 154 male Caucasian workers (all ages) were used to compare lung function test results between different job titles within the study population using minimally exposed workers as an internal reference group. The reference value for each lung function parameters (FEV_1, FVC, PEF), and FEV_1/FVC was calculated based on the age and standing height of an individual and compared with the realized lung function level, using general linear models (PROC GLM), adjusting for smoking habits. Furthermore, pulmonary function parameters (FEV_1, FVC, PEF, FEV_1/FVC and MEF_{50}) in different job titles were investigated by multiple linear regression analysis (PROC REG), adjusting for age, length, and smoking habits. Smoking habits were evaluated using smoking as dichotomous variable (never, former, and current smokers), continuous variable (pack years), and as a combination of both (pack years in current smokers, and pack years in former smokers). Smoking as a dichotomous variable resulted in the best fit and was therefore used in all models.

The accuracy of regression models was examined by using Cook's distance statistic, residual plots and partial regression residual plots. In addition, models were assessed excluding workers older than 65 years of age, participants older than 70 years of age, or workers with severe airway obstruction. Self-reported data on job history in the diacetyl plant were used to assess associations between airway obstruction and duration and/or period of work in the diacetyl plant among process operators. Most workers were unable to quantify the number of days per week or month that they were present in the plant, as most had irregular schedules, shift-work, or only incidental tasks in the diacetyl plant. Therefore, only the cumulative number of years that operators reported to have been working in the plant was calculated. The number of years before and after 1995 was

calculated since modifications of the process were implemented in 1995 which considerably reduced the exposure duration per work shift.
Two-sided P-values of 0.05 or less was considered to represent associations unlikely to be due to chance.

4 Results

4.1 Reconstruction of the exposure profile

Exposure profile for process operators at the diacetyl production plant

During production, process operators were potentially exposed to diacetyl during several tasks:

- quality check (1x/shift; duration 30 min)
- discharge to buffer vessel (1x/shift; duration 15 min)
- check process parameters in the plant (2x/shift; duration 15 min)
- charge batch column (1x/shift; duration 30 minutes; only relevant <1995)
- discharge in 50 kg containers (daily (<1995) or 1x/8 days (>1995); duration 3-5 hours)
- discharge in 2.5 l containers (2x/year; duration 2-3 days)
- cleaning activities finger evaporator (1x/3 wks; duration 2 hours)

Of these tasks, discharge of diacetyl in containers had the highest exposure potential for process operators. As mentioned before, environmental spot samples and some personal task based samples were available for diacetyl and acetaldehyde. The sampling results are described in table 1 and table 2.

Table 1. Results environmental sampling in the diacetyl production plant. All area samples were taken on locations in the plant where operator activities were performed. All sampling results are in mg/m^3.

	N[1]	AM[2]	GM[3]	GSD[4]	Range[5]
Diacetyl[6]					
All samples	26	27.9	8.1	3.9	1.8-351
Samples 1995 to 2001	16	38.7	10.0	4.5	1.9-351
Samples after 2001	10	10.6	5.8	2.9	1.8-51
Acetaldehyde[7]					
All samples	26	6.2	3.1	3.8	0.4-29
Samples 1995 to 2001	16	9.6	7.6	2.1	1.6-29
Samples after 2001	10	0.9	0.7	1.7	0.4-2.3

1 N = number of samples
2 AM = Arithmetic mean
3 GM = Geometric mean
4 GSD = Geometric standard deviation
5 range = lowest and highest sampling results
6 1 mg/m^3 = 0.21 ppm
7 1 mg/m^3 = 0.56 ppm

Table 2. Results of personal task-based sampling during tapping containers of diacetyl.
All sampling results are in mg/m^3.

	N[1]	AM[2]	GM[3]	GSD[4]	Range[5]
Diacetyl[6]					
All samples	4	122.0	38.4	7.5	3-396
Samples 1995 to 2001	3	152.0	40.8	11.7	3-396
Samples after 2001	1	32	32	-	-
Acetaldehyde[7]					
All samples	4	3.8	0.9	7.4	0.2-14
Samples 1995 to 2001	3	4.8	0.8	11.6	0.2-14
Samples after 2001	1	1.0	1.0	-	-

[1] N = number of samples
[2] AM = Arithmetic mean
[3] GM = Geometric mean
[4] GSD = Geometric standard deviation
[5] range = lowest and highest sampling results
[6] 1 mg/m^3 = 0.21 ppm
[7] 1 mg/m^3 = 0.56 ppm

Diacetyl exposure levels can not be evaluated in relation to an occupational standard as no Dutch MAC value or international equivalent is available for this substance. Average levels of acetaldehyde are low compared to current Dutch MAC-value of 180 mg/m^3 (Nationale MAC-lijst, 2005) and the intended new MAC-value of 37 mg/m^3 (SER, 2005). On the other hand a detailed comparison can not be made because sampling times have been relatively short and the MAC value is based on an eight hour time weighted average. Background levels have presumably been low, but there is no supporting exposure information to produce valid long term exposure estimates.

When translating these exposure levels into individual exposures it has to be taken into account that production took place in a 5 shift system over the whole production period. The working schedule varied considerably between workers (some worked at the diacetyl plant for 5 subsequent days whereas others where scheduled to work in the diacetyl plant for one day per week). However, it was estimated by the company representatives that all process operators worked on average an equal number of days per year at the diacetyl plant, but some individual differences may have existed between workers.

Other occupational titles potentially exposed to diacetyl

Although exposure to diacetyl was mainly relevant for process operators, there were several other occupational titles potentially exposed to diacetyl.

- Maintenance workers were estimated to be in the diacetyl plant for on average 1 hour per day for regular maintenance or process disturbances;
- Laboratory workers were not physically present in the diacetyl plant but had to handle and analyze process samples in the lab;
- Transport workers (logistics) occasionally had to enter the diacetyl plant for transportation of containers but were not present at those production sites were diacetyl exposure was primarily relevant;
- SHE-workers (Safety, Health & Environment), occasionally had to be in the building for sampling or other surveys
- Miscellaneous jobs e.g. management and research workers.

Formation of exposure groups

All workers were divided into four exposure groups with varying exposure profile to diacetyl for the purposes of the statistical analyses,:

1. Process operators: certainly exposed to diacetyl; relatively high exposure
2. Maintenance workers: likely to be exposed to diacetyl but exposure is highly variable
3. Laboratory workers: potentially exposed to diacetyl but no further qualitative or quantitative information available
4. All other occupational titles: low exposure potential for diacetyl and if relevant always of short duration

Other exposure potentials at the production site

The diacetyl production plant was only one of the plants in operation at the production site. Most production workers, including all operators, also worked in various other plants on the site. As a result, all workers were also potentially exposed to other chemical agents, including irritants. Because the spectrum of possible chemicals is so broad we chose to evaluate confounding exposure on an individual basis for all clinical cases selected during the study. Correction for confounding exposures was not possible in the epidemiological analyses due to the high variability in chemical agents and because of potential information bias.

Creation of a cumulative in exposure proxy

For exposure to diacetyl three crucial moments have to be taken into account:

1. Until the mid 80's diacetyl was produced in batches but afterwards the production was continuous
2. In 1995 several hand operated processes were automated and as a result operators only had to be present in the diacetyl plant for only 2 hours per day instead of 8 hours per day prior to the automation.
3. Early 2001 a project was executed to close the installation. As a result the exposure to diacetyl and other relevant chemical agents were considerably lower after closure as can be seen in table 1 and 2.

These key moments have been translated into exposure multipliers for diacetyl exposure for process operators. The following time episodes were distinguished and a relative estimate for exposure to diacetyl was made with the most recent time episode as reference period by the occupational hygienist on the study team (Houba):

Table 3. A relative estimate for exposure to diacetyl with the most recent time episode as reference category.

Time episode	**exposure multipliers for diacetyl exposure relative to the 2001-2003 period**
1967-1985[3]	8
1986-1994[2]	16
1995-2000[1]	4
2001-2003	1

[1] Exposure in this episode was estimated to be about 4 times higher compared to 2001-2003, based on the sampling results presented in table 3, as judged by the arithmetic mean exposures

[2] Exposure in this episode was estimated to be about 4 times higher compared to 1995-2000, based on the time present in the plant (8 hours versus 2 hours)

[3] Exposure in this episode was estimated to be about 2 times lower compared to 1986-1994, based on the change in production volume.

4.2 Characteristics of the study population

Of the 196 workers who potentially were exposed to diacetyl in the period 1960-2003 at the diacetyl plant, 174 completed a questionnaire and underwent spirometric lung function tests in 2005 (89 percent).

Four workers performed lung function tests after data analysis of the initial 170 participants was already carried out and were excluded (Table 4). Their lung function was within normal limits of the ERS reference values for FEV_1, FVC and FEV_1/FVC.

Table 4. Personal characteristics and spirometric test results of diacetyl plant workers potentially exposed to diacetyl n=170.

	diacetyl plant workers
N	170
Sex	
Male	164
Female	6
Race	
Caucasian	160
Non-caucasian	10
Age - yr (sd)	51 (9.5)
Smoking status (%)	
Current smoker	28
Former smoker	39
Never smoked	33
FEV_1 % pred (sd)	103.4 (17.6)
FVC % pred (sd)	106.8 (15.9)
PEF % pred (sd)	123.0 (21.6)
FEV_1/FVC % (sd)	77.7 (7.4)

Job titles of all participants are shown in Table 5.

The category 'Other jobs' includes management, research (n=8), and a diversity of other jobs (n=7) For data analyses, the job titles 'logistics', 'SHE workers' and 'other jobs' served as an internal reference group as they all had minimal exposure to diacetyl.

Table 5. Job title of participants who underwent spirometric testing (n = 170).

	N (%)
Process operator	100 (59)
SHE worker	3 (2)
Quality Control Lab	19 (11)
Technical service	22 (13)
Logistics	11 (6)
Other jobs	15 (9)

4.3 Bronchiolitis Obliterans Syndrome in workers exposed to Diacetyl

4.3.1 Workers referred for further clinical investigation

Eight workers were referred, 6 of whom were process operators (Table 6). Six workers had a post bronchodilator FEV_1/FVC < 70 % and a FEV_1 < 100 % predicted. In 4 workers FEV_1 was < 80 % predicted. Of these 4 workers with a fixed airway obstruction, three had a FEV_1 < 50% predicted and underwent a HRCT scan. In 2 of them (never smokers), HRCT scan was consistent with the diagnosis bronchiolitis obliterans syndrome, while this diagnosis was strongly considered in the third (current smoker, 14 pack years). The fourth, a former smoker (29 pack years), had a FEV_1 of 72% predicted and refused further investigation for personal reasons. The remaining 2 workers had an FEV_1 ≥ 80 % of predicted, in one of whom lung function normalized after treatment and the diagnosis asthma was made. The other, a former smoker (29 pack years), had no respiratory symptoms. Both workers were not considered eligible for HRCT scan. Two workers with a FEV_1 < 100 % predicted and a post bronchodilator FEV_1/FVC > 70 %, had a MEF_{50} (maximal expiratory flow at 50% FVC) of 3.40 l/s (no. 7) and 2.21 l/s (no. 8) respectively (Table 6). One worker, who had never smoked, underwent HRCT scan (in a general hospital, The Netherlands) which appeared to be normal. The other, a current smoker and smoking over 41 pack years, did not report respiratory symptoms. He was not considered eligible for HRCT scan.

Table 6. Characteristics of workers referred for further clinical investigation.

Case	Age	Job	FEV_1 % pred	FVC % pred	FEV_1/FVC %	Smoking pack years	HRCT	Diagnosis
1	44	operator	85.4	107.3	64.1	10	-	asthma
2	57	TD	92.4	110.5	66.3	29	-	no symptoms
3	52	operator	71.8	89.6	63.8	29	-	unknown
4	55	operator	35.1	65.2	42.9	0	+	BOS
5	72	operator	37.4	65.0	43.8	0	+	BOS
6	59	operator	42.3	57.7	57.8	14	+	BOS
	Workers with small airways obstruction.							
7	30	Qlab	75.1	84.3	72.9	0	+	no symptoms
8	66	operator	82.3	89.2	71.3	41	-	no symptoms

4.3.2 Cases with radiological signs of bronchiolitis obliterans

History

A HRCT scan of the chest was performed in 4 workers. In 2 cases HRCT was compatible with bronchiolitis obliterans, in one case bronchiolitis obliterans was suspected. These three cases will be described in detail below. In one case, a smoker with small airways obstruction, HRCT appeared to be normal.

Both cases in whom HRCT was compatible with bronchiolitis obliterans had never smoked, one case with suspected bronchiolitis obliterans was a current smoker who started smoking at the age of 20 and has been smoking 14 pack years (Table 7). None of the three cases received an initial diagnosis of bronchiolitis obliterans, but were previously diagnosed by a chest physician as chronic obstructive pulmonary disease (COPD) or asthma.

The age of these three workers at the time of diagnosis was 55, 72 and 59 years, respectively. All three cases had been working as a process operator in the diacetyl production plant. Mean duration of employment at the diacetyl plant was 14 years (range 10-16 years). Each of these workers first became symptomatic between 1985 and 1995 after a mean of 5.3 years of employment (range 0 - 14 years). At that time lung function showed airway obstruction in two of them. Mean duration of time between the onset of symptoms and the end of employment was 8.7 years (range 2 - 16 years). All cases reported exertional dyspnoea as the first symptom. Fatigue was present in two cases. Remarkably, coughing was not reported.
Besides exposure at the diacetyl production plant to a range of agents including diacetyl, AMC, acetaldehyde, and acetic acid, confounding exposure in their other work environments within the plant (e.g. chlorine) was reported by all three workers.

Physical examination

Crackles were not recorded on chest examination in any of the cases.

Lung function testing

All three cases showed a fixed severe airway obstruction (Table 7). Post bronchodilator forced expiratory volume in one second (FEV_1) ranged between 35-42% predicted, forced vital capacity (FVC) between 58-65% predicted and FEV_1/FVC between 43-58%. An increased residual volume (RV) as well as an increased RV to TLC (total lung capacity) ratio, together with a decreased FVC indicated hyperinflation in all cases. Diffusing capacity for carbon monoxide (Tlco) was below the normal range in two cases, transfer coefficient for carbon monoxide (Kco) in one case.

Radiology

Chest radiography performed previously showed hyperinflation in 2 cases.
In all three cases we performed high resolution multislice computed tomography (HRCT) using a volumetric acquisition with 16x0.75mm or 64x0.625mm collimation during inspiration and dynamic expiration.
In all three cases slight inhomogeneity of the lung attenuation was already seen on the inspiratory scans. On the expiratory scans, air trapping was visible in all cases. None of the cases demonstrated major centrilobular or paraseptal emphysema compatible with chronic obstructive pulmonary disease (COPD) as seen in smokers.
In case 1, HRCT showed a mosaic pattern with air trapping compatible with bronchiolitis obliterans.
In case 2, the air trapping was less geographic and mainly localized in the lower lobes and the posterior portions of the upper lobe. There was mild bronchial wall thickening and focal accentuation of centrilobular structures. Together with the air trapping these findings can be compatible with bronchiolitis obliterans.
In case 3, the inhomogeneity of the lung parenchyma accentuated on the expiratory scan with air trapping especially in central and ventral portions of the lung. The lung periphery, lung apex and the basal lungs showed a normal increase in lung density during expiration. This effect was combined with an almost complete collapse of the central bronchi (main and lobar bronchi) during expiration. Modest bronchial wall thickening was present. Although the radiological findings in this case may not be fully explained by bronchiolitis obliterans, the present abnormalities can justify the diagnosis.

Thoracic lung biopsy

At the moment this report was published none of the cases underwent thoracic lung biopsy.

Table 7. Characteristics of cases with radiological signs of bronchiolitis obliterans.

	Case no.		
	1	2	3
Age at time of diagnosis	55	72	59
Age at symptom onset years	45	52	39
Sex	M	M	M
Smoking pack-years	0	0	14
Process Operator	+	+	+
Year started at the diacetyl plant	1993	1971	1985
**Year of symptom onset	1994	1985	1985
Year stopped at the diacetyl plant	2003	1987	2001
Acute onset	-	-	-
Cough	-	-	-
Dyspnoea	+	+	+
Wheeze	+	-	+
Phlegm	-	-	-
Fever	+	-	-
Fatigue	+	+	-
Night sweats	-	-	+
Eye irritation	+	-	+
Nasal irritation	-	-	+
Skin irritation	+	-	-
Initial diagnosis	COPD	COPD	Asthma, COPD
* FEV_1 % pred	35.1	37.4	42.3
* FVC % pred	65.2	65.0	57.7
* FEV_1/FVC %	42.9	43.8	57.8
# Reversibility	-	-	-
TLC % pred	110	94	99
RV % pred	157	132	140
RV/TLC % pred	131	125	130
TL_{co} % pred	85	70	61
K_{co} % pred	108	117	76

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity;
TLC: total lung capacity; RV: residual volume; TL_{co}: diffusing capacity for carbon monoxide; K_{co}: transfer coefficient for carbon monoxide ;% pred: percentage of the predicted value.

* post bronchodilator

\# reversibility defined as $\geq$ 9 percent increases in FEV_1 after bronchodilation

** based on medical history

4.4 Prevalence of respiratory symptoms and lung function test abnormalities in exposed workers compared to a general population sample

4.4.1 Prevalence of respiratory symptoms compared to a general population sample

Diacetyl plant workers reported significantly more continuously trouble with breathing (2.6 times) and daily cough (1.6 times), compared with the ECRHS population (Table 8). The prevalence of self-reported asthma attacks (ever), physician-diagnosed asthma attacks, and, asthma attack last year among diacetyl plant workers were 2.0, 2.3 and 4.4 times higher compared with the ECRHS population, respectively.

Table 8. Prevalence (%) and Odds Ratio with 95% confidence interval (CI) of respiratory symptoms in diacetyl plant workers and a general Dutch population sample of the European Community Respiratory Health Survey (ECRHS) (men only, age between 30 and 70).

	General population (n = 1125)	*Diacetyl plant workers (n = 154)*	
	%	%	OR (95% CI)
Trouble with breathing			
Continuously	2.7	6.5	2.6 (1.2-5.4)*
Repeatedly	7.2	11.0	1.7 (1.0-3.0)
Rarely	9.0	18.8	2.4 (1.5-3.9)*
Cough symptoms			
Daily cough	15.4	21.4	1.6 (1.1-2.5)*
Daily cough up phlegm	11.8	15.6	1.5 (0.9-2.4)
*Shortness of breath (*SOB*), dyspnea, wheezing*			
Exercise induced SOB	19.6	23.4	1.3 (0.9-2.0)
Awakened due to SOB	6.1	7.1	1.2 (0.6-2.4)
Wheezing	24.3	20.8	0.9 (0.6-1.4)
Wheezing with SOB	14.8	14.3	1.0 (0.6-1.6)
Awakened due to chest tightness	12.6	14.9	1.2 (0.8-2.0)
Asthma			
Asthma attack (ever)	5.0	9.7	2.0 (1.1-3.7)*
Asthma attack doctor diagnosed	4.6	10.4	2.3 (1.3-4.2)*
Asthma attack last year	1.1	4.6	4.4 (1.7-11.5)*

* $p<0.05$; Odds ratios adjusted for age and smoking habits

4.4.2 Prevalence of lung function test abnormalities

Reversibility was observed in 10 workers. In 14 workers post bronchodilator Forced Expiration Ratio (FEV_1/FVC) was ≤ 70 % of age and standing height adjusted reference value (predicted). Four of whom had an FEV_1 between 80 and 100 percent of age and standing height adjusted reference value and another 4 had an FEV_1 < 80% of age and standing height adjusted reference value. Of 4 workers with an FEV_1 below 80 percent of age and standing height adjusted reference value, 1 had an FEV_1 between 50 and 80 percent of age and standing height adjusted reference value, and 3 had an FEV_1 between 30 and 50 percent of age and standing height adjusted reference value. Two workers had FVC and FEV_1 values < 80 percent of age and standing height adjusted reference value, but a FEV_1/FVC ratios > 80 percent of age and standing height adjusted reference value.

4.4.3 Prevalence of lung function test abnormalities compared to a general population sample

Comparing personal characteristics and spirometric test results of 144 diacetyl plant workers (Caucasian, age between 30 and 70) with a general Dutch population sample (n=1084) of the European Community Respiratory Health Survey (ECRHS) with only men, age between 30 and 70, showed no significant differences except a higher peak expiratory flow rate percentage of the predicted value after adjusting for smoking habits (Table 9).

Table 9. Personal Characteristics and spirometric test results of diacetyl plant workers versus a general Dutch population sample of the European Community Respiratory Health Survey (ECRHS) (Caucasian males only, age between 30 and 70).

	Diacetyl plant workers	*General population*
N	144	1084
Age - yr (sd)	50 (7.7)	50 (11.4)
Smoking status (%)		
Current smoker	30	41
Former smoker	40	40
Never smoked	30	19
FEV_1 % pred (sd)	104.9 (17.5)	104.9 (18.1)
FVC % pred (sd)	108.5 (15.3)	108.4 (15.0)
PEF % pred (sd)	124.1* (22.1)	114.2 (23.2)
FEV_1/FVC % (sd)	77.5 (7.2)	77.9 (8.6)
Subjects (%) with a low FEV_1#	6 (4.2)	65 (6.0)

* p<0.05; adjusted for smoking habits

\# FEV_1<FEV_1 pred-1.645 x SD. SD = 0.51 [Quanjer et al, 1993]

4.5 Relationship between lung function and exposure to diacetyl

All 4 workers with fixed airway obstruction ($FEV_1/FVC \leq 70\%$ and $FEV_1 < 80\%$ of predicted) were process operators (Table 10). In addition, all three workers with severe airway obstruction, two never smokers and one smoker were all operators, and were employed in the period before 1995 (a period with high exposure).

Data of 154 Caucasian workers of the diacetyl production plant were used for further statistical analysis (Table 10). The explained variance of the model was 39% and 44% for FEV and FVC respectively, and, as expected, considerably lower for the flow volume parameters (Table 11a). Regression analyses for each lung function parameter showed that the regression coefficients for age and standing height were comparable with those used in reference regression equations from the European Respitatory Society (ERS). For instance for the FEV_1 lung function decreases approximately 35 mL per annum and increases 6 mL per cm increase in standing height. The coefficients seemed less stable for smoking. Smokers generally had a lower lung function compared to non-smokers, but ex-smokers did not show the expected pattern, a lower lung function in smokers compared to non-smokers. The lower lung function in former smokers was stable when older workers were excluded, although not significantly for FEV_1 and FVC. Also, reductions were seen for both FVC and FEV_1. An analysis with pack years smoked yielded results that were more comparable with what was expected, a negative association with all lung function variables (Table 11b). However, the fit was lower compared to the simple categorization by smoking habits. The different corrections for smoking had no direct impact on the magnitude of the lung function differences between the three job title categories. We therefore corrected for smoking by the simple categorical approach in all further analyses.

Process operators had a significantly lower FEV_1 and FEV_1/FVC expressed as % of age and standing height adjusted reference value, than workers in the internal reference group ($p<0.05$, adjusted for smoking habits age and standing height).

Table 10. Personal Characteristics and lung function test results of the study population (n = 154, only Caucasian males).

	Process operator	*Technical service*	*Quality Control Lab*	*Other jobs (internal reference group)*
N	93	17	16	28
Age - yr (sd)	53 (9.3)	48 (5.4)	46.3 (11.5)	48.2 (9.5)
Smoking status (%)				
Current smoker	30	29	25	25
Former smoker	42	42	38	43
Never smoked	28	29	38	32
FEV_1 % pred sd)	102.2* (19.5)	104.9 (13.7)	101.2 (14.4)	110.0 (16.2)
FVC % pred (sd)	106.7 (16.5)	109.0 (15.1)	104.7 (12.5)	110.7 (15.0)
PEF % pred (sd)	120.9* (22.7)	125.6 (19.0)	121.4 (19.5)	131.5 (23.8)
FEV_1/FVC % (sd)	76.2* (8.3)	77.5 (4.1)	78.2 (8.6)	80.0 (5.2)
Post bronchodilator				
Reversibility > 9% (%)	3(3.2)	5(29.4)	0(0.0)	2(7.1)
FEV_1/FVC $\leq$ 70% (%)	11(11.8)	3(17.7)	0(0.0)	0(0.0)
Airway obstruction[1]	4	0(0.0)	0(0.0)	0(0.0)

* p<0.05; versus '*other jobs*', adjusted for smoking status

Airway obstruction[1] (FEV_1/FVC $\leq$ 70% and FEV_1 <80% pred)

Regression analysis showed that operators and Quality-control workers had significantly lower FEV_1 values ($\pm$ 300 ml) than workers in the internal reference group (Table 11a). This effect was stable and also existed in models when workers older than 65 were excluded (FEV_1: operators −327 ml, p = 0.02; QC lab −407 ml, p = 0.053), or participants older than 70 or workers with severe airway obstruction (FEV_1: operators −282 ml, p = 0.03; QC lab −330 ml, p = 0.07; PEF: operators −947 $ml \cdot s^{-1}$, p = 0.02). were excluded. Differences were somewhat larger when pre-dilator values were used with a smaller p-value (not shown).

Table 11a. Multiple linear regression analysis results of pulmonary function parameters of 154 workers exposed to diacetyl. Smoking status expressed in current and former smokers.

Determinant	FEV_1 (ml)		FVC (ml)		FEV_1/FVC %		MEF50 l/s	
	β	SE	β	SE	β	SE	β	SE
Intercept	-534	1609	-5392*	1813	148*	18	7.20†	3,67
Age	-35*	6	-30*	7	-0.27*	0.07	-0.05*	0.014
Length	3596*	818	6730*	922	-30*	9	0.25	1.87
Smoking status (%)¶								
Current smoker	-290*	137	-298†	154	-0.72	1.51	-0.34	0.31
Former smoker	226†	130	229	147	1.64	1.44	0.51†	0.30
Jobs§								
Process operator	-287*	143	-162	161	-3.26*	1.57	-0.54†	0.33
Quality Control Lab	-356†	206	-362	232	-1.41	2.27	-0.75	0.47
Technical service	-212	200	-47	225	-2.71	2.20	-0.75	0.46
Adjusted R^2 (%)	39		44		12		12	

* $p<0.05$; † $p<0.10$
SE: standard error
¶ never smoked as reference group
§ The job titles 'logistics', 'SHE workers' and 'other jobs' as reference group

Table 11b. Multiple linear regression analysis results of pulmonary function parameters of 154 workers exposed to diacetyl. Smoking status expressed in pack years.

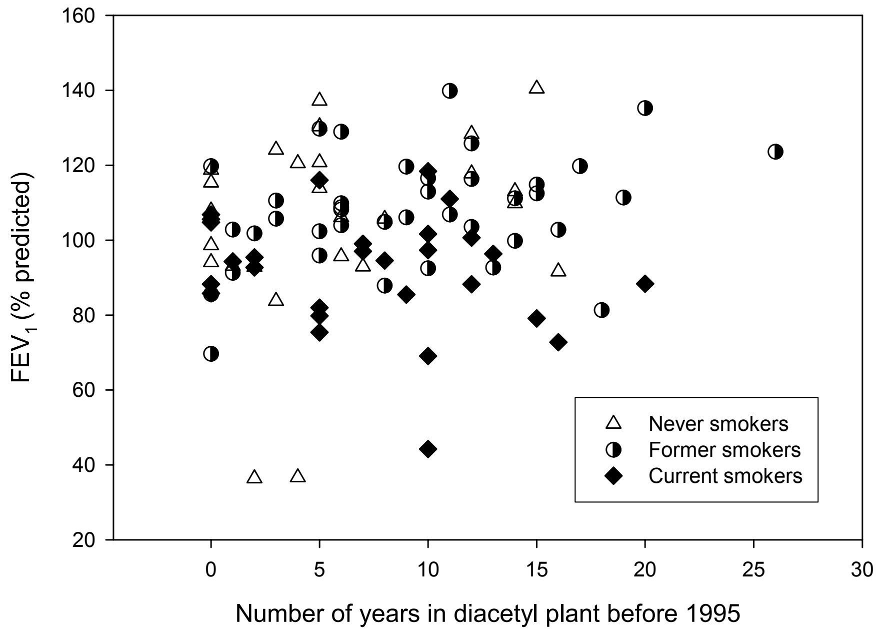
Determinant	FEV_1 (ml)		FVC (ml)		FEV_1/FVC %		MEF50 ml/s	
	β	SE	β	SE	β	SE	β	SE
Intercept	-2202	1668	-6917*	1875	139*	18	3900	3728
Age	-27*	6	-23*	7	-0.23*	0.07	-40*	14
Length	4335*	852	7385*	957	-26*	9	1767	1904
Smoking status								
Pack years	-7†	4	-5	4	-0.03	0.04	-10	9
Jobs								
Process operator	-232	151	-112	170	-3.13†	1.62	-416	338
Quality Control Lab	-369†	214	-356	241	-1.75	2.30	-762	479
Technical service	-172	213	-8	240	-2.45	2.29	-595	477
Adjusted R^2 (%)	35		40		11		8	

* $p<0.05$; † $p<0.10$
SE: standard error

To assess the effect of exposure duration, the same regression analysis was done. In addition, for process-operators a distinction was made in operators who were working at the diacetyl plant before 1995 (a period with high exposure, n=79) and a group that started working after 1995 at the plant (n=14).

Figure 1 and 2 show that there does not appear to be an association between airway obstruction and the number of years that operators had tasks in the diacetyl plant. However, three subjects with severe obstruction were all process operators who worked in the plant before 1995.
Also no relationship exists between the percentage of age and standing height adjusted reference value of FEV_1 and the year that operators commenced tasks in the diacetyl plant (Figure 3).

Figure 1. Percentage of predicted FEV_1 value in relation to smoking habits and the number of years before 1995 * that process operators (n=93) had tasks in the diacetyl plant.



* If the number of years is 0, process operators started their tasks in the diacetyl plant after 1995.

Figure 2. Percentage of predicted FEV_1 value in relation to smoking habits and the total number of years that process operators (n=93) had tasks in the diacetyl plant.

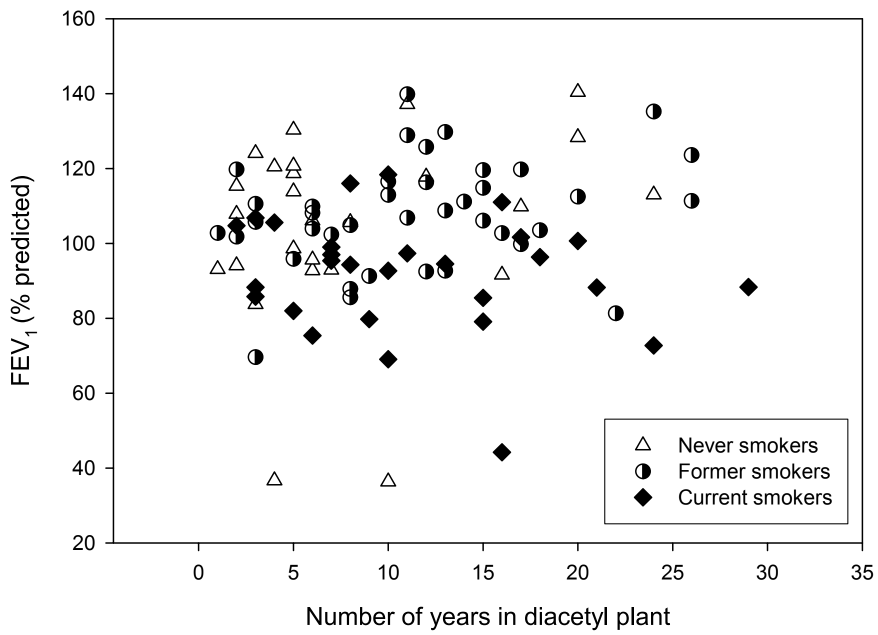


Figure 3. Percentage of predicted FEV_1 value in relation to smoking habits and the first year that process operators (n=93) had tasks in the diacetyl plant.

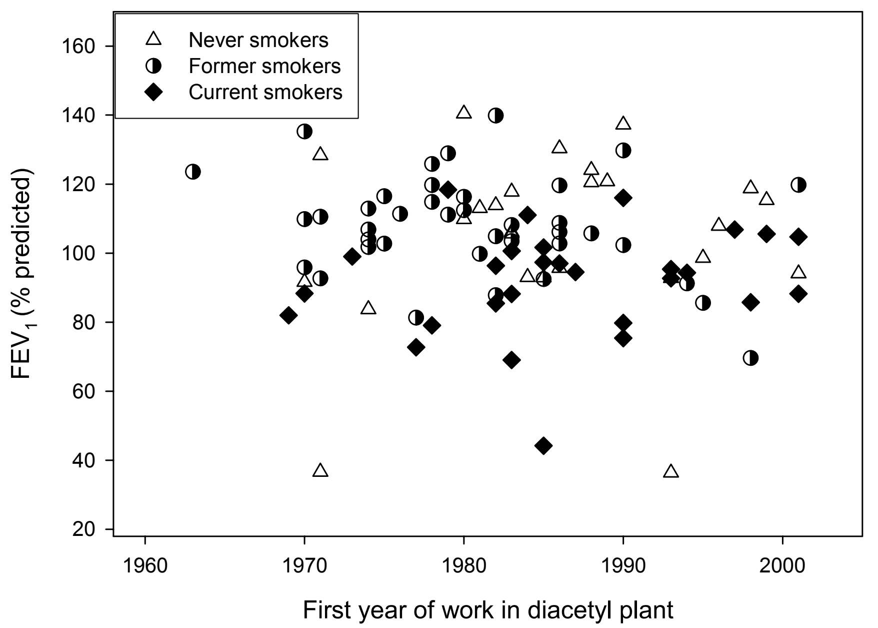
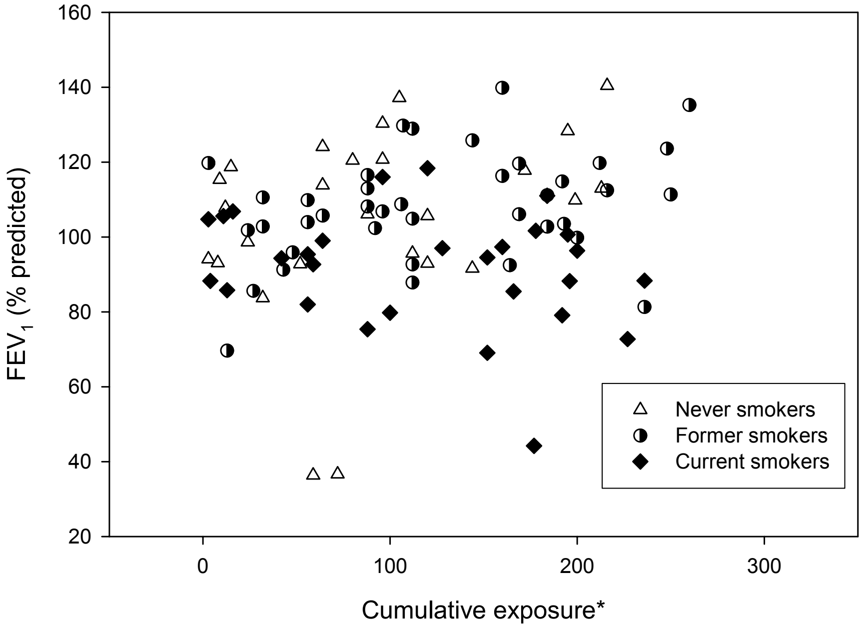


Figure 4. Percentage of predicted FEV_1 value in relation to smoking habits and estimated cumulative exposure to diacetyl for process operators (n = 93).



Relative cumulative exposure was estimated for process operators according to Table 3 as 8 times the number of years between 1967 and 1985 + 16 times the number of years between 1986 and 1994 + 4 times the number of years between 1995 and 2000 + the number of years between 2001 and 2003 that a worker had tasks in the diacetyl plant. The association between the relative cumulative exposure and FEV_1 (% predicted) was studied and presented in figure 4 which shows no clear association. Pearson's correlation coefficient was 0.15 (p=0.16). When correlation coefficients were calculated by smoking habits, FEV_1 of never smokers and former smokers was positively related with cumulative exposure (r = 0.32, p= 0.11 and r = 0.33, p=0.04, respectively), whereas FEV_1 of current smokers was negatively associated with exposure (r = -0.25, p = 0.20). Multiple linear regression of FEV_1 (actual value) with age, standing height, smoking, and cumulative exposure as explanatory variables showed a positive association between FEV_1 and cumulative exposure. According to this model, the FEV_1 increases with 19.4 ml per 10 cumulative exposure units ($p<0.05$). The explained variance of this model was 41% (adjusted R^2). This effect also existed when the three operators with severe airway obstruction were excluded (16.7 ml per 10 exposure units, $p<0.05$, R^2 45%).

5 Discussion

5.1 Cases of bronchiolitis obliterans syndrome

In this study we found three cases of bronchiolitis obliterans syndrome among 196 potentially exposed former workers of a diacetyl production plant. The three cases in this study were typified by irreversible fixed airways obstruction. Two workers, who had never smoked, showed a typical pattern with air trapping on HRCT compatible with bronchiolitis obliterans. The third case, a smoker, had an unusual pattern of air trapping combined with a nearly complete collapse of the central bronchial tree during expiration. The radiological findings in the last case do probably not fully account for bronchiolitis obliterans.

Bronchiolitis obliterans syndrome has been reported in workers in popcorn plants who were exposed to butter flavorings containing diacetyl [Akpinar-Elci et al, 2004]. Akpinar-Elci et al. described nine cases of bronchiolitis obliterans syndrome among approximately 450 former workers of a popcorn production plant [Akpinar-Elci et al, 2004]. The clinical characteristics, physiological and imaging features of those cases were consistent with the cases identified in this study. In the study of Akpinar-Elci et al., three cases underwent thoracoscopic lung biopsy. In the pathological examination of one case, tissue changes supported a diagnosis of constrictive bronchiolitis. Biopsy samples from two other cases were not diagnostic of bronchiolitis. These two other cases showed severe obstructive pulmonary defects and mosaic patterns on HRCT, and the negative biopsy results were probably due to a sampling problem. In the present study thoracoscopic lung biopsies were not performed.

Although bronchiolitis obliterans is a histologic diagnosis, the typical features on HRCT may obviate the need for biopsy in those with occupational exposure to a recognized agent. Moreover, the risk of surgical intervention and sampling error should be weighed against the need for a correct diagnosis and consequences for treatment.

Prevalence figures for bronchiolitis obliterans syndrome in the general population are not available.

In a population-based study Coultas et al. a prevalence of Interstitial Lung Diseases (ILD) was observed (including bronchiolitis obliterans syndrome) of 81 per 100.000 in males. They calculated also the prevalence of several subgroups of ILD. In the subgroup with environmental and occupational related ILD the prevalence was 21 per 100.000 [Coultas et al., 1994].

Parmet & von Essen [Parmet & von Essen 2002] estimated the occurrence of bronchiolitis obliterans syndrome in the general population to be between 1:40.000 and 1:100.000 using King as a reference [King, 1998].
The likelihood of observing 3 workers at this particular plant out of a total of 196 (equals 1531:100.000) is considerably higher than would be expected if we would apply the figures for the occurrence of bronchiolitis obliterans syndrome in the general population to this workforce. This is indicative of an elevated risk related to working in this workforce.
However, these general population figures may be an underestimation since bronchiolitis obliterans syndrome is easily misclassified as COPD [Akpinar-Elci et al, 2004], even when HRCT may be normal, at least during inspiration. In the present study two bronchiolitis obliterans cases were initially diagnosed as COPD. One case was initially diagnosed as asthma by the general physician but later on as COPD.
The risk in this study is actually even higher if one realizes that all cases had been working as a process operator at the diacetyl production plant out of a total of 100. A complication is that a direct comparison of bronchiolitis obliterans syndrome in this work force with the general population can not be made because of differences in the nature of the information available. For instance, general population information comes from respiratory clinics to which individuals were referred by GPs. In this study, a protocolized approach was used in an epidemiological context. However, despite these complications, which may affect direct comparisons, the likelihood of observing three bronchiolitis obliterans syndrome cases in a working force of approximately only 100 workers over a working life period is low, given the order of magnitude of the background prevalence.

Although no direct evidence can be obtained from this study, the distribution of bronchiolitis obliterans syndrome among process operators at the diacetyl production plant provides strong suspicion that they have been exposed to an agent that caused bronchiolitis obliterans syndrome in their work environment.

5.2 Epidemiological study

Compared with the ECRHS population, workers at the diacetyl production plant reported significantly more respiratory symptoms and self reported asthma. This was not corroborated by spirometric abnormalities. Several explanations for these differences may exist. Explanations of the excess rates of actual respiratory symptoms among workers could be the exposure to (irritating) chemicals in their current job. These symptoms need not necessarily be related to exposures in the past. Another explanation could be that ECRHS population also included non-active workers who were not fit enough to be active in the workforce. Therefore, the internal reference group in the present study may be more appropriate. Also, slight differences might have occurred in the way spirometric parameters have been measured in this study compared to the ECRH Survey.

Within the study population of the diacetyl production plant two groups of workers (process operators and quality-control workers) seem to have more spirometric abnormalities compared with the internal reference group. Quality-control workers had significantly lower FEV_1 values than workers in the internal reference group, but none of the Quality-control workers received a diagnosis of bronchiolitis obliterans syndrome or had fixed airways obstruction. We were unable to explain this observation, because quantitative exposure information for this group of workers is lacking.

Process operators had a significantly lower FEV_1 and Forced Expiration Ratio. However, a relation between lung function abnormalities and exposure to diacetyl could not be established.

Neither period of employment at the diacetyl plant, nor the number of years that operators had tasks in the diacetyl plant was associated with airway obstruction. In addition, no relationship exists between the age and standing height adjusted reference value of FEV_1 and the year that operators commenced tasks in the diacetyl plant. An association with the cumulative exposure proxy was not present. These findings seem not consistent with the results of the study of Kreiss et al.[Kreiss et al., 2002], where the estimated cumulative exposure to diacetyl was correlated with chronic effects on lung function, in terms of both the rate of abnormalities on spirometry and the average decreases in FEV_1 in quartiles of increasing cumulative exposure. However, specific differences between this study and the one by Kreiss et al., may account for the different results found in both studies. First, the population in this study was engaged in different chemical processes with different qualitative exposure profiles. Second, the exposure to diacetyl in this study population changed considerably over time, and it is likely that changes have occurred that were not captured by the limited number of exposure measurements available. Third, the time spent in this process changed over time as well. Since workers rotated between different plants, the time spent in the diacetyl plant had to be estimated on the basis of historic questionnaires and has been estimated only very crudely. Therefore, estimates of the cumulative exposure are very likely to be subject to considerable measurement error resulting in exposure misclassification. It is likely that exposure misclassification is larger in this study than the study by Kreiss et al., because of the more complex and dynamic processes and work environments in this chemical production industry relative to a more static popcorn production industry. The observation of relationships between lung function and exposure was restricted to severe airway obstruction [Kreiss et al. 2002]. On the other hand Akpinar-Elci [Akpinar-Elci et al., 2005] found in their study no significant differences between the high-exposure and low-exposure groups for the spirometric lung function parameters.Kreiss et al. [Kreiss et al., 2002], hypothesize that diacetyl may be a cause of respiratory disease or a marker of the causative exposures in the study population at the popcorn plant. They also indicated that respiratory effects are more likely to be explained by peak exposures. If true, this makes the cumulative exposure a poor proxy of bronchiolitis obliterans syndrome risk in any exposed population. In an animal model, concentrations of butter flavoring vapors containing diacetyl that can occur during the manufacture of foods were associated with epithelial injury in the nasal passages and pulmonary airways of rats [Hubbs et al, 2002]. This finding suggests a causal relationship between exposure to butter flavoring vapors containing diacetyl and airway injury. But in a author reply of Kreiss et al. to a comment [Taubert, 2002] it was noted that other agents within the workplace may have contributed to the clinical bronchiolitis obliterans syndrome as seen in the workforce at the popcorn plant. This was based on experiments, yet unpublished that indicate that airway injury in rats exposed to butter flavoring vapors was more severe than in rats exposed to diacetyl alone.

Indication exist that also other agents, including acetaldehyde may be associated with bronchiolitis obliterans syndrome occurrence [NIOSH 2004a]

The spectrum of airborne Volatile Organic Compounds (VOC’s) in the popcorn factories has been established in several studies [Kreiss et al, 2002; Simoes et al, 2002; NIOSH, 2003; NIOSH, 2004b].The results of semi-quantitative air sampling analyses in several areas of the popcorn plants showed approximately 100 different Volatile Organic Compounds (VOC’s). The most important compounds identified in the mixing area have been identified as diacetyl, methyl ethyl ketone, acetoin (or acetylmethylcarbinol), methanol, and ethanol [NIOSH, 2003]. Diacetyl was the predominant compound isolated from air samples [Kreiss et al, 2002; Akpinar-Elci, 2004; NIOSH, 2003].Both diacetyl and acetoin have been produced at the plant under study.Comparing popcorn plants with the diacetyl production plant setting, concentrations of diacetyl in production areas were, as far as can be evaluated from available information, at least during some

periods in time in the same order of magnitude as observed in the popcorn industry. However, it seems plausible that because of the differences in process (production versus intermediary use) clear differences exist with regard to the qualitative exposure profile and process temperature, or may exist with regard to exposure peaks and duration of exposure, but detailed information to corroborate this is lacking.
As in the study by Kreiss et al., diacetyl may either be a cause of respiratory disease or a marker of the causative exposures. The spectrum of potential causative agents is smaller in this production plant than in the popcorn processing plant.

5.3 Study limitations

There is a high participation rate, and selection bias due to selective recruitment, especially among retired workers, seems unlikely. However, the number of participants who were minimally or highly exposed were small. This limited statistical power in internal and external comparisons even after correction for confounding variables in multiple regression modeling. There was no evidence of confounding, either by smoking status or age, although relationships between smoking habits and lung function did not always behave as expected.
Most workers have at some point in time been exposed to several other chemicals including irritants, and this might potentially have confounded the effect of diacetyl exposure in the population study. As argued earlier, historic exposures have been measured with a considerable measurement error and it is likely that exposure misclassification has occurred, obscuring exposure response relationships. Moreover, our analyses of exposure have not addressed the potential importance of short-term or peak exposures among workers since information to do such analyses was lacking.
All workers who were alive and potentially exposed to diacetyl in the plant were traced. In this cohort 10 workers have died before the onset of this study. Their cause of death was not investigated. It is not clear if BO cases occurred among deceased and to what extent this might have contributed to the findings in this study.

5.4 Résumé

Our study suggests an excess rate of bronchiolitis obliterans syndrome cases in a diacetyl production plant, which is consistent with findings in the literature of bronchiolitis obliterans syndrome cases associated with butter flavoring exposure and in particular diacetyl in popcorn workers. This supports the conclusion that an agent in the diacetyl production process has caused bronchiolitis obliterans syndrome in exposed process operators at this plant. The exact causal agent responsible for the health effect and which has been present in the production plant remains unclear and cannot be established from this study.

6 Conclusion

Answers to the specific research questions

Are there cases of bronchiolitis obliterans syndrome in the group of workers exposed to Diacetyl?

We found three cases of bronchiolitis obliterans syndrome, all process operators, in the group of workers who were potentially exposed to diacetyl in the period 1960-2003.

What is the prevalence of respiratory symptoms and lung function abnormalities in exposed workers compared to a general population sample?

Compared with the ECRHS population, workers who were potentially exposed to diacetyl reported significantly more continuously trouble with breathing, daily cough and asthma. This was not corroborated by spirometric abnormalities.

Does a relationship between bronchiolitis obliterans syndrome cases and exposure to diacetyl exist?

A relation between lung function abnormalities and exposure to diacetyl could not be established.

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Alphabetical List by Abbreviation

AM	Arithmetic mean
AMC	Acetyl Methyl Carbinol
BO	Bronchiolitis Obliterans
BOS	Bronchiolitis Obliterans Syndrome
COPD	Chronic Obstructive Pulmonary Disease
DNPH	Dinitrophenylhydrazine
ECRHS	European Community Respiratory Health Survey
ECSC	European Community for Steel and Coal
ERS	European Respiratory Society
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GM	Geometric mean
GSD	Geometric standard deviation
HRCT	High Resolution Computed Tomography
ILD	Interstitial Lung Diseases
MAC	Maximal Accepted Concentration
MDI	Metered Dose Inhaler
MEF_{50}	Maximal Expiratory Flow at 50% FVC
Kco	Transfer coefficient for carbon monoxide
N	Number
NIOSH	National Institute for Occupational Safety and Health
PEF	Peak Expiratory Flow
ppm	Parts per million
% pred	Percentage of the predicted value
Qlab	Quality Control Lab
RADS	Reactive Airways Dysfunction Syndrome
RV	Residual Volume
SD	Standard Deviation
SE	Standard Error
SHE-workers	Safety, Health & Environment-workers
TD	Technische Dienst = Maintenance workers
TLC	Total lung capacity
Tlco	Transfer coefficient for carbon monoxide
VOC	Volatile Organic Compounds