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Chronic respiratory health risk associated with powdered toner exposure in the longitudinal observation of occupationally exposed Japanese workers

Toshio Nakadate(1), Yuko Yamano(1), Takenori Yamauchi(1), Shigeko Okubo(1), Daichi Nagashima(1)

(1) Department of Hygiene and Preventive Medicine, Showa University School of Medicine, Tokyo, Japan

Corresponding author: Toshio Nakadate

    Department of Hygiene and Preventive Medicine,

     Showa University School of Medicine

    1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

    e-mail: nakadate@med.showa-u.ac.jp

    Tel: +81-3-3784-8137   Fax: +81-3-3784-8251

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Abstract

Objective: We aimed to estimate the chronic health risk to humans associated with routine toner dust exposure in copier-industry workers under current actual work conditions, since little epidemiological evidence exists regarding the chronic respiratory effects of inhaled powdered toner exposure in humans.

Design: A prospective observational cohort study of occupational population.

Methods: Changes in chest radiogram, spirometry measurements, and serum and urine biomarkers of biomedical responses to extrinsic stress, as well as subjective symptoms were longitudinally observed for up to 10 years in Japanese copier-industry workers responsible for the manufacturing, maintenance or recycling of powdered toner or toner-using machines. A total of 694 subjects who did not change their work category during the follow-up and were free from chronic respiratory diseases at the baseline survey provided reliable results on at least 3 survey occasions during 3 years or more of follow-up.

Results: Typical fibrosis findings associated with pneumoconiosis was not observed on chest radiograms. No significant differences associated with toner exposure were noted in the frequency of new incidence of either non-specific findings on chest radiogram or serum fibrosis biomarkers (KL6 and SPD). However the exposed subjects tended to show increases in the frequency of respiratory symptoms and reduced spirometry results during the follow-up compared with the control group, although significant differences were only seen in chronic

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cough.

Conclusions: Under the current reasonably controlled work environmental conditions, lung fibrotic changes caused by inhaled dust exposure, including powdered toner, appear to be relatively uncommon; however, nonspecific temporal irritation causing subjective symptoms and inflammatory responses might exist.

Key words: Dusts, Longitudinal studies, Respiratory, Epidemiology

Strengths and limitations of this study

- An occupational cohort of workers in business machine industry exposed to printing toner dust in various types of work was followed for up to 10 years regarding their respiratory health status on an annual basis, mainly focusing on lung fibrotic changes.

- The incidence of newly emerging lung fibrotic changes was compared between the exposed workers and the non-exposed controls using wide-range health outcomes including chest X-ray findings, lung function results, and serum and urine biomarkers, as well as subjective symptoms.

- The total dust exposure during toner handling was measured in a representative sample of workers, but the fraction of toner particles in the whole dust exposure cannot be estimated accurately.

- Conventional chest radiography was utilized to evaluate lung fibrotic changes in an epidemiological research setting; however, computed tomography (CT) might have been more sensitive to early minimal fibrotic changes.

- Healthy worker bias might exist because some portion of the cohort ceased participation during the follow-up due to resigning, retiring, or transferring, although no toner-related health problems, such as lung fibrosis, were reported among these subjects.

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Introduction

Possible harmful effects of inhaled photocopier toner dust have been a matter of concern

since the publication of several case reports suggesting the existence of toner-related

pulmonary disorders [1][2][3]. However, little evidence is available regarding the potential

for toner dust inhalation in occupational and general home environments where photocopier

machines and laser printing devices are in use.

    We reported the results of a cross-sectional study of toner-handling workers engaged

in several types of toner exposure work [4]. Although the results suggested the limited

possibility of adverse effects due to toner dust inhalation, it was also suggested that a study

with longitudinal design would be useful for a more detailed examination of the hypothesized

association between toner dust exposure and adverse health effects under current actual work

conditions.

    We therefore conducted a longitudinal study of toner-handling workers for 10 years in

a business machine-producing company and affiliated companies involved in maintaining and

recycling these machines in order to obtain longitudinal evidence to determine a possible

health risk associated with a practical level of toner dust exposure under current actual work

conditions, with particular focus on chronic fibrotic changes in the lungs.

    This is the first report of this study and mainly presents the results of analyses of the

possible chronic health effects, including lung fibrotic changes as the principal research target,

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associated with toner dust exposure under current actual work conditions based on

longitudinally observed health data.

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Methods

Subjects

The subjects were employees of seven branches of a group of companies that produced, maintained, and recycled photocopiers, laser printers, and related products for printing with the use of powdered toner. We identified four categories of toner-handling work among the subjects. Toner production (TPD) represents work associated with any process in the course to producing powdered toner, such as mixing and molding raw materials, crushing toner mass, and filling and packing the products. Designing and development of machines (DDM) represents work associated with developing new models of toner-using business machines, in which various types of toner-handling procedures are involved. Maintenance (MTN) represents the work associated with maintaining photocopiers and laser printers in users' offices and homes. Recycling (RCL) represents work associated with processing recycled toner cartridges.

The survey on the health status of the subjects started in the fall of 2003 and was repeated until 2013 basically on an annual basis in the same manner as the first one in 2003. Throughout the entire study period, a total of 1176 toner handling workers (587 for TPD, 207 for DDM, 346 for MTN, and 36 for RCL) participated in at least one of these surveys. However, some participants ceased participation during an early stage of the study for various reasons other than toner-related health problems (e.g. resigning, retiring, or transferring), and

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others started participating in a later stage of the study. Therefore, we defined the subjects

with a sufficient follow-up duration for a longitudinal data analysis in this paper as those who

started participating by the spring of 2005 and were followed-up for three years or more with

at least three surveys throughout the entire study period. In addition, subjects were those who

had not changed their work category (concerning the presence or absence of toner handling)

during their follow-up.

The number of toner workers who met the above criteria was 489. As a reference

population for comparison with these toner-handling workers, an additional 309 control

subjects who had never participated in any types of toner-handling work described above also

participated in this study by the spring of 2005. Among them, the 221 who met the same

criteria as exposed workers stated above were analyzed. Control subjects were recruited at

each participating branch and amounted to roughly half the number of toner-handling

workers.

Health status indices

Subjects' health status was evaluated on an annual basis from various health-related aspects

using physiological, biochemical, and radiographic examination tools as well as via the

assessment of subjective symptoms and clinical signs. This evaluation focused mainly on

chronic respiratory deterioration, including pulmonary fibrotic conditions, and biological

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responses to inhaled potentially harmful substances. Standardized methods were utilized for

health status measurement to keep the measurement bias as small as possible. The biomedical

indices examined in this study are described below.

Chest X-ray findings

Chest radiography was carried out to obtain a standardized anterior-posterior X-ray film by

using prescribed standard procedures to detect mineral-dust pneumoconiosis in Japan [5].

Each X-ray film was read by one of three readers with sufficient experience in the

radiographic diagnosis of pneumoconiosis while referencing the standard films for the

diagnosis [6]. The readers were privy to no information regarding the exposure status of the

subjects. The severity of fibrotic findings in the lung fields was classified into 12 grades,

from 0/- to 3/+, according to the distribution and density of the small opacities. This

classification was compatible with that of the International Labor Organization (ILO) [7].

However, we noted none of the fibrotic findings typically observed in mineral dust

pneumoconiosis cases with 1/1 or a more severe grade profusion during early surveys.

Therefore, we additionally recorded mild, non-specific findings, such as sparse irregular

opacity, unclear interstitial shadow, or disrupted vascular shadow in radiographs. These

findings were not only potentially related to early effects of toner toxicity but also might be

caused by acute or sub-acute non-specific irritation or infection, which might be reversible.

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Questionnaire survey

Detailed information regarding respiratory symptoms, allergic symptoms, past and present medical history, and lifetime smoking history was obtained using a translated version of a self-administered questionnaire standardized by the American Thoracic Society (ATS-DLD-78A) [8], with slight modification and several additional questions on allergies of the eye, nose, and skin. The responses were certified by several trained interviewers, and supplemental interviews were held when necessary. The lifetime work history was also confirmed during this interview.

Spirometry

Subjects were asked to repeat the forced expiratory maneuver up to a maximum of five times in the standing position in order to obtain acceptable and reproducible spirometry results. The mechanical specifications of the spirometer used (DISCOM-21FX2; CHEST Co. Ltd., Tokyo, Japan) met the standards stipulated by ATS [9]. Routine BTPS correction and back-extrapolation were carried out. The calibration of the spirometer, spirometry measurements, and evaluation of the results were based on prescribed procedures described in detail elsewhere [10].

The percent predicted values standardized on sex, age, and height were calculated for

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FVC (%FVC) and FEV1 (%FEV1) in each subject based on the prediction equations

published by the Japan Thoracic Society [11]. We also calculated the percentage FEV1 by

FVC (FEV1%). The values of %FVC and %FEV1 were defined as reduced when they were

smaller than 80%. Similarly, FEV1% was considered to be reduced if it was less than 70%.

All figures were calculated from the best maneuver yielding the largest sum of FVC and

FEV1 for each subject [12].

Serum and urine biomarkers

Peripheral venous blood and urine (10 and 20 ml, respectively) were obtained from each

subject at the time of every survey. Serum was immediately separated from whole blood

sample using a routine procedure. Then, serum and urine samples were deeply frozen at

-80 °C until analyses were carried out.

As the indices of biological responses to inhaled extrinsic irritants like toner particles,

we employed the following biomarkers: highly sensitive C-reactive protein (CRP),

non-specific immunoglobulin E (IgE), sialylated carbohydrate antigen KL-6 (KL6), and lung

surfactant protein D (SPD) in serum, and 8-hydrovy deoxy guanosine (8OHdG) in urine. In

Japan, serum CRP, IgE, KL6, and SPD levels are routine clinical laboratory test items

assessed in common medical practice. The analyses of those results were therefore carried out

according to the appropriate Standard Operation Procedures (SOP) in SRL Inc., Tokyo.,

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Japan which has been providing high quality testing under the certification of the Japan

Accreditation Board, a public service juridical foundation for ensuring the reliability of

clinical laboratory testing in Japan.

Urine 8OHdG was measured using an ELISA kit (New 8OHdG Check ELISA Kit;

Japan Institute for the Control of Aging) and was adjusted for urine condensation using the

urine creatinine concentration as the denominator.

The result of the measurement of each index was judged to be out of the normal range

when the value exceeded the following figures: 1500 ng/ml for CRP, 173 IU/ml for IgE,

500 U/ml for KL6, 110 ng/ml for SPD, and 20 ng/mg creatinine for 8OHdG.

Exposure assessment

We directly measured the personal exposure levels in individual toner-handling workers.

Workers were asked to wear a portable personal dust sampler that continuously sampled the

air in the workplace environment to collect suspended dust throughout a given period of

toner-related work. The concentration of total and respirable dust exposed to the workers was

calculated as a time-weighted average value for 8 h (TWA-8h) based on the amount of dust

collected on the filter and the total volume of air inspired by the sampling pump.

Measurements were conducted basically twice a year for at least 5 workers in each

toner-handling procedure, yielding a total of 2042 measurements (1751 for TPD, 81 for DDM,

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88 for MTN, and 122 for RCL) throughout the study period. Those values obtained were evaluated with reference to the recommendations by the Japan Association of Industrial Health (JAIH) [13]. We were unable to obtain reliable estimates of the amount of toner dust fraction in the whole dust cluster collected on the sampling filter, mainly because of large variability of the values depending on the types of toner handled.

Analyses

- Cross-sectional evaluation of baseline data

For each subject, we considered the results obtained in the first year of participation as the baseline data, indicating the condition at the beginning of the longitudinal observation of this study. The relative frequency of subjects with positive results was calculated for each health-related item described above (prevalence) and compared statistically between the exposed subjects as a whole and the control subjects.

- Longitudinal evaluation of chronic fibrotic findings

For the longitudinal analysis, we mainly focused on the results associated with chronic respiratory toxicity, including the fibrotic potential of toner dust inhalation exposure. To this end, we analyzed the changes in chest radiographic findings during the follow-up period. We also used three pulmonary function indices (%FVC, %FEV1, and FEV1%) and two serum

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biomarkers (KL6 and SPD, both of which are recognized as useful markers of active-phase

fibrotic conditions in the lungs) [14]. In addition, we selected three chronic respiratory

symptoms associated with chronic respiratory diseases, such as fibrotic lung changes, namely

chronic cough, chronic phlegm, and breathlessness. Chronic cough, as well as chronic

phlegm, was considered to be present when the symptom existed for three months or more in

a year. Breathlessness was considered to be present when a subject walked slower than their

counterparts of the same age on level ground.

In the longitudinal data analysis, we evaluated the frequency of abnormalities that

newly emerged during the follow-up observation (incidence). Abnormalities were considered

to be present when the respiratory symptom or the X-ray findings were newly noted or when

the pulmonary function or biomarker values exceeded the normal range during follow-up.

Therefore, those subjects who already had abnormalities at their baseline survey were

excluded from the longitudinal data analysis.

Statistical analyses

For comparisons by exposure categories, the chi-squared test and Fisher's exact test as well as

a multiple logistic regression analysis were performed for categorical variables, and a simple

t-test and an analysis of covariance were performed for numeric variables, with the alpha

error level set at 0.05. All statistical tests and estimations were carried out using the SAS

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statistical package on a personal computer (PC-SAS Version 9.2; SAS Institute Inc., NC,

U.S.A.).

Ethical considerations

The objectives and outline of the study were explained to the subjects at the beginning of the

study, and written informed consent was obtained from each participant. All information

obtained in this study was handled in accordance with the guidelines for epidemiological

studies authorized in Japan [15]. This study was conducted under the approval of the

Institutional Review Board on Medical Ethics of Showa University School of Medicine

(Authorization No. 201).

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Results

As stated earlier, we selected 710 workers (489 exposed and 221 controls) as the subjects for

this longitudinal data analysis. Among them, 16 subjects reported that they had suffered from

chronic respiratory diseases prior to the beginning of this study (chronic obstructive

pulmonary disease and/or pulmonary tuberculosis). These subjects were therefore excluded,

and the remaining 694 subjects were ultimately analyzed. The average number of surveys in

which those subjects participated was 6.8, and the average follow-up duration (elapsed time

from the first observation to the final one) was 7.4 years. No significant difference was

observed in those average values between the exposed and control groups.

Several background characteristics at the baseline point were compared among the

exposure categories in Table 1. The male to female ratio was comparable between the

exposed and control groups, although the absolute number of female subjects was quite small

compared to males. Significant differences were observed between the exposed and control

groups in mean age and smoking habit classification at the baseline survey, with the exposed

group being younger and more likely to smoke than the controls. Seventy-six of 360 current

smokers at baseline quit smoking during their follow-up period, whereas only 1 out of 220

never smokers at the point of baseline started smoking during the follow-up. As for body size,

the mean values of standing height and body mass index (BMI) were comparable between the

exposed and control groups.

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Table 2 summarizes the result of personal exposure measurement during the entire study period, showing the median and the 10th and 90th percentile values of the TWA-8h concentration of total and respirable dust exposure. The TPD and RCL workers tended to show higher percentile values both in the total and respirable TWA-8h than the DDM and MTN workers. As suggested by those summary percentile values, the individual TWA-8h values distributed across a rather wide range, and some of them exceeded the permissible limit recommended by JAIH (4 mg/m^3 as total and 1 mg/m^3 as respirable carbon black dust, and 8 mg/m^3 as total and 2 mg/m^3 as respirable general dust) [13]. However, the overall median TWA-8h values of both total and respirable dust were well below the JAIH recommendations, even among the TPD and RCL workers. No marked changes were observed in the percentile values during the 10-year follow-up, suggesting that the work environment was relatively stable throughout the study period in terms of dust exposure to the workers as a whole.

Table 3 summarizes the prevalence of the biomedical indices studied at the baseline survey according to the exposure categories. The odds ratios (ORs) and their 95% confidence intervals (CIs) of the exposed to the control group are also shown after adjustment for possible confounding variables. The exposed group tended to show higher prevalence in respiratory symptom indices, although significant differences were observed only in chronic phlegm and wheeze grade 2. The prevalence values for CRP and IgE abnormalities were also

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significantly larger in the exposed group than in the control group, although the results of

KL6 and SPD were comparable between the two groups. The difference in CRP remained

significant even after adjustment for BMI, a well-known contributor to the serum CRP level.

Subjects with %FVC, %FEV1 or FEV1% values outside of the reference range were

relatively rare. There were no cases of clear pneumoconiosis with 1/1 or a more severe grade

on baseline chest X-ray films of the subjects analyzed, although non-specific findings were

noted in 3.4% of the exposed and 1.8% of the control subjects at baseline. No significant

difference between the two groups was noted in the prevalence at baseline of either the

pulmonary function or chest X-ray indices.

Table 4 compared the frequency of newly emerging abnormalities observed during the

follow-up period between the exposed and the control groups for selected biomedical indices

in order to examine the degree of chronic respiratory deterioration, including lung fibrotic

changes. After carefully evaluating the chest X-ray films accumulated during the follow-up

period, we observed no cases with 1/1 or a more severe grade of pneumoconiosis. In addition,

the incidence of non-specific findings during the follow-up was comparable between the

exposed and control groups. Furthermore, two clinically useful biomarkers of lung fibrosis

(KL6 and SPD) showed rather low incidence values for newly emerging abnormalities, which

were comparable between the exposed and control groups. However, regarding symptoms

and the pulmonary function, the exposed group tended to have a higher incidence of

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abnormalities more frequently than the control group. The ORs estimated by logistic regression analyses mostly exceeded 1.5, and after adjustment for age and smoking status during the follow-up period, chronic cough emerged significantly more frequently in the exposed subjects than in the control group.

Table 5 summarizes the significant independent contribution of confounding factors in the multivariate analyses described in Tables 3 and 4 as estimated ORs for the health-related outcomes. Only statistically significant results are presented with their 95% CIs. Smoking was considered to increase the incidence of chronic phlegm, SPD abnormality, and reduced %FVC and %FEV1 during follow-up, as well as the prevalence of many symptoms and a reduced pulmonary function at baseline. Similar increases in ORs with age were also observed for the incidence during the follow-up and the prevalence at baseline of some of the examined indices. A clear independent association between serum CRP abnormalities and obesity was also observed.

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Discussion

Photocopiers and laser printers using powdered toner are widely used in offices and homes, but the powdered toner for printing and emissions from these machines may become air pollutants in office and home environments [16][17]. McGarry et al. reported the condition of particulate air pollution resulting from the use of laser printers in several actual office environments [18]. However, only a few epidemiological reports have studied the possible health effects of everyday office pollutants, including toner dust used in photocopiers and laser printers. We previously reported [4] the results of an epidemiologic study on the health-related parameters associated with toner-handling procedures in Japanese workers. There seemed to be only a limited possibility that cumulative toner exposure could cause severe chronic health disorders, as long as a properly-controlled work environment was maintained. Khatri et al. [19] conducted a human volunteer exposure study in an actual current office environment and found that several biomarkers, including 8OHdG as a marker of oxidative stress [20], changed in relation with the particulate air pollutants emitted by photocopiers and laser printers, although those changes were acute or sub-acute and not chronic in nature. However, the results reported thus far were all obtained in cross-sectional studies, and longitudinal study results are needed to fully examine the health impact of particulate air pollutants associated with office machines, as far as chronic health effects are concerned. Quite recently, Ikegami et al. [21] reported no adverse health effects due to toner

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dust exposure in their longitudinal observation of Japanese toner-handling workers in what

seems to our knowledge to be the only longitudinal study reported thus far. Therefore, as a

subsequent study of our cross-sectional one mentioned above [4], we conducted a

longitudinal study to further evaluate the possible health effects of toner dust exposure,

focusing mainly on the chronic respiratory effects, such as lung fibrotic changes. To

determine the actual risk in the current real-world environment, we studied an actual working

population. Personal exposure measurement results showed that the current level of exposure

to total and respirable dust in toner-handling workers was on average well below the

permissible exposure levels of general mineral dust and carbon black dust recommended by

JAIH [13], even among TPD and RCL workers, in whom relatively high exposure was

observed. We also used several biomedical indices to detect possible harmful effects on the

respiratory system: namely imaging findings by chest X-ray, KL6 and SPD levels in serum as

clinically utilized biomarkers on lung fibrosis, spirometry results to evaluate the lung

functional status, and subjective symptoms obtained through standardized methods.

In a cross-sectional analysis of the baseline results, the exposed group tended to have

subjective symptoms more frequently than in the control group, and similar group differences

were observed in the frequency of abnormal values in the majority of the biomedical indices

examined. In addition, the difference between the two groups reached a statistically

significant level in several items, even after controlling for confounders. This tendency was

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basically consistent with what we had previously reported, with exposed workers complaining of symptoms more frequently than control workers despite having a comparable frequency of pulmonary function and chest radiography abnormalities. However, the cross-sectional evaluation at the baseline might have been influenced by a number of factors prior to the start of this study. Therefore, newly emerging abnormalities during follow-up should be examined in subjects without any abnormalities at the baseline observation, as far as the chronic health effects are concerned. One interesting finding of the cross-sectional analysis was the higher prevalence of CRP abnormalities, a sensitive marker of acute inflammatory status in the body, among the exposed subjects compared with control subjects. It seems unlikely that the elevated CRP values were significantly influenced by events in the past due to its nature as an acute inflammatory biomarker. In addition, the difference seemed to be independent of inflammatory conditions caused by obesity or smoking, as both were used as adjustment factors in the logistic regression model. These findings suggest that acute inflammatory conditions might be associated with current toner exposure. At the same time, an increase in the serum IgE level was also frequently found in the exposed subjects. In Japan, an allergy to cedar pollen is commonly observed every spring, so cedar pollenosis may be related to the findings for CRP and IgE.

In the longitudinal analyses of biomedical indices, we evaluated the frequency of a newly emerging abnormality during the follow-up period by excluding the subjects from the

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analysis if they already had the finding at baseline. Among the indices associated with lung

fibrotic changes, we found closely similar results between the exposed and control groups

regarding the new incidence of chest radiographic findings and serum biomarker

abnormalities. These results suggest that the fibrogenic potential of dust exposure associated

with toner-handling work seems to be minimal, if any at all, as far as the current working

environment is concerned.

In contrast, the estimated ORs for %FVC and FEV1% abnormality adjusted for

confounders showed relatively large values exceeding 1.5, although their increases were not

statistically significant. However, it seems difficult to attribute certain biological or medical

reasons to the moderate but insignificant elevation in ORs for %FVC and FEV1%

abnormality because the incidence of reduced %FEV1 was comparable between the exposed

and control subjects. Some fluctuation around normal ranges might be partially responsible

for the observed elevation in OR values. In the case of chronic cough, the exposed group

showed a larger incidence than the control group. This finding was not associated with the

findings for chronic phlegm, which showed a comparable incidence after adjusting for the

significant contribution of smoking. This suggests that the significant increase in the rate of

chronic cough cannot be attributed to mucus hypersecretion. Given the findings at baseline

mentioned earlier, it might be possible to hypothesize that non-specific irritation leading to

subjective symptoms, fluctuation in the pulmonary function, and inflammatory response were

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caused to some extent by exposure associated with toner-handling work.

Several limitations associated with the present study warrant mention. First, the number of subjects of this study (694 in total) might be insufficient to detect minimal health deteriorations. However, several well-established health risks were reconfirmed in this study, including the increase in the incidence of mucus hypersecretion symptoms and the pulmonary function deterioration due to smoking, as well as the increase in the incidence of non-specific chest X-ray findings with age. A significant positive association between obesity and serum CRP concentration was also observed. These results suggest that this study had a reasonably sufficient sample size, at least for health risks at a generally detectable level, despite not being highly sensitive. A meta-analysis of the studies with a common methodological basis will be useful for detecting minimal effects of toner dust. Second, we obtained measurements on personal exposure concentration for each work category as an exposure assessment. However, the results showed large variability. In addition, we were unable to obtain reliable data on the relative content of toner as a fraction of the whole dust sample. We therefore cannot reliably estimate the amount of personal exposure to toner dust for each individual subject. An analysis of the dose-response relationship will require a more precise exposure assessment for toner dust. Third, we simply utilized conventional chest radiography and did not use computed tomography (CT) to evaluate lung fibrotic changes, because we should minimize the amount of X-rays that the participants were exposed to in an epidemiological

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research setting. We were therefore unable to distinguish the non-specific findings in cases

where extremely-early-phase of toner-related changes might have been included. Advanced

radiography techniques will be useful for the more precise evaluation of lung fibrotic

deterioration caused by toner dust inhalation.

Conclusion

Under the current reasonably controlled work environmental conditions, lung fibrotic

changes caused by inhaled dust exposure, including powdered toner, appear to be relatively

uncommon; however, nonspecific temporal irritation causing subjective symptoms and

inflammatory responses might exist. These longitudinal findings are consistent with the

results observed in a cross-sectional study conducted previously.

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Acknowledgments

Footnotes

Contributors: TN designed the study; TN and YY executed the data collection in annual surveys; YY, TY, SO and DN contributed to the data preparation and analyses; TN wrote the initial manuscript. All of the authors contributed to the revision of the manuscript and approved the final form of the manuscript for submission.

Competing interest: The authors report no conflicts of interest for the submitted work.

Data sharing statement: No additional data available.

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Table 1. Background characteristics of the subjects according to the exposure category.

Category of exposure condition

Characteristics	Control	Exposed[*]					$p^{\dagger}$
		Total	TPD	DDM	MTN	RCL	
Number surveyed	217	477	175	145	150	7	
Sex (M/F)	205/12	461/16	167/8	141/4	149/1	4/3	0.18
Age (years)	39.1(8.2)	34.1(8.1)	32.9(10.3)	35.4(6.5)	34.2(6.3)	32.4(7.6)	<0.0001
Height (cm)	170.1(6.6)	170.3(6.4)	169.0(6.1	171.3(6.4)	171.4(6.4)	161.5(6.8)	0.63
BMI (kg/m^2)	23.0(3.1)	23.0(3.7)	23.2(4.6)	22.4(2.6)	23.4(3.2)	19.9(1.5)	0.999
Smoking habit[‡]							0.026
Never	70(32)	150(31)	38(22)	69(48)	38(25)	5(71)	
Former	47(22)	67(14)	17(10)	26(18)	24(16)	0	
Current	100(46)	260(55)	120(68)	50(34)	88(59)	2(29)	

* TPD: toner production, DDM: machine design and development, MTN: maintenance, RCL:

recycling, BMI: body mass index

†Probability under the hypothesis that there are no differences between the control group and

the exposed group as a whole.

‡Percentage of column total in parentheses.

Values are presented the number of subjects for sex and smoking habit and the arithmetic

mean (standard deviations) for age, height, and BMI at the baseline survey.

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Table 2. Exposure levels of toner-handling workers by their working category and year of measurement estimated by personal exposure measurements

	Total dust (mg/m^3)			Respirable dust (mg/m^3)		
	10th %ile	Median	90th %ile	10th %ile	Median	90th %ile
Total	0.08	0.49	2.38	0.03	0.05	0.36
Work category*						
TPD	0.09	0.5	2.14	0.03	0.09	0.35
DDM	0.04	0.08	0.38	0.03	0.03	0.04
MTN	0.07	0.17	0.8	0.03	0.03	0.15
RCL	0.51	1.46	5.6	0.02	0.04	0.64
Year of measurement						
2004	0.11	0.54	2.44	0.03	0.07	0.43
2005	0.07	0.43	1.96	0.03	0.05	0.3
2006	0.08	0.62	2.26	0.03	0.06	0.37
2007	0.1	0.74	3.34	0.03	0.05	0.47
2008	0.08	0.58	2.8	0.03	0.06	0.49

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2009	0.09	0.49	2.58	0.02	0.06	0.31
2010	0.09	0.48	2.35	0.02	0.05	0.28
2011	0.08	0.44	1.96	0.02	0.06	0.37
2012	0.09	0.4	1.68	0.02	0.04	0.22
2013	0.06	0.26	1.82	0.02	0.04	0.16

* TPD: toner production, DDM: machine design and development, MTN: maintenance, RCL:

recycling

Values are presented as the median and the 10th and 90th percentiles (%ile) for personal

exposure measurement data.

Table 3. The comparison of the baseline status in select biomedical indices at the point of the first examination for each subject between the exposed subjects as a whole and the controls

Biomedical indices	Univariate analysis Prevalence (%)			Multivariate analysis Logistic regression model	
	Exposed	Controls	p*	OR[†]	95%CI[†]
Respiratory symptoms[‡]					
Cough #1 (7A)	19.4	14.8	0.14	1.35	(0.85-2.14)
Cough #2 (7B)	14.8	11.1	0.19	1.41	(0.84-2.37)
Chronic cough (7E)	5.5	4.2	0.46	1.6	(0.70-3.64)
Phlegm #1 (8A)	23.7	20.4	0.34	1.23	(0.81-1.87)
Phlegm #2 (8B)	13.5	10.7	0.29	1.45	(0.85-2.48)
Chronic phlegm (8C)	8.9	4.2	0.03	2.62	(1.20-5.70)
Exacerbation (9A)	10.5	7	0.14	1.56	(0.84-2.91)
Wheeze #1 (10A1)	9.5	5.6	0.09	1.51	(0.76-2.99)
Wheeze #2[§] (10A2)	2.7	0	0.01	-	-
Chronic Wheeze (10A3)	0	0	-	-	-
Breathlessness #1 (13A)	9.7	11.6	0.45	1	(0.58-1.73)

Breathlessness #2§ (13B)	2.2	1.4	0.76	2.27	(0.58-8.89)
Breathlessness #3§ (13C)	0.4	0.9	0.59	0.96	(0.13-7.20)
Biomarker abnormality					
CRP	12.0	6.0	0.02	2.02	(1.05-3.88)
IgE	31.7	20.8	0.003	1.66	(1.12-2.48)
8OHdG§	1.1	1.8	0.47	0.68	(0.17-2.78)
KL6§	0.4	0	1	-	-
SPD	3.2	3.4	0.89	0.91	(0.35-2.37)
Reduced pulmonary function					
%FVC	5.3	4.2	0.55	1.07	(0.47-2.42)
%FEV1	8.4	4.7	0.08	1.97	(0.95-4.14)
FEV1%§	0.6	0.5	1	2.54	(0.25-25.6)
Chest X-ray findings					
Fibrotic change ≥1/1	0	0	1	-	-
Non-specific findings	3.4	1.8	0.27	2.47	(0.78-7.81)

* Probability under the hypothesis that there are no differences between the control group and exposed group as a whole examined by the chi-squared test or Fisher's exact probability method.

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† Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the

control group, regarding the positive findings at the baseline survey

‡ The ATS-DLD-78A questionnaire code is shown in the parentheses

Fisher's exact probability method was applied.

\#   Grade of the severity of the symptom

The figures in the column of prevalence represent the proportion of subjects with symptoms

or positive findings in each index. The results of multivariate analyses were adjusted for the

age and smoking status of the subjects. Body mass index was also used as an adjustment

factor in the case of CRP.

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Table 4. A comparison of the cumulative frequency of incidence in select biomedical indices associated with lung fibrotic changes that newly emerged during the follow-up period

	Univariate analysis			Multivariate analysis	
	Incidence			Logistic regression model	
	Exposed	Controls	$p*$	OR[†]	95%CI[†]
Respiratory symptoms[‡]					
Chronic cough (7C)	11.3	6.3	0.04	2.26	(1.13-4.53)
Chronic phlegm (8C)	14	9.6	0.12	1.6	(0.91-2.83)
Breathlessness #2 (13B)	6	4.7	0.48	1.69	(0.73-3.94)
Serum biomarkers					
KL6[§]	2.1	1.8	1	1.27	(0.38-4.29)
SPD	2.4	2.4	1	1.06	(0.34-3.28)
Pulmonary function					
%FVC	12.8	8.2	0.08	1.51	(0.83-2.73)
%FEV1	13.5	13.5	1	1.01	(0.60-1.70)
FEV1%	3.8	2.8	0.5	1.99	(0.75-5.30)

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Chest X-ray

Non-specific findings   5.6   5.2   0.8   1.39   (0.65-2.97)

* Probability under the hypothesis that there are no differences between the control group and exposed group as a whole examined by the chi-squared test or Fisher's exact probability method

† Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the control group, retarding the newly emerging abnormalities of biomedical indices

‡ The ATS-DLD-78A questionnaire code is shown in the parentheses.

§ Fisher's exact probability method was applied.

\#   Grade of the severity of the symptom

The figures in the column of incidence represent the proportion of subjects starting to show symptoms or positive findings during the follow-up period among those who did not have the symptom or positive finding at baseline. The results of multivariate analyses were adjusted for the age and smoking status of the subjects.

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Table 5. Independent contribution of confounding factors in the multivariate analyses of biomedical indices

	Smoking		Age		BMI
	Prevalence[*]	Incidence[†]	Prevalence[*]	Incidence[†]	Prevalence[*]
	OR(95% CI)[‡]	OR(95% CI)[‡]	OR(95%Cis)[‡]	OR(95% CI)[‡]	OR(95% CI)[‡]
Cough #1 (7A)[§]	1.68(1.12-2.51)				
Cough #2 (7B)[§]	1.62(1.03-2.54)				
Chronic cough (7E)[§]	3.34(1.49-7.48)		1.63(1.08-2.45)		
Phlegm #1 (8A)[§]	2.38(1.63-3.47)				
Phlegm #2 (8B)[§]	2.62(1.60-4.29)		1.38(1.05-1.82)		
Chronic phlegm (8C)[§]	3.69(1.85-7.35)	1.80(1.10-2.96)	1.53(1.08-2.16)		

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Breathlessness #1 (13A)[§]   1.59(1.17-2.16)

Breathlessness #2†(13B)[§]   2.64(1.36-5.12)

Breathlessness #3†(13C)[§]   5.62(1.51-20.85)

CRP abnormality   1.32(1.17-1.49)

SPD abnormality   4.10(1.15-14.58)

Reduced %FVC   3.13(1.09-8.97)

Reduced %FEV1   3.82(1.38-10.58)

Reduced FEV1%   3.92(1.20-12.87)2.11(1.27-3.52)

Non-specific X-ray   1.59(1.06-2.38)

 findings

* An analysis of the prevalence at baseline.

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† An analysis of the new incidence during the follow-up observation

‡Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the control group, regarding the prevalence and incidence

of biomedical indices.

§ The ATS-DLD-78A questionnaire code is shown in the parentheses.

Grade of the severity of the symptom

Values are presented as the OR (95% CI) estimated using the logistic regression models shown in Tables 3 and 4. Only statistically significant

results are shown.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6-7
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8-9
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*10-13*
Bias	9	Describe any efforts to address potential sources of bias	9, 10-12
Study size	10	Explain how the study size was arrived at	8-9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	14-15
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	15
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	8-9
		(*e*) Describe any sensitivity analyses	20, 41-43
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	17
		(b) Give reasons for non-participation at each stage	17
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	17-19, 32-38
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	17
Outcome data	15*	Report numbers of outcome events or summary measures over time	19-20, 39-40
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	19-20, 39-40
		(*b*) Report category boundaries when continuous variables were categorized	11, 13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	20, 41-43
Discussion			
Key results	18	Summarise key results with reference to study objectives	23-24
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	25-26
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	28

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Chronic respiratory health risk associated with powdered toner exposure in the longitudinal observation of occupationally exposed Japanese workers

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Chronic respiratory health risk associated with powdered toner exposure in the longitudinal observation of occupationally exposed Japanese workers

Toshio Nakadate(1), Yuko Yamano(1), Takenori Yamauchi(1), Shigeko Okubo(1), Daichi Nagashima(1)

(1) Department of Hygiene and Preventive Medicine, Showa University School of Medicine, Tokyo, Japan

Corresponding author: Toshio Nakadate

Department of Hygiene and Preventive Medicine,

Showa University School of Medicine

1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

e-mail: nakadate@med.showa-u.ac.jp

Tel: +81-3-3784-8137   Fax: +81-3-3784-8251

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Abstract

Background: Little epidemiological evidence exists regarding the chronic respiratory effects

of inhaled powdered toner exposure in humans, although several case reports have suggested

the existence of lung disorders that might be related to exposure to toner dust.

Objective: We aimed to estimate the chronic health risk to humans associated with routine

toner dust exposure in copier-industry workers under current actual work conditions.

Design: A prospective observational cohort study of occupational population.

Methods: Changes in chest radiogram, spirometry measurements, and serum and urine

biomarkers of biomedical responses to extrinsic stress, as well as subjective symptoms were

longitudinally observed for up to 10 years in Japanese copier-industry workers responsible

for the manufacturing, maintenance or recycling of powdered toner or toner-using machines.

A total of 694 subjects who did not change their work category during the follow-up and were

free from chronic respiratory diseases at the baseline survey provided reliable results on at

least 3 survey occasions during 3 years or more of follow-up.

Results: Typical fibrosis findings associated with pneumoconiosis was not observed on chest

radiograms. No significant differences associated with toner exposure were noted in the

frequency of new incidence of either non-specific findings on chest radiogram or serum

fibrosis biomarkers (KL6 and SPD). However the exposed subjects tended to show increases

in the frequency of respiratory symptoms and reduced spirometry results during the follow-up

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compared with the control group, although significant differences were only seen in chronic

cough.

Conclusions: Under the current reasonably controlled work environmental conditions, lung

fibrotic changes caused by inhaled dust exposure, including powdered toner, appear to be

relatively uncommon; however, nonspecific temporal irritation causing subjective symptoms

and inflammatory responses might exist.

Key words: Dust, Longitudinal studies, Respiratory, Epidemiology

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Strengths and limitations of this study

- An occupational cohort of workers in business machine industry exposed to printing toner dust in various types of work was followed for up to 10 years regarding their respiratory health status on an annual basis, mainly focusing on lung fibrotic changes.

- The incidence of newly emerging lung fibrotic changes was compared between the exposed workers and the non-exposed controls using wide-range health outcomes including chest X-ray findings, lung function results, and serum and urine biomarkers, as well as subjective symptoms.

- The total dust exposure during toner handling was measured in a representative sample of workers, but the fraction of toner particles in the whole dust exposure cannot be estimated accurately.

- Conventional chest radiography was utilized to evaluate lung fibrotic changes in an epidemiological research setting; however, computed tomography (CT) might have been more sensitive to early minimal fibrotic changes.

- Healthy worker bias might exist because some portion of the cohort ceased participation during the follow-up due to resigning, retiring, or transferring, although no toner-related health problems, such as lung fibrosis, were reported among these subjects.

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Introduction

Possible harmful effects of inhaled photocopier toner dust have been a matter of concern

since the publication of several case reports suggesting the existence of toner-related

pulmonary disorders [1][2][3]. However, little evidence is available regarding the potential

for toner dust inhalation in occupational and general home environments where photocopier

machines and laser printing devices are in use.

We reported the results of a cross-sectional study of toner-handling workers engaged

in several types of toner exposure work [4]. Although the results suggested the limited

possibility of adverse effects due to toner dust inhalation, it was also suggested that a study

with longitudinal design would be useful for a more detailed examination of the hypothesized

association between toner dust exposure and adverse health effects under current actual work

conditions.

We therefore conducted a longitudinal study of toner-handling workers for 10 years in

a business machine-producing company and affiliated companies involved in maintaining and

recycling these machines in order to obtain longitudinal evidence to determine a possible

health risk associated with a practical level of toner dust exposure under current actual work

conditions, with particular focus on chronic fibrotic changes in the lungs.

This is the first report of this study and mainly presents the results of analyses of the

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possible chronic health effects, including lung fibrotic changes as the principal research target,

associated with toner dust exposure under current actual work conditions based on

longitudinally observed health data.

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Methods

Subjects

The subjects were employees of seven branches of a group of companies that produced, maintained, and recycled photocopiers, laser printers, and related products for printing with the use of powdered toner. We identified four categories of toner-handling work among the subjects. Toner production (TPD) represents work associated with any process in the course to producing powdered toner, such as mixing and molding raw materials, crushing toner mass, and filling and packing the products. Designing and development of machines (DDM) represents work associated with developing new models of toner-using business machines, in which various types of toner-handling procedures are involved. Maintenance (MTN) represents the work associated with maintaining photocopiers and laser printers in users' offices and homes. Recycling (RCL) represents work associated with processing recycled toner cartridges. The air environment was generally well-maintained using local ventilation instruments according to the regulations in Japan. The workers were therefore encouraged to wear respiratory protection masks in only limited situations where heavy exposure might occur, such as when cleaning the mixing tanks or performing maintenance on/repairing equipment.

The survey on the health status of the subjects started in the fall of 2003 and was

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repeated until 2013 basically on an annual basis in the same manner as the first one in 2003.

Throughout the entire study period, a total of 1176 toner handling workers (587 for TPD, 207

for DDM, 346 for MTN, and 36 for RCL) participated in at least one of these surveys.

However, some participants ceased participation during an early stage of the study for various

reasons other than toner-related health problems (e.g. resigning, retiring, or transferring), and

others started participating in a later stage of the study. Therefore, we defined the subjects

with a sufficient follow-up duration for a longitudinal data analysis in this paper as those who

started participating by the spring of 2005 and were followed-up for three years or more with

at least three surveys throughout the entire study period. In addition, subjects were those who

had not changed their work category (concerning the presence or absence of toner handling)

during their follow-up. The flowchart in Fig. 1 shows the number of participants included in

or excluded from the analysis based on the above-mentioned criteria.

The number of toner workers who met the above criteria was 489. As a reference

population for comparison with these toner-handling workers, an additional 309 control

subjects who had never participated in any types of toner-handling work described above also

participated in this study by the spring of 2005. Among them, the 221 who met the same

criteria as exposed workers stated above were analyzed. Control subjects were recruited at

each participating branch and amounted to roughly half the number of toner-handling

workers. The control subjects basically worked in different buildings from the ones where the

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exposed workers were engaged in toner-handling work. While the control subjects

occasionally happened to enter the factory area, such accidental exposure was quite limited.

Health status indices

Subjects' health status was evaluated on an annual basis from various health-related aspects

using physiological, biochemical, and radiographic examination tools as well as via the

assessment of subjective symptoms and clinical signs. This evaluation focused mainly on

chronic respiratory deterioration, including pulmonary fibrotic conditions, and biological

responses to inhaled potentially harmful substances. Standardized methods were utilized for

health status measurement to keep the measurement bias as small as possible. The biomedical

indices examined in this study are described below.

Chest X-ray findings

Chest radiography was carried out to obtain a standardized anterior-posterior X-ray film by

using prescribed standard procedures to detect mineral-dust pneumoconiosis in Japan [5].

Each X-ray film was read by one of three readers with sufficient experience in the

radiographic diagnosis of pneumoconiosis while referencing the standard films for the

diagnosis [6]. The readers were privy to no information regarding the exposure status of the

subjects. The severity of fibrotic findings in the lung fields was classified into 12 grades,

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from 0/- to 3/+, according to the distribution and density of the small opacities. This

classification was compatible with that of the International Labor Organization (ILO) [7].

However, we noted none of the fibrotic findings typically observed in mineral dust

pneumoconiosis cases with 1/1 or a more severe grade profusion during early surveys.

Therefore, we additionally recorded mild, non-specific findings, such as sparse irregular

opacity, unclear interstitial shadow, or disrupted vascular shadow in radiographs. These

findings were not only potentially related to early effects of toner toxicity but also might be

caused by acute or sub-acute non-specific irritation or infection, which might be reversible.

Questionnaire survey

Detailed information regarding respiratory symptoms, allergic symptoms, past and present

medical history, and lifetime smoking history was obtained using a translated version of a

self-administered questionnaire standardized by the American Thoracic Society

(ATS-DLD-78A) [8], with slight modification and several additional questions on allergies of

the eye, nose, and skin. The responses were certified by several trained interviewers, and

supplemental interviews were held when necessary. Subjects were considered to have a

symptom when they gave an affirmative response to questions about the specific symptom.

The lifetime work history was also confirmed during this interview.

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Spirometry

Subjects were asked to repeat the forced expiratory maneuver up to a maximum of five times in the standing position in order to obtain acceptable and reproducible spirometry results. The mechanical specifications of the spirometer used (DISCOM-21FX2; CHEST Co. Ltd., Tokyo, Japan) met the standards stipulated by ATS [9]. Routine BTPS correction and back-extrapolation were carried out. The calibration of the spirometer, spirometry measurements, and evaluation of the results were based on prescribed procedures described in detail elsewhere [10].

The percent predicted values standardized on sex, age, and height were calculated for forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and FEV1/FVC in each subject based on the prediction equations published by the Japanese Respiratory Society [11]. The values of FVC, FEV1, and FEV1/FVC were defined as reduced when they were smaller than the lower value of the confidence interval of the authorized reference equations described above. All figures were calculated from the best maneuver yielding the largest sum of FVC and FEV1 for each subject [12].

For the analysis of the longitudinal changes in spirometry indices, we calculated the height-squared proportional values of FVC (FVC/HT^2) and FEV1 ($FEV1/HT^2$) to adjust for differences in the body size. The annual decline in FVC/HT^2 and $FEV1/HT^2$ were calculated for each subject as a regression coefficient using a simple linear regression equation of those

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values against age during the follow-up period.

Serum and urine biomarkers

Peripheral venous blood and urine (10 and 20 ml, respectively) were obtained from each

subject at the time of every survey. Serum was immediately separated from whole blood

sample using a routine procedure. Then, serum and urine samples were deeply frozen at

-80 °C until analyses were carried out.

As the indices of biological responses to inhaled extrinsic irritants like toner particles,

we employed the following biomarkers: highly sensitive C-reactive protein (CRP),

non-specific immunoglobulin E (IgE), sialylated carbohydrate antigen KL-6 (KL6), and lung

surfactant protein D (SPD) in serum, and 8-hydrovy deoxy guanosine (8OHdG) in urine. In

Japan, serum CRP, IgE, KL6, and SPD levels are routine clinical laboratory test items

assessed in common medical practice. The analyses of those results were therefore carried out

according to the appropriate Standard Operation Procedures (SOP) in SRL Inc., Tokyo.,

Japan which has been providing high quality testing under the certification of the Japan

Accreditation Board, a public service juridical foundation for ensuring the reliability of

clinical laboratory testing in Japan.

Urine 8OHdG was measured using an ELISA kit (New 8OHdG Check ELISA Kit;

Japan Institute for the Control of Aging) and was adjusted for urine condensation using the

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urine creatinine concentration as the denominator.

The result of the measurement of each index was judged to be out of the normal range when the value exceeded the following figures: 1500 ng/ml for CRP, 173 IU/ml for IgE, 500 U/ml for KL6, 110 ng/ml for SPD, and 20 ng/mg creatinine for 8OHdG.

Exposure assessment

We directly measured the personal exposure levels in individual toner-handling workers. The average diameter of the toner particles contained in the products handled by the workers in this study was about 10 μm. Workers were asked to wear a portable personal dust sampler (NWPS-254 sampler with a T60A20 sampling filter, SIBATA SCIENTIFIC TECHNOLOGY Ltd., Tokyo, Japan) that continuously sampled the air in the workplace environment at 2.5 L/min air flow driven by a MP-2N or MP-3Σ pump (SIBATA SCIENTIFIC TECHNOLOGY Ltd., Tokyo, Japan). The sampling head was located around the neck of each subject to collect particles suspended in the air throughout a given period of toner-related work. The respirable fraction was separated using filters designed to cut 50% of 4-μm-diameter particles. The concentration of total and respirable dust exposed to the workers was calculated as a time-weighted average value for 8 h (TWA-8h) based on the amount of dust collected on the filter and the total volume of air inspired by the sampling pump. Measurements were conducted basically twice a year for at least 5 workers in each toner-handling procedure,

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yielding a total of 2042 measurements (1751 for TPD, 81 for DDM, 88 for MTN, and 122 for

RCL) throughout the study period. Those values obtained were evaluated with reference to

the recommendations by the Japan Association of Industrial Health (JAIH) [13]. We also

analyzed several chemical components that were generated when the toner was heated at a

temperature of around 180 degrees centigrade as representative components of powdered

toner using gas chromatography and mass spectrometry (GC-MS) in order to estimate the

toner exposure separately from other types of dusts suspended in the air. However, we were

unable to obtain reliable estimates of the amount of toner dust fraction in the whole dust

cluster collected on the sampling filter, mainly because of large variability of the values

depending on the types of toner handled.

Analyses

- Cross-sectional evaluation of baseline data

For each subject, we considered the results obtained in the first year of participation as the

baseline data, indicating the condition at the beginning of the longitudinal observation of this

study. The relative frequency of subjects with positive results was calculated for each

health-related item described above (prevalence) and compared statistically between the

exposed subjects as a whole and the control subjects. The significance of differences in the

prevalence between the exposed subjects and controls was assessed using a chi-squared test

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and Fisher's exact test as appropriate. A multiple logistic regression analysis was used to

obtain the OR estimates adjusted for possible confounding factors.

- Longitudinal evaluation of chronic fibrotic findings

For the longitudinal analysis, we mainly focused on the results associated with chronic

respiratory toxicity, including the fibrotic potential of toner dust inhalation exposure. To this

end, we analyzed the changes in chest radiographic findings during the follow-up period. We

also used three pulmonary function indices (FVC, FEV1, and FEV1/FVC) and two serum

biomarkers (KL6 and SPD, both of which are recognized as useful markers of active-phase

fibrotic conditions in the lungs) [14]. In addition, we selected three chronic respiratory

symptoms associated with chronic respiratory diseases, such as fibrotic lung changes, namely

chronic cough, chronic phlegm, and breathlessness. Chronic cough, as well as chronic

phlegm, was considered to be present when the symptom existed for three months or more in

a year. Breathlessness was considered to be present when a subject walked slower than their

counterparts of the same age on level ground.

In the longitudinal data analysis, we evaluated the frequency of abnormalities that

newly emerged during the follow-up observation (incidence). Abnormalities were considered

to be present when the respiratory symptom or the X-ray findings were newly noted or when

the pulmonary function or biomarker values exceeded the normal range during follow-up.

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Therefore, those subjects who already had abnormalities at their baseline survey were excluded from the longitudinal data analysis. The incidence was compared between the exposed workers as a whole and the control subjects, as well as by the work categories. In a similar manner to the cross-sectional analyses, the incidence was compared using a chi-squared test or Fisher's exact test as appropriate, and the OR estimates adjusted for confounders were calculated using a multiple logistic regression analysis.

Statistical analyses

For comparisons by exposure categories, the chi-squared test and Fisher's exact test as well as a multiple logistic regression analysis were performed for categorical variables, and a simple *t*-test and an analysis of covariance were performed for numeric variables, with the alpha error level set at 0.05. All statistical tests and estimations were carried out using the SAS statistical package on a personal computer (PC-SAS Version 9.2; SAS Institute Inc., Cary, NC, USA).

Ethical considerations

The objectives and outline of the study were explained to the subjects at the beginning of the study, and written informed consent was obtained from each participant. All information obtained in this study was handled in accordance with the guidelines for epidemiological

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studies authorized in Japan [15]. This study was conducted under the approval of the

Institutional Review Board on Medical Ethics of Showa University School of Medicine

(Authorization No. 201).

Patient involvement

Patients were not involved in this study.

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Results

As stated earlier, we selected 710 workers (489 exposed and 221 controls) as the subjects for

this longitudinal data analysis. Among them, 16 subjects reported that they had suffered from

chronic respiratory diseases prior to the beginning of this study (chronic obstructive

pulmonary disease and/or pulmonary tuberculosis). These subjects were therefore excluded,

and the remaining 694 subjects were ultimately analyzed (Fig. 1). The average number of

surveys in which those subjects participated was 6.8, and the average follow-up duration

(elapsed time from the first observation to the final one) was 7.4 years. No significant

difference was observed in those average values between the exposed and control groups.

Several background characteristics at the baseline point, as well as the results of

personal exposure measurements during the entire study period as the 10th and 90th

percentile values of the TWA-8h concentration, were compared among the exposure

categories in Table 1. The male to female ratio was comparable between the exposed and

control groups. However, the absolute number of female subjects was quite small compared

to males, with only 28 women analyzed. Significant differences were observed between the

exposed and control groups in mean age and smoking habit classification at the baseline

survey, with the exposed group being younger and more likely to smoke than the controls.

Seventy-six of 360 current smokers at baseline quit smoking during their follow-up period,

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whereas only 1 out of 220 never smokers at the point of baseline started smoking during the follow-up. As for body size, the mean values of standing height and body mass index (BMI) were comparable between the exposed and control groups.

The TPD and RCL workers tended to show higher percentile values both in the total and respirable TWA-8h than the DDM and MTN workers. As suggested by those summary percentile values, the individual TWA-8h values distributed across a rather wide range, and some of them exceeded the permissible limit recommended by JAIH (4 mg/m^3 as total and 1 mg/m^3 as respirable carbon black dust, and 8 mg/m^3 as total and 2 mg/m^3 as respirable general dust) [13]. However, the overall median TWA-8h values of both total and respirable dust were well below the JAIH recommendations, even among the TPD and RCL workers. Fig. 2 shows the time-dependent change in those summary statistics of personal exposure measurement during the study period. No marked trends were observed in the percentile values during the 10-year follow-up, suggesting that the work environment was relatively stable throughout the study period in terms of dust exposure to the workers as a whole.

Table 2 summarizes the prevalence of the biomedical indices studied at the baseline survey according to the exposure categories. The odds ratios (ORs) and their 95% confidence intervals (CIs) of the exposed to the control group are also shown after adjustment for possible confounding variables. The exposed group tended to show higher prevalence in respiratory symptom indices, although significant differences were observed only in chronic

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phlegm and wheeze grade 2. The prevalence values for CRP and IgE abnormalities were also

significantly larger in the exposed group than in the control group, although the results of

KL6 and SPD were comparable between the two groups. The difference in CRP remained

significant even after adjustment for BMI, a well-known contributor to the serum CRP level.

Only a small number of subjects demonstrated values outside of the reference ranges of FVC,

FEV1 or FEV1/FVC, and the prevalence showed values ranging from 2 to 6 %. There were

no cases of clear pneumoconiosis with 1/1 or a more severe grade on baseline chest X-ray

films of the subjects analyzed, although non-specific findings were noted in 3.4% of the

exposed and 1.8% of the control subjects at baseline. No significant difference between the

two groups was noted in the prevalence at baseline of either the pulmonary function or chest

X-ray indices.

Table 3 compares the frequency of newly emerging abnormalities observed during the

follow-up period between the exposed and control groups for selected biomedical indices in

order to examine the degree of chronic respiratory deterioration, including lung fibrotic

changes. This table also includes the results of comparisons according to work categories,

although no RCL workers were included in this analysis because the number of such workers

was too small; namely, only seven workers. After carefully evaluating the chest X-ray films

accumulated during the follow-up period, we observed no cases with 1/1 or a more severe

grade of pneumoconiosis. In addition, the incidence of non-specific findings during the

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follow-up was comparable between the exposed and control groups. Furthermore, two

clinically useful biomarkers of lung fibrosis (KL6 and SPD) showed rather low incidence

values for newly emerging abnormalities, which were comparable between the exposed and

control groups. However, regarding symptoms and the pulmonary function, the exposed

group tended to have a higher incidence of abnormalities more frequently than the control

group. The ORs estimated by logistic regression analyses mostly exceeded 1.5, and after

adjustment for age and smoking status during the follow-up period, chronic cough emerged

significantly more frequently in the exposed subjects than in the control group. In the

analyses according to work categories, an increase in the OR value of chronic cough was

observed in all exposed work categories that were analyzed, and reaching a statistically

significant level in DDM and MTN workers. The TPD, the highest exposure category, tended

to show a more consistent elevation of OR values versus the controls than other work

categories.

Table 4 shows the comparison of FVC/HT^2 and $FEV1/HT^2$ decline during the

follow-up period among the exposure categories. Comparable values were obtained for both

indices between the exposed as a whole and the control groups. After adjustment for the

significant contribution of age, toner exposure was not a significant factor influencing the

decline in the pulmonary function.

Table 5 summarizes the significant independent contribution of confounding factors in

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the multivariate analyses described in Tables 2 and 3 as estimated ORs for the health-related

outcomes. Only statistically significant results are presented with their 95% CIs. Smoking

was considered to increase the incidence of chronic phlegm, SPD abnormality, and reduced

FVC and FEV1 during follow-up, as well as the prevalence of many symptoms and a reduced

pulmonary function at baseline. Similar increases in ORs with age were also observed for the

incidence during the follow-up and the prevalence at baseline of some of the examined

indices. A clear independent association between serum CRP abnormalities and obesity was

also observed.

Discussion

Photocopiers and laser printers using powdered toner are widely used in offices and homes,

but the powdered toner for printing and emissions from these machines may become air

pollutants in office and home environments [16][17]. McGarry et al. reported the condition of

particulate air pollution resulting from the use of laser printers in several actual office

environments [18]. As several case reports have suggested the existence of chronic

pulmonary disorders possibly associated with inhaled toner dust [1][2][3], many aspects of

toner-related health effects have been reported. Khatri et al. [19] conducted a human

volunteer exposure study in an actual current office environment and found that several

biomarkers, including 8OHdG as a marker of oxidative stress [20], changed in relation with

the particulate air pollutants emitted by photocopiers and laser printers, although those

changes were acute or sub-acute and not chronic in nature. Elango et al. [21] reported the

result of a cross-sectional study on photocopier center workers in India, showing the

significant elevation of serum inflammatory biomarkers among the exposed workers

compared with the controls. Kasi et al. [22] suggested possible genotoxicity associated with

photocopier-related exposure in a survey of photocopier operators and maintenance workers.

Yanagi et al. [23] found no marked difference in the pulmonary function due to toner-related

work in their cross-sectional analysis of workers at a business equipment manufacturer. In

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their critical review, Pirela et al. [24] stated that particulate matter, including nanoparticles

emitted from photocopiers and laser printers during operations, could exert biological effects

on the respiratory system, such as oxidative stress and inflammatory responses.

Compared with human volunteer or cross-sectional epidemiological studies, the

evidence obtained by longitudinal epidemiological studies is quite limited, but longitudinal

study results are needed to fully examine the health impact of particulate air pollutants

associated with office machines, as far as chronic health effects are concerned. In a cohort

study on toner and photocopier manufacturing workers conducted over four years, no

evidence was obtained regarding the adverse effects on the pulmonary function or chest

X-ray findings associated with toner-handling work [25][26], although an increased

prevalence of breathlessness was observed in association with toner-handling work [27].

However, those studies did not evaluate the incidence of abnormalities based on

longitudinally observed data. Therefore, we conducted a longitudinal study to further

evaluate the possible health effects of toner dust exposure as a subsequent study of our

cross-sectional one mentioned above [4] with a focus mainly on the chronic respiratory

effects, such as lung fibrotic changes. To determine the actual risk in the current real-world

environment, we studied an actual working population. Personal exposure measurement

results showed that the current level of exposure to total and respirable dust in toner-handling

workers was on average well below the permissible exposure levels of general mineral dust

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and carbon black dust recommended by JAIH [13], even among TPD and RCL workers, in whom relatively high exposure was observed. We also used several biomedical indices to detect possible harmful effects on the respiratory system: namely imaging findings by chest X-ray, KL6 and SPD levels in serum as clinically utilized biomarkers on lung fibrosis, spirometry results to evaluate the lung functional status, and subjective symptoms obtained through standardized methods.

In a cross-sectional analysis of the baseline results, the exposed group tended to have subjective symptoms more frequently than in the control group, and similar group differences were observed in the frequency of abnormal values in the majority of the biomedical indices examined. In addition, the difference between the two groups reached a statistically significant level in several items, even after controlling for confounders. This tendency was basically consistent with what we had previously reported, with exposed workers complaining of symptoms more frequently than control workers despite having a comparable frequency of pulmonary function and chest radiography abnormalities. Although the presence of consistent difference between the groups might suggest the existence of certain subclinical changes, the cross-sectional evaluation at the baseline might have been influenced by a number of factors prior to the start of this study. Therefore, newly emerging abnormalities during follow-up should be examined in subjects without any abnormalities at the baseline observation, as far as the chronic health effects are concerned. One interesting finding of the

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cross-sectional analysis was the higher prevalence of CRP abnormalities, a sensitive marker

of acute inflammatory status in the body, among the exposed subjects compared with control

subjects. It seems unlikely that the elevated CRP values were significantly influenced by

events in the past due to its nature as an acute inflammatory biomarker. In addition, the

difference seemed to be independent of inflammatory conditions caused by obesity or

smoking, as both were used as adjustment factors in the logistic regression model. These

findings suggest that acute inflammatory conditions might be associated with current toner

exposure. At the same time, an increase in the serum IgE level was also frequently found in

the exposed subjects. In Japan, an allergy to cedar pollen is commonly observed every spring,

so cedar pollenosis may be related to the findings for CRP and IgE. However, as there are

multiple factors affecting the CRP [28] as well as the IgE levels, we were unable to specify

the reason for that group difference.

In the longitudinal analyses of biomedical indices, we evaluated the frequency of a

newly emerging abnormality during the follow-up period by excluding the subjects from the

analysis if they already had the finding at baseline. Among the indices associated with lung

fibrotic changes, we found closely similar results between the exposed and control groups

regarding the new incidence of chest radiographic findings and serum biomarker

abnormalities. These results suggest that the fibrogenic potential of dust exposure associated

with toner-handling work seems to be minimal, if any at all, as far as the current working

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environment is concerned.

In contrast, the estimated ORs for FEV1/FVC abnormality adjusted for confounders showed a relatively a large value exceeding 1.5, although the increase was not statistically significant. A statistically significant increase in the OR of TPD versus the controls was observed in FVC and FEV1 abnormalities. However, the overall differences in the incidence of reduced FVC and FEV1 were relatively small between the exposed subjects and control subjects. Furthermore, the annual declines in the FVC/HT^2 and $FEV1/HT^2$ of the exposed subjects during the follow-up period were comparable to those of the control subjects after controlling for the age and smoking status during the follow-up. Thus, it seems difficult to attribute certain biological or medical reasons to the moderate, but a insignificant elevation in ORs that was seen regarding the pulmonary function index abnormality. Some fluctuation around normal ranges might be partially responsible for the observed elevation in OR values. In the case of chronic cough, the exposed group showed a larger incidence than the control group. This finding was not associated with the findings for chronic phlegm, which showed a comparable incidence after adjusting for the significant contribution of smoking. This suggests that the significant increase in the rate of chronic cough cannot be attributed to mucus hypersecretion. Given the findings at baseline mentioned earlier, it might be possible to hypothesize that non-specific irritation leading to subjective symptoms, fluctuation in the pulmonary function, and inflammatory response were caused to some extent by exposure

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associated with toner-handling work.

However, of note: in all indices selected for the longitudinal incidence analyses, the OR value adjusted for confounders exceeded the unit value without exception, suggesting mostly non-significant but consistent elevation of the incidence in the exposed workers. Therefore, further epidemiological studies are needed in order to identify mild and nonspecific effects in a large-scale sample with sufficient statistical power. In this context, a similar tendency toward a consistent OR elevation was observed in TPD work, which was associated with the greatest exposure of total and respirable dust in the personal exposure measurement. However, it was difficult to make any conclusions regarding this consistency in relation to the toner exposure levels, because no information was obtained regarding the exact percentage of toner included in the total dust sampled. The toner particle exposure should be measured separately from other substances in future studies.

Several limitations associated with the present study warrant mention. First, the number of subjects of this study (694 in total) might be insufficient to detect minimal and subclinical health deteriorations, although this study seems to have reasonably sufficient power to detect at least well-established health risks, such as aging, smoking, and obesity. A meta-analysis of the studies with a common methodological basis will be useful for detecting minimal effects of toner dust. Second, we obtained measurements on personal exposure concentration for each work category as an exposure assessment. However, the results

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showed large variability. In addition, we were unable to obtain reliable data on the relative

content of toner as a fraction of the whole dust sample. We therefore cannot reliably estimate

the amount of personal exposure to toner dust for each individual subject. An analysis of the

dose-response relationship will require a more precise exposure assessment for toner dust.

Third, we simply utilized conventional chest radiography and did not use computed

tomography (CT) to evaluate lung fibrotic changes, because we should minimize the amount

of X-rays that the participants were exposed to in an epidemiological research setting. We

were therefore unable to distinguish the non-specific findings in cases where

extremely-early-phase of toner-related changes might have been included. Advanced

radiography techniques will be useful for the more precise evaluation of lung fibrotic

deterioration caused by toner dust inhalation.

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Conclusion

Under the current reasonably controlled work environmental conditions, lung fibrotic changes caused by inhaled dust exposure, including powdered toner, appear to be relatively uncommon; however, nonspecific temporal irritation causing subjective symptoms and inflammatory responses might exist. Further epidemiological research will be useful for clarifying the possible health effects of toner dust exposure fully, especially the minimal and non-specific health changes in a study setting with a sufficient sample size to detect these changes.

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Footnotes

Contributors: TN designed the study; TN and YY executed the data collection in annual surveys; YY, TY, SO and DN contributed to the data preparation and analyses; TN wrote the initial manuscript. All of the authors contributed to the revision of the manuscript and approved the final form of the manuscript for submission.

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Figure legends

Fig. 1 The number of subjects analyzed based on the inclusion criteria of this study

Fig. 2. The exposure levels of toner-handling workers by year of measurement estimated

by personal exposure measurements. The values are presented as the median and the 10th

and 90th percentiles (%ile) for personal exposure measurement data.

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Table 1. Background characteristics of the subjects according to the exposure category.

Category of exposure condition

Characteristics	Control	Exposed[*]					p[†]
		Total	TPD	DDM	MTN	RCL	
Number surveyed	217	477	175	145	150	7	
Sex (M/F)	205/12	461/16	167/8	141/4	149/1	4/3	0.18
Age (years)	39.1(8.2)	34.1(8.1)	32.9(10.3)	35.4(6.5)	34.2(6.3)	32.4(7.6)	<0.0001
Height (cm)	170.1(6.6)	170.3(6.4)	169.0(6.1	171.3(6.4)	171.4(6.4)	161.5(6.8)	0.63
BMI (kg/m^2)	23.0(3.1)	23.0(3.7)	23.2(4.6)	22.4(2.6)	23.4(3.2)	19.9(1.5)	0.999
Smoking habit[‡]							0.026
Never	70(32)	150(31)	38(22)	69(48)	38(25)	5(71)	
Former	47(22)	67(14)	17(10)	26(18)	24(16)	0	
Current	100(46)	260(55)	120(68)	50(34)	88(59)	2(29)	
Personal exposure (mg/m^3)							-
Number of samples		2042	1751	81	88	122	
Total dust							

10th % ile	0.08	0.09	0.04	0.07	0.51
Median	0.49	0.50	0.08	0.17	1.46
90th % ile	2.38	2.14	0.38	0.80	5.60
Respirable dust					
10th % ile	0.03	0.03	0.03	0.03	0.02
Median	0.05	0.09	0.03	0.03	0.04
90th % ile	0.36	0.35	0.04	0.15	0.64

* TPD: toner production, DDM: machine design and development, MTN: maintenance, RCL:

recycling, BMI: body mass index

†Probability under the hypothesis that there are no differences between the control group and

the exposed group as a whole.

‡Percentage of column total in parentheses.

Values are presented as the number of subjects for sex and smoking habit and the arithmetic

mean (standard deviations) for age, height, and BMI at the baseline survey.

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Table 2. The comparison of the baseline status in select biomedical indices at the point of the first examination for each subject between the exposed subjects as a whole and the controls

| Biomedical indices | Univariate analysis | | | Multivariate analysis | |
| | Prevalence (%) | | | Logistic regression model | |
	Exposed	Controls	p*	OR[†]	95%CI[†]
Respiratory symptoms[‡]					
Cough #1 (7A)	19.4	14.8	0.14	1.35	(0.85-2.14)
Cough #2 (7B)	14.8	11.1	0.19	1.41	(0.84-2.37)
Chronic cough (7E)	5.5	4.2	0.46	1.6	(0.70-3.64)
Phlegm #1 (8A)	23.7	20.4	0.34	1.23	(0.81-1.87)
Phlegm #2 (8B)	13.5	10.7	0.29	1.45	(0.85-2.48)
Chronic phlegm (8E)	8.9	4.2	0.03	2.62	(1.20-5.70)
Exacerbation (9A)	10.5	7	0.14	1.56	(0.84-2.91)
Wheeze #1 (10A1)	9.5	5.6	0.09	1.51	(0.76-2.99)
Wheeze #2[§] (10A2)	2.7	0	0.01	-	-
Chronic Wheeze (10A3)	0	0	-	-	-
Breathlessness #1 (13A)	9.7	11.6	0.45	1	(0.58-1.73)

Breathlessness #2[§] (13B)	2.2	1.4	0.76	2.27	(0.58-8.89)
Breathlessness #3[§] (13C)	0.4	0.9	0.59	0.96	(0.13-7.20)
Biomarker abnormality					
CRP	12.0	6.0	0.02	2.02	(1.05-3.88)
IgE	31.7	20.8	0.003	1.66	(1.12-2.48)
8OHdG[§]	1.1	1.8	0.47	0.68	(0.17-2.78)
KL6[§]	0.4	0	1	-	-
SPD	3.2	3.4	0.89	0.91	(0.35-2.37)
Reduced pulmonary function					
FVC	6.5	6.0	0.76	1.00	(0.49-2.00)
FEV1	6.1	2.8	0.06	2.09	(0.83-5.27)
FEV1/FVC[§]	2.1	5.1	0.03	0.44	(0.18-1.09)
Chest X-ray findings					
Fibrotic change $\geq$1/1	0	0	1	-	-
Non-specific findings	3.4	1.8	0.27	2.47	(0.78-7.81)

* Probability under the hypothesis that there are no differences between the control group and exposed group as a whole examined by the chi-squared test or Fisher's exact probability method.

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† Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the

control group, regarding the positive findings at the baseline survey

‡ The ATS-DLD-78A questionnaire code is shown in the parentheses

§ Fisher's exact probability method was applied.

Grade of symptom severity: cough #1 and phlegm #1, usually having the symptom; cough

#2 and phlegm #2, 4 to 6 times a day on 4 or more days a week; wheeze #1, when having a

cold; wheeze #2, occasionally apart from cold; breathlessness #1, when hurrying on the level

or walking up a slight hill; breathlessness #2, having to walk slower than people your age on

the level; breathlessness #3, having to stop for breath when walking at your own pace.

The figures in the column of prevalence represent the proportion of subjects with symptoms

or positive findings in each index. The results of multivariate analyses were adjusted for the

age and smoking status of the subjects. Body mass index was also used as an adjustment

factor in the case of CRP.

Table 3. A comparison of the cumulative frequency of incidence in select biomedical indices associated with lung fibrotic changes that newly emerged during the follow-up period

	Univariate analysis			Multivariate analysis			
	Incidence			Logistic regression model			
	Total			Total	TPD	DDM	MTN
	Exposed	Controls	*p**	OR(95%CI)[†]	OR (95%CI)[†]	OR (95%CI)[†]	OR (95%CI)[†]
Respiratory symptoms[‡]							
Chronic cough (7E)	11.3	6.3	0.04	2.26(1.13-4.53)	1.88(0.82-4.31)	2.70(1.21-5.99)	2.31(1.01-5.25)
Chronic phlegm (8E)	14.0	9.6	0.12	1.6(0.91-2.83)	1.46(0.74-2.88)	1.65(0.81-3.37)	1.68(0.83-3.40)

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Breathlessness #2 (13B)	6.0	4.7	0.48	1.69(0.73-3.94)	1.51(0.54-4.19)	0.94(0.27-3.25)	2.62(1.00-6.87)
Serum biomarkers							
KL6§	2.1	1.8	1.00	1.27(0.38-4.29)	2.09(0.54-8.05)	0.44(0.05-4.09)	1.25(0.26-5.94)
SPD	2.4	2.4	1.00	1.06(0.34-3.28)	0.87(0.21-3.54)	1.62(0.40-6.48)	0.94(0.21-4.24)
Pulmonary function							
FVC	15.3	13.2	0.50	1.22(0.73-2.05)	2.60(1.44-4.71)	0.54(0.24-1.21)	0.84(0.42-1.68)
FEV1	11.4	8.5	0.26	1.38(0.76-2.49)	2.43(1.24-4.76)	0.68(0.27-1.69)	1.19(0.55-2.57)
FEV1/FVC	9.9	6.8	0.20	1.59(0.81-3.09)	1.88(0.87-4.09)	1.16(0.48-2.85)	1.72(0.77-3.85)
Chest X-ray							
Non-specific findings	5.6	5.2	0.8	1.39(0.65-2.97)	1.38(0.53-3.59)	1.75(0.70-4.37)	0.98(0.34-2.80)

TPD: toner production, DDM: machine design and development, MTN: maintenance, RCL: recycling

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* Probability under the hypothesis that there are no differences between the control group and exposed group as a whole examined by the

chi-squared test or Fisher's exact probability method

† Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the control group, retarding the newly emerging

abnormalities of biomedical indices

‡ The ATS-DLD-78A questionnaire code is shown in the parentheses.

§ Fisher's exact probability method was applied.

\#   Grade of the severity of the symptom

The figures in the column of incidence represent the proportion of subjects starting to show symptoms or positive findings during the follow-up

period among those who did not have the symptom or positive finding at baseline. The results of multivariate analyses were adjusted for the age

and smoking status of the subjects.

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Table 4. (New) Annual decline in spirometric indices according to exposure category.

	Category of exposure condition (number of subjects)						Statistical test of the mean values†	
	Control	Exposed*						
		Total	TPD	DDM	MTN	RCL	*p*	
Spirometric indices	(N=217)	(N=477)	(N=175)	(N=145)	(N=150)	(N=7)	Unadjusted	Adjusted‡
Δ FVC/HT^2 (ml/m^2)	-6.2(21.4)	-4.0(19.1)	-0.7(25.8)	-2.5(12.5)	-9.0(14.3)	-5.8(13.3)	0.19	0.50
Δ FEV1/HT^2 (ml/m^2)	-8.8(16.3)	-7.5(13.9)	-6.5(16.3)	-7.3(10.7)	-9.0(12.5)	-6.7(32.6)	0.33	0.30

* TPD: Toner production, DDM: Machine design and development, MTN: Maintenance, RCL: Recycling

† Comparison between the control and the exposed as a whole

‡ Adjusted for age, sex, and smoking habit at the baseline.

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Values are the arithmetic means and standard deviations at the point of baseline survey.

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Table 5. Independent contribution of confounding factors in the multivariate analyses of biomedical indices

	Smoking		Age		BMI
	Prevalence[*]	Incidence[†]	Prevalence[*]	Incidence[†]	Prevalence[*]
	OR(95% CI)[‡]	OR(95% CI)[‡]	OR(95%Cis)[‡]	OR(95% CI)[‡]	OR(95% CI)[‡]
Cough #1 (7A)[§]	1.68(1.12-2.51)				
Cough #2 (7B)[§]	1.62(1.03-2.54)				
Chronic cough (7E)[§]	3.34(1.49-7.48)		1.63(1.08-2.45)		
Phlegm #1 (8A)[§]	2.38(1.63-3.47)				
Phlegm #2 (8B)[§]	2.62(1.60-4.29)		1.38(1.05-1.82)		
Chronic phlegm (8C)[§]	3.69(1.85-7.35)	1.80(1.10-2.96)	1.53(1.08-2.16)		

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Breathlessness #1 (13A)[§]   1.59(1.17-2.16)

Breathlessness #2†(13B)[§]   2.64(1.36-5.12)

Breathlessness #3†(13C)[§]   5.62(1.51-20.85)

CRP abnormality   1.32(1.17-1.49)

5   SPD abnormality   4.10(1.15-14.58)

Reduced FVC   3.13(1.09-8.97)

Reduced FEV1   3.82(1.38-10.58)

Reduced FEV1/FVC   3.92(1.20-12.87)2.11(1.27-3.52)

Non-specific X-ray   1.59(1.06-2.38)

10   findings

* An analysis of the prevalence at baseline.

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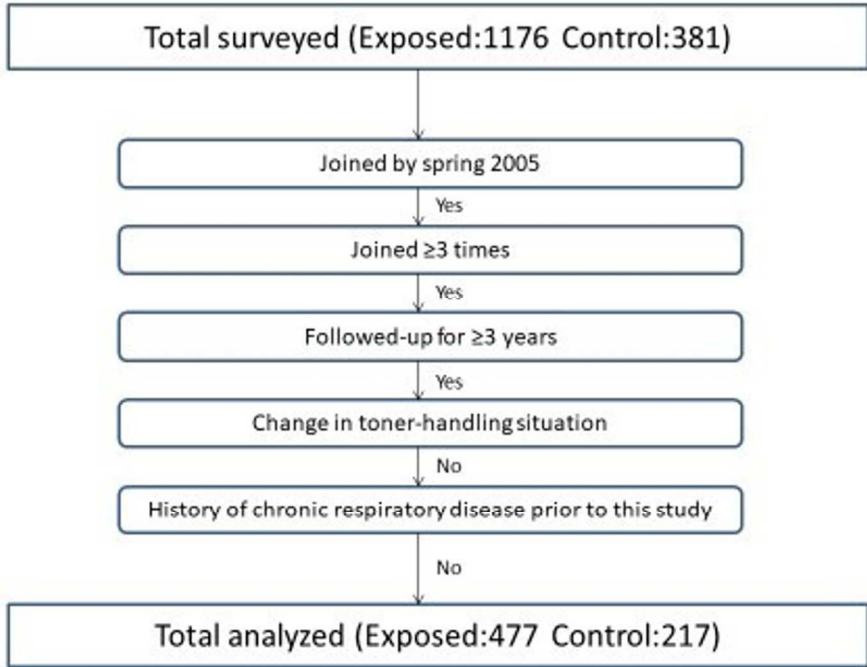† An analysis of the new incidence during the follow-up observation

‡Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the control group, regarding the prevalence and incidence

of biomedical indices.
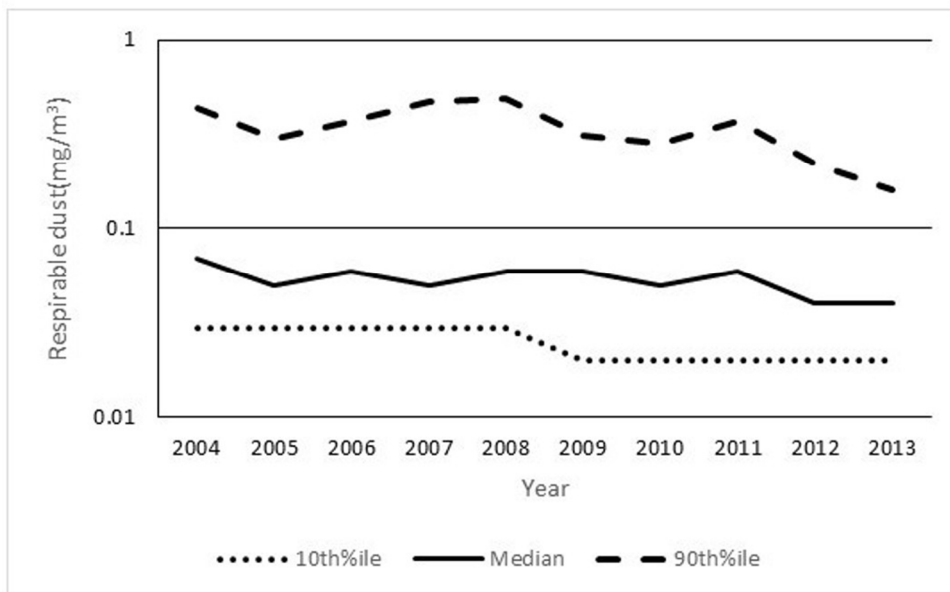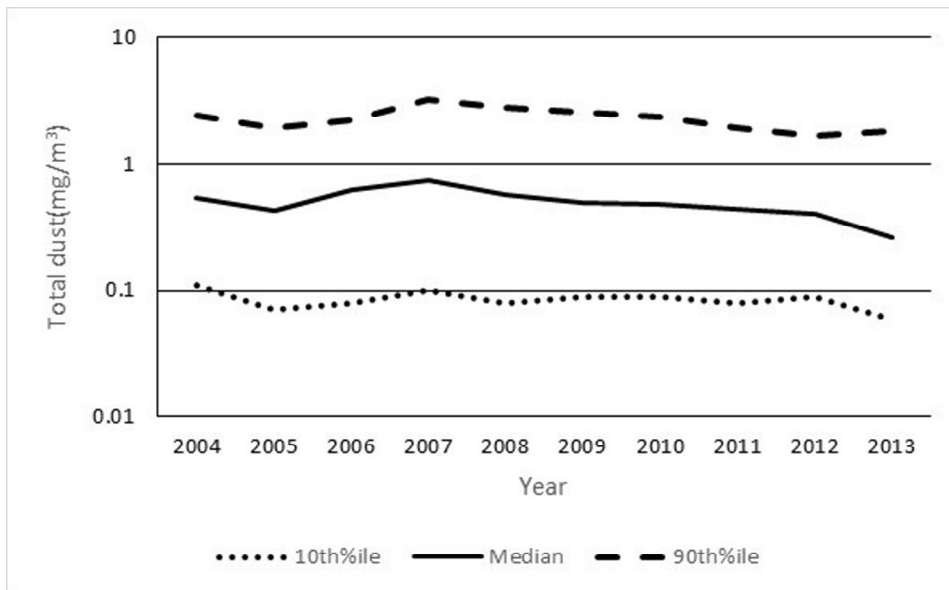
§ The ATS-DLD-78A questionnaire code is shown in the parentheses.

5 # Grade of the severity of the symptom

Values are presented as the OR (95% CI) estimated using the logistic regression models shown in Tables 3 and 4. Only statistically significant

results are shown.

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Total surveyed (Exposed:1176 Control:381)

Joined by spring 2005

Yes

Joined ≥3 times

Yes

Followed-up for ≥3 years

Yes

Change in toner-handling situation

No

History of chronic respiratory disease prior to this study

No

Total analyzed (Exposed:477 Control:217)

119x90mm (300 x 300 DPI)

90x112mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6-7
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8-10
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10-15
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*10-15*
Bias	9	Describe any efforts to address potential sources of bias	9, 10-13
Study size	10	Explain how the study size was arrived at	8-9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	15-17
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	17
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	8-9
		(*e*) Describe any sensitivity analyses	22-23, 48-50
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8-9, 19
		(b) Give reasons for non-participation at each stage	8-9, 19
		(c) Consider use of a flow diagram	51(Fib.1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	19-20, 38-39, 52(Fig.2)
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	19
Outcome data	15*	Report numbers of outcome events or summary measures over time	21-22, 43-47
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	21-22, 43-47
		(*b*) Report category boundaries when continuous variables were categorized	12, 14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	22-23, 43-45, 48-50
Discussion			
Key results	18	Summarise key results with reference to study objectives	27, 31
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	29-30
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	32

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Assessing the chronic respiratory health risk associated with inhalation exposure to powdered toner for printing in actual working conditions: A cohort study on occupationally exposed workers over 10 years

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Assessing the chronic respiratory health risk associated with inhalation exposure to powdered toner for printing in actual working conditions: A cohort study on occupationally exposed workers over 10 years

Toshio Nakadate(1), Yuko Yamano(1), Takenori Yamauchi(1), Shigeko Okubo(1), Daichi Nagashima(1)

(1) Department of Hygiene, Public Health, and Preventive Medicine, Showa University School of Medicine, Tokyo, Japan

Corresponding author: Toshio Nakadate

Department of Hygiene, Public Health, and Preventive Medicine,

Showa University School of Medicine

1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

e-mail: nakadate@med.showa-u.ac.jp

Tel: +81-3-3784-8137   Fax: +81-3-3784-8251

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Abstract: 276

Text (Introduction, Methods, Results, Discussion, Conclusion): 5259

1

Table: 5, Figure 2

References: 28

2

Abstract

Background: Little epidemiological evidence exists regarding the chronic respiratory effects

of inhaled powdered toner exposure in humans, although several case reports have suggested

the existence of lung disorders that might be related to exposure to toner dust.

Objective: We aimed to estimate the chronic health risk to humans associated with routine

toner dust exposure in copier-industry workers under current actual work conditions.

Design: A prospective observational cohort study of occupational population.

Methods: Changes in chest radiogram, spirometry measurements, and serum and urine

biomarkers of biomedical responses to extrinsic stress, as well as subjective symptoms were

longitudinally observed for up to 10 years in Japanese copier-industry workers responsible

for the manufacturing, maintenance or recycling of powdered toner or toner-using machines.

A total of 694 subjects who did not change their work category during the follow-up and were

free from chronic respiratory diseases at the baseline survey provided reliable results on at

least 3 survey occasions during 3 years or more of follow-up.

Results: Typical fibrosis findings associated with pneumoconiosis was not observed on chest

radiograms. No significant differences associated with toner exposure were noted in the

frequency of new incidence of either non-specific findings on chest radiogram or serum

fibrosis biomarkers (KL6 and SPD). However the exposed subjects tended to show increases

in the frequency of respiratory symptoms and reduced spirometry results during the follow-up

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compared with the control group, although significant differences were only seen in chronic

cough.

Conclusions: Under the current reasonably controlled work environmental conditions, lung

fibrotic changes caused by inhaled dust exposure, including powdered toner, appear to be

relatively uncommon; however, nonspecific temporal irritation causing subjective symptoms

and inflammatory responses might exist.

Key words: Dust, Longitudinal studies, Respiratory, Epidemiology

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Strengths and limitations of this study

- An occupational cohort of workers in business machine industry exposed to printing toner dust in various types of work was followed for up to 10 years regarding their respiratory health status on an annual basis, mainly focusing on lung fibrotic changes.

- The incidence of newly emerging lung fibrotic changes was compared between the exposed workers and the non-exposed controls using wide-range health outcomes including chest X-ray findings, lung function results, and serum and urine biomarkers, as well as subjective symptoms.

- The total dust exposure during toner handling was measured in a representative sample of workers, but the fraction of toner particles in the whole dust exposure cannot be estimated accurately.

- Conventional chest radiography was utilized to evaluate lung fibrotic changes in an epidemiological research setting; however, computed tomography (CT) might have been more sensitive to early minimal fibrotic changes.

- Healthy worker bias might exist because some portion of the cohort ceased participation during the follow-up due to resigning, retiring, or transferring, although no toner-related health problems, such as lung fibrosis, were reported among these subjects.

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Introduction

Possible harmful effects of inhaled photocopier toner dust have been a matter of concern

since the publication of several case reports suggesting the existence of toner-related

pulmonary disorders [1][2][3]. However, little evidence is available regarding the potential

for toner dust inhalation in occupational and general home environments where photocopier

machines and laser printing devices are in use.

We reported the results of a cross-sectional study of toner-handling workers engaged

in several types of toner exposure work [4]. Although the results suggested the limited

possibility of adverse effects due to toner dust inhalation, it was also suggested that a study

with longitudinal design would be useful for a more detailed examination of the hypothesized

association between toner dust exposure and adverse health effects under current actual work

conditions.

We therefore conducted a longitudinal study of toner-handling workers for 10 years in

a business machine-producing company and affiliated companies involved in maintaining and

recycling these machines in order to obtain longitudinal evidence to determine a possible

health risk associated with a practical level of toner dust exposure under current actual work

conditions, with particular focus on chronic fibrotic changes in the lungs.

This is the first report of this study and mainly presents the results of analyses of the

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possible chronic health effects, including lung fibrotic changes as the principal research target,

associated with toner dust exposure under current actual work conditions based on

longitudinally observed health data.

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Methods

Subjects

The subjects were employees of seven branches of a group of companies that produced, maintained, and recycled photocopiers, laser printers, and related products for printing with the use of powdered toner. We identified four categories of toner-handling work among the subjects. Toner production (TPD) represents work associated with any process in the course to producing powdered toner, such as mixing and molding raw materials, crushing toner mass, and filling and packing the products. Designing and development of machines (DDM) represents work associated with developing new models of toner-using business machines, in which various types of toner-handling procedures are involved. Maintenance (MTN) represents the work associated with maintaining photocopiers and laser printers in users' offices and homes. Recycling (RCL) represents work associated with processing recycled toner cartridges. The air environment was generally well-maintained using local ventilation instruments according to the regulations in Japan. The workers were therefore encouraged to wear respiratory protection masks in only limited situations where heavy exposure might occur, such as when cleaning the mixing tanks or performing maintenance on/repairing equipment.

The survey on the health status of the subjects started in the fall of 2003 and was

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repeated until 2013 basically on an annual basis in the same manner as the first one in 2003.

Throughout the entire study period, a total of 1176 toner handling workers (587 for TPD, 207

for DDM, 346 for MTN, and 36 for RCL) participated in at least one of these surveys.

However, some participants ceased participation during an early stage of the study for various

reasons other than toner-related health problems (e.g. resigning, retiring, or transferring), and

others started participating in a later stage of the study. Therefore, we defined the subjects

with a sufficient follow-up duration for a longitudinal data analysis in this paper as those who

started participating by the spring of 2005 and were followed-up for three years or more with

at least three surveys throughout the entire study period. In addition, subjects were those who

had not changed their work category (concerning the presence or absence of toner handling)

during their follow-up. The flowchart in Fig. 1 shows the number of participants included in

or excluded from the analysis based on the above-mentioned criteria.

The number of toner workers who met the above criteria was 487. As a reference

population for comparison with these toner-handling workers, an additional 309 control

subjects who had never participated in any types of toner-handling work described above also

participated in this study by the spring of 2005. Among them, the 223 who met the same

criteria as exposed workers stated above were analyzed. Control subjects were recruited at

each participating branch and amounted to roughly half the number of toner-handling

workers. The control subjects basically worked in different buildings from the ones where the

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exposed workers were engaged in toner-handling work. While the control subjects

occasionally happened to enter the factory area, such accidental exposure was quite limited.

Health status indices

Subjects' health status was evaluated on an annual basis from various health-related aspects

using physiological, biochemical, and radiographic examination tools as well as via the

assessment of subjective symptoms and clinical signs. This evaluation focused mainly on

chronic respiratory deterioration, including pulmonary fibrotic conditions, and biological

responses to inhaled potentially harmful substances. Standardized methods were utilized for

health status measurement to keep the measurement bias as small as possible. The biomedical

indices examined in this study are described below.

Chest X-ray findings

Chest radiography was carried out to obtain a standardized anterior-posterior X-ray film by

using prescribed standard procedures to detect mineral-dust pneumoconiosis in Japan [5].

Each X-ray film was read by one of three readers with sufficient experience in the

radiographic diagnosis of pneumoconiosis while referencing the standard films for the

diagnosis [6]. The readers were privy to no information regarding the exposure status of the

subjects. The severity of fibrotic findings in the lung fields was classified into 12 grades,

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from 0/- to 3/+, according to the distribution and density of the small opacities. This classification was compatible with that of the International Labor Organization (ILO) [7]. However, we noted none of the fibrotic findings typically observed in mineral dust pneumoconiosis cases with 1/1 or a more severe grade profusion during early surveys. Therefore, we additionally recorded mild, non-specific findings, such as sparse irregular opacity, unclear interstitial shadow, or disrupted vascular shadow in radiographs. These findings were not only potentially related to early effects of toner toxicity but also might be caused by acute or sub-acute non-specific irritation or infection, which might be reversible.

Questionnaire survey

Detailed information regarding respiratory symptoms, allergic symptoms, past and present medical history, and lifetime smoking history was obtained using a translated version of a self-administered questionnaire standardized by the American Thoracic Society (ATS-DLD-78A) [8], with slight modification and several additional questions on allergies of the eye, nose, and skin. The responses were certified by several trained interviewers, and supplemental interviews were held when necessary. Subjects were considered to have a symptom when they gave an affirmative response to questions about the specific symptom. The lifetime work history was also confirmed during this interview.

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Spirometry

Subjects were asked to repeat the forced expiratory maneuver up to a maximum of five times

in the standing position in order to obtain acceptable and reproducible spirometry results. The

mechanical specifications of the spirometer used (DISCOM-21FX2; CHEST Co. Ltd., Tokyo,

Japan) met the standards stipulated by ATS [9]. Routine BTPS correction and

back-extrapolation were carried out. The calibration of the spirometer, spirometry

measurements, and evaluation of the results were based on prescribed procedures described

in detail elsewhere [10].

The percent predicted values standardized on sex, age, and height were calculated for

forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and FEV1/FVC

in each subject based on the prediction equations published by the Japanese Respiratory

Society [11]. The values of FVC, FEV1, and FEV1/FVC were defined as reduced when they

were smaller than the lower value of the confidence interval of the authorized reference

equations described above. All figures were calculated from the best maneuver yielding the

largest sum of FVC and FEV1 for each subject [12].

For the analysis of the longitudinal changes in spirometry indices, we calculated the

height-squared proportional values of FVC (FVC/HT^2) and FEV1 ($FEV1/HT^2$) to adjust for

differences in the body size. The annual decline in FVC/HT^2 and $FEV1/HT^2$ were calculated

for each subject as a regression coefficient using a simple linear regression equation of those

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values against age during the follow-up period.

Serum and urine biomarkers

Peripheral venous blood and urine (10 and 20 ml, respectively) were obtained from each

subject at the time of every survey. Serum was immediately separated from whole blood

sample using a routine procedure. Then, serum and urine samples were deeply frozen at

-80 °C until analyses were carried out.

As the indices of biological responses to inhaled extrinsic irritants like toner particles,

we employed the following biomarkers: highly sensitive C-reactive protein (CRP),

non-specific immunoglobulin E (IgE), sialylated carbohydrate antigen KL-6 (KL6), and lung

surfactant protein D (SPD) in serum, and 8-hydrovy deoxy guanosine (8OHdG) in urine. In

Japan, serum CRP, IgE, KL6, and SPD levels are routine clinical laboratory test items

assessed in common medical practice. The analyses of those results were therefore carried out

according to the appropriate Standard Operation Procedures (SOP) in SRL Inc., Tokyo.,

Japan which has been providing high quality testing under the certification of the Japan

Accreditation Board, a public service juridical foundation for ensuring the reliability of

clinical laboratory testing in Japan.

Urine 8OHdG was measured using an ELISA kit (New 8OHdG Check ELISA Kit;

Japan Institute for the Control of Aging) and was adjusted for urine condensation using the

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urine creatinine concentration as the denominator.

The result of the measurement of each index was judged to be out of the normal range

when the value exceeded the following figures: 1500 ng/ml for CRP, 173 IU/ml for IgE,

500 U/ml for KL6, 110 ng/ml for SPD, and 20 ng/mg creatinine for 8OHdG.

Exposure assessment

We directly measured the personal exposure levels in individual toner-handling workers.

The average diameter of the toner particles contained in the products handled by the workers

in this study was about 10 μm. Workers were asked to wear a portable personal dust sampler

(NWPS-254 sampler with a T60A20 sampling filter, SIBATA SCIENTIFIC TECHNOLOGY

Ltd., Tokyo, Japan) that continuously sampled the air in the workplace environment at 2.5

L/min air flow driven by a MP-2N or MP-3Σ pump (SIBATA SCIENTIFIC TECHNOLOGY

Ltd., Tokyo, Japan). The sampling head was located around the neck of each subject to

collect particles suspended in the air throughout a given period of toner-related work. The

respirable fraction was separated using filters designed to cut 50% of 4-μm-diameter particles.

The concentration of total and respirable dust exposed to the workers was calculated as a

time-weighted average value for 8 h (TWA-8h) based on the amount of dust collected on the

filter and the total volume of air inspired by the sampling pump. Measurements were

conducted basically twice a year for at least 5 workers in each toner-handling procedure,

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yielding a total of 2042 measurements (1751 for TPD, 81 for DDM, 88 for MTN, and 122 for

RCL) throughout the study period. Those values obtained were evaluated with reference to

the recommendations by the Japan Association of Industrial Health (JAIH) [13]. We also

analyzed several chemical components that were generated when the toner was heated at a

temperature of around 180 degrees centigrade as representative components of powdered

toner using gas chromatography and mass spectrometry (GC-MS) in order to estimate the

toner exposure separately from other types of dusts suspended in the air. However, we were

unable to obtain reliable estimates of the amount of toner dust fraction in the whole dust

cluster collected on the sampling filter, mainly because of large variability of the values

depending on the types of toner handled.

Analyses

- Cross-sectional evaluation of baseline data

For each subject, we considered the results obtained in the first year of participation as the

baseline data, indicating the condition at the beginning of the longitudinal observation of this

study. The relative frequency of subjects with positive results was calculated for each

health-related item described above (prevalence) and compared statistically between the

exposed subjects as a whole and the control subjects. The significance of differences in the

prevalence between the exposed subjects and controls was assessed using a chi-squared test

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and Fisher's exact test as appropriate. A multiple logistic regression analysis was used to

obtain the OR estimates adjusted for possible confounding factors.

- Longitudinal evaluation of chronic fibrotic findings

For the longitudinal analysis, we mainly focused on the results associated with chronic

respiratory toxicity, including the fibrotic potential of toner dust inhalation exposure. To this

end, we analyzed the changes in chest radiographic findings during the follow-up period. We

also used three pulmonary function indices (FVC, FEV1, and FEV1/FVC) and two serum

biomarkers (KL6 and SPD, both of which are recognized as useful markers of active-phase

fibrotic conditions in the lungs) [14]. In addition, we selected three chronic respiratory

symptoms associated with chronic respiratory diseases, such as fibrotic lung changes, namely

chronic cough, chronic phlegm, and breathlessness. Chronic cough, as well as chronic

phlegm, was considered to be present when the symptom existed for three months or more in

a year. Breathlessness was considered to be present when a subject walked slower than their

counterparts of the same age on level ground.

In the longitudinal data analysis, we evaluated the frequency of abnormalities that

newly emerged during the follow-up observation (incidence). Abnormalities were considered

to be present when the respiratory symptom or the X-ray findings were newly noted or when

the pulmonary function or biomarker values exceeded the normal range during follow-up.

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Therefore, those subjects who already had abnormalities at their baseline survey were

excluded from the longitudinal data analysis. The incidence was compared between the

exposed workers as a whole and the control subjects, as well as by the work categories. In a

similar manner to the cross-sectional analyses, the incidence was compared using a

chi-squared test or Fisher's exact test as appropriate, and the OR estimates adjusted for

confounders were calculated using a multiple logistic regression analysis.

Statistical analyses

For comparisons by exposure categories, the chi-squared test and Fisher's exact test as well as

a multiple logistic regression analysis were performed for categorical variables, and a simple

t-test and an analysis of covariance were performed for numeric variables, with the alpha

error level set at 0.05. All statistical tests and estimations were carried out using the SAS

statistical package on a personal computer (PC-SAS Version 9.2; SAS Institute Inc., Cary, NC,

USA).

Ethical considerations

The objectives and outline of the study were explained to the subjects at the beginning of the

study, and written informed consent was obtained from each participant. All information

obtained in this study was handled in accordance with the guidelines for epidemiological

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studies authorized in Japan [15]. This study was conducted under the approval of the

Institutional Review Board on Medical Ethics of Showa University School of Medicine

(Authorization No. 201).

Patient involvement

Patients were not involved in this study.

Results

As stated earlier, we selected 710 workers (489 exposed and 221 controls) as the subjects for

this longitudinal data analysis. Among them, 16 subjects reported that they had suffered from

chronic respiratory diseases prior to the beginning of this study (chronic obstructive

pulmonary disease and/or pulmonary tuberculosis). These subjects were therefore excluded,

and the remaining 694 subjects were ultimately analyzed (Fig. 1). The average number of

surveys in which those subjects participated was 6.8, and the average follow-up duration

(elapsed time from the first observation to the final one) was 7.4 years. No significant

difference was observed in those average values between the exposed and control groups.

Several background characteristics at the baseline point, as well as the results of

personal exposure measurements during the entire study period as the 10th and 90th

percentile values of the TWA-8h concentration, were compared among the exposure

categories in Table 1. The male to female ratio was comparable between the exposed and

control groups. However, the absolute number of female subjects was quite small compared

to males, with only 28 women analyzed. Significant differences were observed between the

exposed and control groups in mean age and smoking habit classification at the baseline

survey, with the exposed group being younger and more likely to smoke than the controls.

Seventy-six of 360 current smokers at baseline quit smoking during their follow-up period,

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whereas only 1 out of 220 never smokers at the point of baseline started smoking during the follow-up. As for body size, the mean values of standing height and body mass index (BMI) were comparable between the exposed and control groups.

The TPD and RCL workers tended to show higher percentile values both in the total and respirable TWA-8h than the DDM and MTN workers. As suggested by those summary percentile values, the individual TWA-8h values distributed across a rather wide range, and some of them exceeded the permissible limit recommended by JAIH (4 mg/m^3 as total and 1 mg/m^3 as respirable carbon black dust, and 8 mg/m^3 as total and 2 mg/m^3 as respirable general dust) [13]. However, the overall median TWA-8h values of both total and respirable dust were well below the JAIH recommendations, even among the TPD and RCL workers. Fig. 2 shows the time-dependent change in those summary statistics of personal exposure measurement during the study period. No marked trends were observed in the percentile values during the 10-year follow-up, suggesting that the work environment was relatively stable throughout the study period in terms of dust exposure to the workers as a whole.

Table 2 summarizes the prevalence of the biomedical indices studied at the baseline survey according to the exposure categories. The odds ratios (ORs) and their 95% confidence intervals (CIs) of the exposed to the control group are also shown after adjustment for possible confounding variables. The exposed group tended to show higher prevalence in respiratory symptom indices, although significant differences were observed only in chronic

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phlegm and wheeze grade 2. The prevalence values for CRP and IgE abnormalities were also

significantly larger in the exposed group than in the control group, although the results of

KL6 and SPD were comparable between the two groups. The difference in CRP remained

significant even after adjustment for BMI, a well-known contributor to the serum CRP level.

Only a small number of subjects demonstrated values outside of the reference ranges of FVC,

FEV1 or FEV1/FVC, and the prevalence showed values ranging from 2 to 6 %. There were

no cases of clear pneumoconiosis with 1/1 or a more severe grade on baseline chest X-ray

films of the subjects analyzed, although non-specific findings were noted in 3.4% of the

exposed and 1.8% of the control subjects at baseline. No significant difference between the

two groups was noted in the prevalence at baseline of either the pulmonary function or chest

X-ray indices.

Table 3 compares the frequency of newly emerging abnormalities observed during the

follow-up period between the exposed and control groups for selected biomedical indices in

order to examine the degree of chronic respiratory deterioration, including lung fibrotic

changes. This table also includes the results of comparisons according to work categories,

although no RCL workers were included in this analysis because the number of such workers

was too small; namely, only seven workers. After carefully evaluating the chest X-ray films

accumulated during the follow-up period, we observed no cases with 1/1 or a more severe

grade of pneumoconiosis. In addition, the incidence of non-specific findings during the

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follow-up was comparable between the exposed and control groups. Furthermore, two

clinically useful biomarkers of lung fibrosis (KL6 and SPD) showed rather low incidence

values for newly emerging abnormalities, which were comparable between the exposed and

control groups. However, regarding symptoms and the pulmonary function, the exposed

group tended to have a higher incidence of abnormalities more frequently than the control

group. The ORs estimated by logistic regression analyses mostly exceeded 1.5, and after

adjustment for age and smoking status during the follow-up period, chronic cough emerged

significantly more frequently in the exposed subjects than in the control group. In the

analyses according to work categories, an increase in the OR value of chronic cough was

observed in all exposed work categories that were analyzed, and reaching a statistically

significant level in DDM and MTN workers. The TPD, the highest exposure category, tended

to show a more consistent elevation of OR values versus the controls than other work

categories.

Table 4 shows the comparison of FVC/HT^2 and $FEV1/HT^2$ decline during the

follow-up period among the exposure categories. Comparable values were obtained for both

indices between the exposed as a whole and the control groups. After adjustment for the

significant contribution of age, toner exposure was not a significant factor influencing the

decline in the pulmonary function. In the comparison among the three exposed work

categories the MTN workers showed a larger annual decline in both FVC/HT^2 and $FEV1/HT^2$

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than TPD and DDM workers, although the differences were not statistically significant after

adjusting for age.

Table 5 summarizes the significant independent contribution of confounding factors in

the multivariate analyses described in Tables 2 and 3 as estimated ORs for the health-related

outcomes. Only statistically significant results are presented with their 95% CIs. Smoking

was considered to increase the incidence of chronic phlegm, SPD abnormality, and reduced

FVC and FEV1 during follow-up, as well as the prevalence of many symptoms and a reduced

pulmonary function at baseline. Similar increases in ORs with age were also observed for the

incidence during the follow-up and the prevalence at baseline of some of the examined

indices. A clear independent association between serum CRP abnormalities and obesity was

also observed.

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Discussion

Photocopiers and laser printers using powdered toner are widely used in offices and homes, but the powdered toner for printing and emissions from these machines may become air pollutants in office and home environments [16][17]. McGarry et al. reported the condition of particulate air pollution resulting from the use of laser printers in several actual office environments [18]. As several case reports have suggested the existence of chronic pulmonary disorders possibly associated with inhaled toner dust [1][2][3], many aspects of toner-related health effects have been reported. Khatri et al. [19] conducted a human volunteer exposure study in an actual current office environment and found that several biomarkers, including 8OHdG as a marker of oxidative stress [20], changed in relation with the particulate air pollutants emitted by photocopiers and laser printers, although those changes were acute or sub-acute and not chronic in nature. Elango et al. [21] reported the result of a cross-sectional study on photocopier center workers in India, showing the significant elevation of serum inflammatory biomarkers among the exposed workers compared with the controls. Kasi et al. [22] suggested possible genotoxicity associated with photocopier-related exposure in a survey of photocopier operators and maintenance workers. Yanagi et al. [23] found no marked difference in the pulmonary function due to toner-related work in their cross-sectional analysis of workers at a business equipment manufacturer. In their critical review, Pirela et al. [24] stated that particulate matter, including nanoparticles

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emitted from photocopiers and laser printers during operations, could exert biological effects

on the respiratory system, such as oxidative stress and inflammatory responses.

Compared with human volunteer or cross-sectional epidemiological studies, the

evidence obtained by longitudinal epidemiological studies is quite limited, but longitudinal

study results are needed to fully examine the health impact of particulate air pollutants

associated with office machines, as far as chronic health effects are concerned. In a cohort

study on toner and photocopier manufacturing workers conducted over four years, no

evidence was obtained regarding the adverse effects on the pulmonary function or chest

X-ray findings associated with toner-handling work [25][26], although an increased

prevalence of breathlessness was observed in association with toner-handling work [27].

However, those studies did not evaluate the incidence of abnormalities based on

longitudinally observed data. Therefore, we conducted a longitudinal study to further

evaluate the possible health effects of toner dust exposure as a subsequent study of our

cross-sectional one mentioned above [4] with a focus mainly on the chronic respiratory

effects, such as lung fibrotic changes. To determine the actual risk in the current real-world

environment, we studied an actual working population. Personal exposure measurement

results showed that the current level of exposure to total and respirable dust in toner-handling

workers was on average well below the permissible exposure levels of general mineral dust

and carbon black dust recommended by JAIH [13], even among TPD and RCL workers, in

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whom relatively high exposure was observed. We also used several biomedical indices to detect possible harmful effects on the respiratory system: namely imaging findings by chest X-ray, KL6 and SPD levels in serum as clinically utilized biomarkers on lung fibrosis, spirometry results to evaluate the lung functional status, and subjective symptoms obtained through standardized methods.

In a cross-sectional analysis of the baseline results, the exposed group tended to have subjective symptoms more frequently than in the control group, and similar group differences were observed in the frequency of abnormal values in the majority of the biomedical indices examined. In addition, the difference between the two groups reached a statistically significant level in several items, even after controlling for confounders. This tendency was basically consistent with what we had previously reported, with exposed workers complaining of symptoms more frequently than control workers despite having a comparable frequency of pulmonary function and chest radiography abnormalities. Although the presence of consistent difference between the groups might suggest the existence of certain subclinical changes, the cross-sectional evaluation at the baseline might have been influenced by a number of factors prior to the start of this study. Therefore, newly emerging abnormalities during follow-up should be examined in subjects without any abnormalities at the baseline observation, as far as the chronic health effects are concerned. One interesting finding of the cross-sectional analysis was the higher prevalence of CRP abnormalities, a sensitive marker

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of acute inflammatory status in the body, among the exposed subjects compared with control

subjects. It seems unlikely that the elevated CRP values were significantly influenced by

events in the past due to its nature as an acute inflammatory biomarker. In addition, the

difference seemed to be independent of inflammatory conditions caused by obesity or

smoking, as both were used as adjustment factors in the logistic regression model. These

findings suggest that acute inflammatory conditions might be associated with current toner

exposure. At the same time, an increase in the serum IgE level was also frequently found in

the exposed subjects. In Japan, an allergy to cedar pollen is commonly observed every spring,

so cedar pollenosis may be related to the findings for CRP and IgE. However, as there are

multiple factors affecting the CRP [28] as well as the IgE levels, we were unable to specify

the reason for that group difference.

In the longitudinal analyses of biomedical indices, we evaluated the frequency of a

newly emerging abnormality during the follow-up period by excluding the subjects from the

analysis if they already had the finding at baseline. Among the indices associated with lung

fibrotic changes, we found closely similar results between the exposed and control groups

regarding the new incidence of chest radiographic findings and serum biomarker

abnormalities. These results suggest that the fibrogenic potential of dust exposure associated

with toner-handling work seems to be minimal, if any at all, as far as the current working

environment is concerned.

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In contrast, the estimated ORs for FEV1/FVC abnormality adjusted for confounders showed a relatively a large value exceeding 1.5, although the increase was not statistically significant. A statistically significant increase in the OR of TPD versus the controls was observed in FVC and FEV1 abnormalities. However, the overall differences in the incidence of reduced FVC and FEV1 were relatively small between the exposed subjects and control subjects. Furthermore, the annual declines in the FVC/HT^2 and $FEV1/HT^2$ of the exposed subjects during the follow-up period were comparable to those of the control subjects after controlling for the age and smoking status during the follow-up. Thus, it seems difficult to attribute certain biological or medical reasons to the moderate, but a insignificant elevation in ORs that was seen regarding the pulmonary function index abnormality. Some fluctuation around normal ranges might be partially responsible for the observed elevation in OR values. In the case of chronic cough, the exposed group showed a larger incidence than the control group. This finding was not associated with the findings for chronic phlegm, which showed a comparable incidence after adjusting for the significant contribution of smoking. This suggests that the significant increase in the rate of chronic cough cannot be attributed to mucus hypersecretion. Given the findings at baseline mentioned earlier, it might be possible to hypothesize that non-specific irritation leading to subjective symptoms, fluctuation in the pulmonary function, and inflammatory response were caused to some extent by exposure associated with toner-handling work.

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However, of note: in all indices selected for the longitudinal incidence analyses, the OR value adjusted for confounders exceeded the unit value without exception, suggesting mostly non-significant but consistent elevation of the incidence in the exposed workers. Therefore, further epidemiological studies are needed in order to identify mild and nonspecific effects in a large-scale sample with sufficient statistical power. In this context, a similar tendency toward a consistent OR elevation was observed in TPD work, which was associated with the greatest exposure of total and respirable dust in the personal exposure measurement. In the comparison of the pulmonary function decline, the largest annual losses of FVC/HT^2 and $FEV1/HT^2$ were seen in the MTN category, where the degree of dust exposure was smaller than that for TPD. However, it was difficult to perform a detailed analysis by the work exposure categories with regard to the degree of toner exposures, as no information was obtained regarding the exact percentage of toner included in the total dust sampled. The toner particle exposure should be measured separately from other substances in future studies in order to enable detailed analyses of the dose-response relationship as well as to facilitate focusing on specific work categories, such as TPD and MTN.

Several limitations associated with the present study warrant mention. First, the number of subjects of this study (694 in total) might be insufficient to detect minimal and subclinical health deteriorations, although this study seems to have reasonably sufficient power to detect at least well-established health risks, such as aging, smoking, and obesity. A

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meta-analysis of the studies with a common methodological basis will be useful for detecting

minimal effects of toner dust. Second, we obtained measurements on personal exposure

concentration for each work category as an exposure assessment. However, the results

showed large variability. In addition, we were unable to obtain reliable data on the relative

content of toner as a fraction of the whole dust sample. We therefore cannot reliably estimate

the amount of personal exposure to toner dust for each individual subject. An analysis of the

dose-response relationship will require a more precise exposure assessment for toner dust.

Third, we simply utilized conventional chest radiography and did not use computed

tomography (CT) to evaluate lung fibrotic changes, because we should minimize the amount

of X-rays that the participants were exposed to in an epidemiological research setting. We

were therefore unable to distinguish the non-specific findings in cases where

extremely-early-phase of toner-related changes might have been included. Advanced

radiography techniques will be useful for the more precise evaluation of lung fibrotic

deterioration caused by toner dust inhalation.

Conclusion

Under the current reasonably controlled work environmental conditions, lung fibrotic changes caused by inhaled dust exposure, including powdered toner, appear to be relatively uncommon; however, nonspecific temporal irritation causing subjective symptoms and inflammatory responses might exist. Further epidemiological research will be useful for clarifying the possible health effects of toner dust exposure fully, especially the minimal and non-specific health changes in a study setting with a sufficient sample size to detect these changes.

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Figure legends

Fig. 1 The number of subjects analyzed based on the inclusion criteria of this study

Fig. 2. The exposure levels of toner-handling workers by year of measurement estimated

by personal exposure measurements. The values are presented as the median and the 10th

and 90th percentiles (%ile) for personal exposure measurement data.

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Table 1. Background characteristics of the subjects according to the exposure category.

Category of exposure condition

Characteristics	Control	Exposed[*]					p[†]
		Total	TPD	DDM	MTN	RCL	
Number surveyed	217	477	175	145	150	7	
Sex (M/F)	205/12	461/16	167/8	141/4	149/1	4/3	0.18
Age (years)	39.1(8.2)	34.1(8.1)	32.9(10.3)	35.4(6.5)	34.2(6.3)	32.4(7.6)	<0.0001
Height (cm)	170.1(6.6)	170.3(6.4)	169.0(6.1	171.3(6.4)	171.4(6.4)	161.5(6.8)	0.63
BMI (kg/m^2)	23.0(3.1)	23.0(3.7)	23.2(4.6)	22.4(2.6)	23.4(3.2)	19.9(1.5)	0.999
Smoking habit[‡]							0.026
Never	70(32)	150(31)	38(22)	69(48)	38(25)	5(71)	
Former	47(22)	67(14)	17(10)	26(18)	24(16)	0	
Current	100(46)	260(55)	120(68)	50(34)	88(59)	2(29)	
Personal exposure (mg/m^3)							-
Number of samples		2042	1751	81	88	122	
Total dust							

10th % ile	0.08	0.09	0.04	0.07	0.51
Median	0.49	0.50	0.08	0.17	1.46
90th % ile	2.38	2.14	0.38	0.80	5.60

Respirable dust

10th % ile	0.03	0.03	0.03	0.03	0.02
Median	0.05	0.09	0.03	0.03	0.04
90th % ile	0.36	0.35	0.04	0.15	0.64

* TPD: toner production, DDM: machine design and development, MTN: maintenance, RCL:

recycling, BMI: body mass index

†Probability under the hypothesis that there are no differences between the control group and

the exposed group as a whole.

‡Percentage of column total in parentheses.

Values are presented as the number of subjects for sex and smoking habit and the arithmetic

mean (standard deviations) for age, height, and BMI at the baseline survey.

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Table 2. The comparison of the baseline status in select biomedical indices at the point of the first examination for each subject between the exposed subjects as a whole and the controls

| Biomedical indices | Univariate analysis | | | Multivariate analysis | |
| | Prevalence (%) | | | Logistic regression model | |
	Exposed	Controls	p*	OR[†]	95%CI[†]
Respiratory symptoms[‡]					
Cough #1 (7A)	19.4	14.8	0.14	1.35	(0.85-2.14)
Cough #2 (7B)	14.8	11.1	0.19	1.41	(0.84-2.37)
Chronic cough (7E)	5.5	4.2	0.46	1.6	(0.70-3.64)
Phlegm #1 (8A)	23.7	20.4	0.34	1.23	(0.81-1.87)
Phlegm #2 (8B)	13.5	10.7	0.29	1.45	(0.85-2.48)
Chronic phlegm (8E)	8.9	4.2	0.03	2.62	(1.20-5.70)
Exacerbation (9A)	10.5	7	0.14	1.56	(0.84-2.91)
Wheeze #1 (10A1)	9.5	5.6	0.09	1.51	(0.76-2.99)
Wheeze #2[§] (10A2)	2.7	0	0.01	-	-
Chronic Wheeze (10A3)	0	0	-	-	-
Breathlessness #1 (13A)	9.7	11.6	0.45	1	(0.58-1.73)

Breathlessness #2[§] (13B)	2.2	1.4	0.76	2.27	(0.58-8.89)
Breathlessness #3[§] (13C)	0.4	0.9	0.59	0.96	(0.13-7.20)
Biomarker abnormality					
CRP	12.0	6.0	0.02	2.02	(1.05-3.88)
IgE	31.7	20.8	0.003	1.66	(1.12-2.48)
8OHdG[§]	1.1	1.8	0.47	0.68	(0.17-2.78)
KL6[§]	0.4	0	1	-	-
SPD	3.2	3.4	0.89	0.91	(0.35-2.37)
Reduced pulmonary function					
FVC	6.5	6.0	0.76	1.00	(0.49-2.00)
FEV1	6.1	2.8	0.06	2.09	(0.83-5.27)
FEV1/FVC[§]	2.1	5.1	0.03	0.44	(0.18-1.09)
Chest X-ray findings					
Fibrotic change ≥1/1	0	0	1	-	-
Non-specific findings	3.4	1.8	0.27	2.47	(0.78-7.81)

* Probability under the hypothesis that there are no differences between the control group and exposed group as a whole examined by the chi-squared test or Fisher's exact probability method.

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† Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the

control group, regarding the positive findings at the baseline survey

‡ The ATS-DLD-78A questionnaire code is shown in the parentheses

§ Fisher's exact probability method was applied.

Grade of symptom severity: cough #1 and phlegm #1, usually having the symptom; cough

#2 and phlegm #2, 4 to 6 times a day on 4 or more days a week; wheeze #1, when having a

cold; wheeze #2, occasionally apart from cold; breathlessness #1, when hurrying on the level

or walking up a slight hill; breathlessness #2, having to walk slower than people your age on

the level; breathlessness #3, having to stop for breath when walking at your own pace.

The figures in the column of prevalence represent the proportion of subjects with symptoms

or positive findings in each index. The results of multivariate analyses were adjusted for the

age and smoking status of the subjects. Body mass index was also used as an adjustment

factor in the case of CRP.

Table 3. A comparison of the cumulative frequency of incidence in select biomedical indices associated with lung fibrotic changes that newly emerged during the follow-up period

	Univariate analysis			Multivariate analysis			
	Incidence			Logistic regression model			
	Total			Total	TPD	DDM	MTN
	Exposed	Controls	p*	OR(95%CI)[†]	OR (95%CI)[†]	OR (95%CI)[†]	OR (95%CI)[†]
Respiratory symptoms[‡]							
Chronic cough (7E)	11.3	6.3	0.04	2.26(1.13-4.53)	1.88(0.82-4.31)	2.70(1.21-5.99)	2.31(1.01-5.25)
Chronic phlegm (8E)	14.0	9.6	0.12	1.6(0.91-2.83)	1.46(0.74-2.88)	1.65(0.81-3.37)	1.68(0.83-3.40)

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Breathlessness #2 (13B)	6.0	4.7	0.48	1.69(0.73-3.94)	1.51(0.54-4.19)	0.94(0.27-3.25)	2.62(1.00-6.87)
Serum biomarkers							
KL6§	2.1	1.8	1.00	1.27(0.38-4.29)	2.09(0.54-8.05)	0.44(0.05-4.09)	1.25(0.26-5.94)
SPD	2.4	2.4	1.00	1.06(0.34-3.28)	0.87(0.21-3.54)	1.62(0.40-6.48)	0.94(0.21-4.24)
Pulmonary function							
FVC	15.3	13.2	0.50	1.22(0.73-2.05)	2.60(1.44-4.71)	0.54(0.24-1.21)	0.84(0.42-1.68)
FEV1	11.4	8.5	0.26	1.38(0.76-2.49)	2.43(1.24-4.76)	0.68(0.27-1.69)	1.19(0.55-2.57)
FEV1/FVC	9.9	6.8	0.20	1.59(0.81-3.09)	1.88(0.87-4.09)	1.16(0.48-2.85)	1.72(0.77-3.85)
Chest X-ray							
Non-specific findings	5.6	5.2	0.8	1.39(0.65-2.97)	1.38(0.53-3.59)	1.75(0.70-4.37)	0.98(0.34-2.80)

TPD: toner production, DDM: machine design and development, MTN: maintenance, RCL: recycling

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* Probability under the hypothesis that there are no differences between the control group and exposed group as a whole examined by the chi-squared test or Fisher's exact probability method

† Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the control group, retarding the newly emerging abnormalities of biomedical indices

‡ The ATS-DLD-78A questionnaire code is shown in the parentheses.

§ Fisher's exact probability method was applied.

Grade of the severity of the symptom

The figures in the column of incidence represent the proportion of subjects starting to show symptoms or positive findings during the follow-up period among those who did not have the symptom or positive finding at baseline. The results of multivariate analyses were adjusted for the age and smoking status of the subjects.

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Table 4. Annual decline in spirometric indices according to exposure category.

Spirometric indices	Category of exposure condition (number of subjects)						Statistical test of the mean values†	
	Control	Exposed*						
		Total	TPD	DDM	MTN	RCL	p	
	(N=217)	(N=477)	(N=175)	(N=145)	(N=150)	(N=7)	Unadjusted	Adjusted‡
Δ FVC/HT^2 (ml/m^2)	-6.2(21.4)	-4.0(19.1)	-0.7(25.8)	-2.5(12.5)	-9.0(14.3)	-5.8(13.3)	0.19	0.50
Δ FEV1/HT^2 (ml/m^2)	-8.8(16.3)	-7.5(13.9)	-6.5(16.3)	-7.3(10.7)	-9.0(12.5)	-6.7(32.6)	0.33	0.30

* TPD: Toner production, DDM: Machine design and development, MTN: Maintenance, RCL: Recycling

† Comparison between the control and the exposed as a whole

‡ Adjusted for age, sex, and smoking habit at the baseline.

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Values are the arithmetic means and standard deviations at the point of baseline survey.

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Table 5. Independent contribution of confounding factors in the multivariate analyses of biomedical indices

	Smoking		Age		BMI
	Prevalence[*]	Incidence[†]	Prevalence[*]	Incidence[†]	Prevalence[*]
	OR(95% CI)[‡]	OR(95% CI)[‡]	OR(95%Cis)[‡]	OR(95% CI)[‡]	OR(95% CI)[‡]
Cough #1 (7A)[§]	1.68(1.12-2.51)				
Cough #2 (7B)[§]	1.62(1.03-2.54)				
Chronic cough (7E)[§]	3.34(1.49-7.48)		1.63(1.08-2.45)		
Phlegm #1 (8A)[§]	2.38(1.63-3.47)				
Phlegm #2 (8B)[§]	2.62(1.60-4.29)		1.38(1.05-1.82)		
Chronic phlegm (8C)[§]	3.69(1.85-7.35)	1.80(1.10-2.96)	1.53(1.08-2.16)		

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Breathlessness #1 (13A)[§]   1.59(1.17-2.16)

Breathlessness #2†(13B)[§]   2.64(1.36-5.12)

Breathlessness #3†(13C)[§]   5.62(1.51-20.85)

CRP abnormality   1.32(1.17-1.49)

5   SPD abnormality   4.10(1.15-14.58)

Reduced FVC   3.13(1.09-8.97)

Reduced FEV1   3.82(1.38-10.58)

Reduced FEV1/FVC   3.92(1.20-12.87)2.11(1.27-3.52)

Non-specific X-ray   1.59(1.06-2.38)

10   findings

* An analysis of the prevalence at baseline.

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† An analysis of the new incidence during the follow-up observation

‡Odds ratio (OR) and its 95% confidence intervals (95% CI) of the exposed group to the control group, regarding the prevalence and incidence

of biomedical indices.

§ The ATS-DLD-78A questionnaire code is shown in the parentheses.

5   # Grade of the severity of the symptom

Values are presented as the OR (95% CI) estimated using the logistic regression models shown in Tables 3 and 4. Only statistically significant

results are shown.

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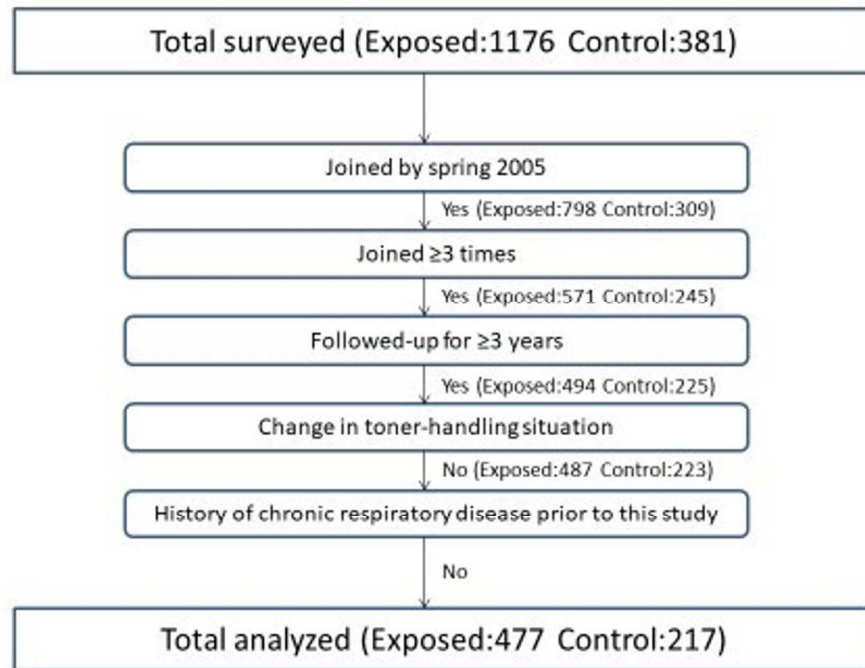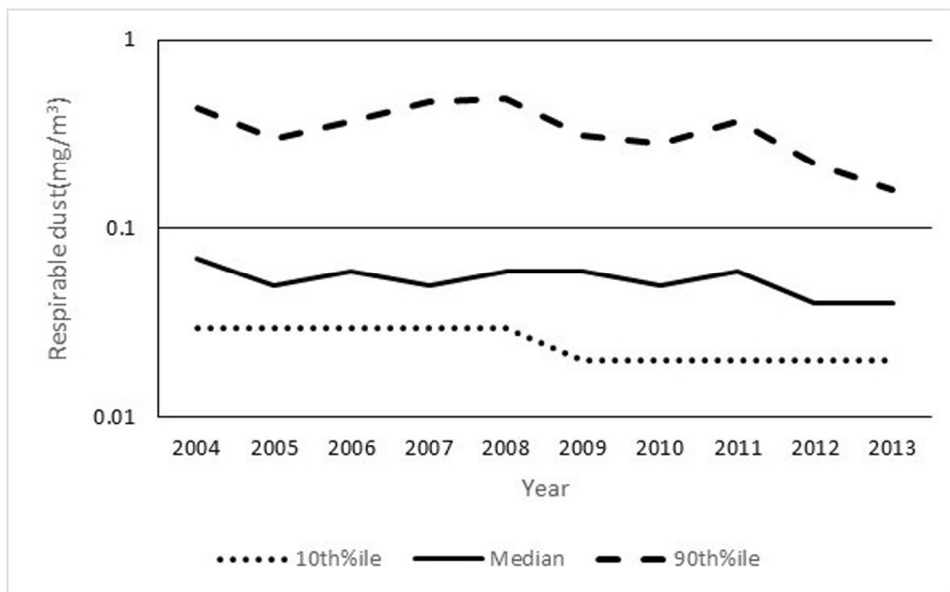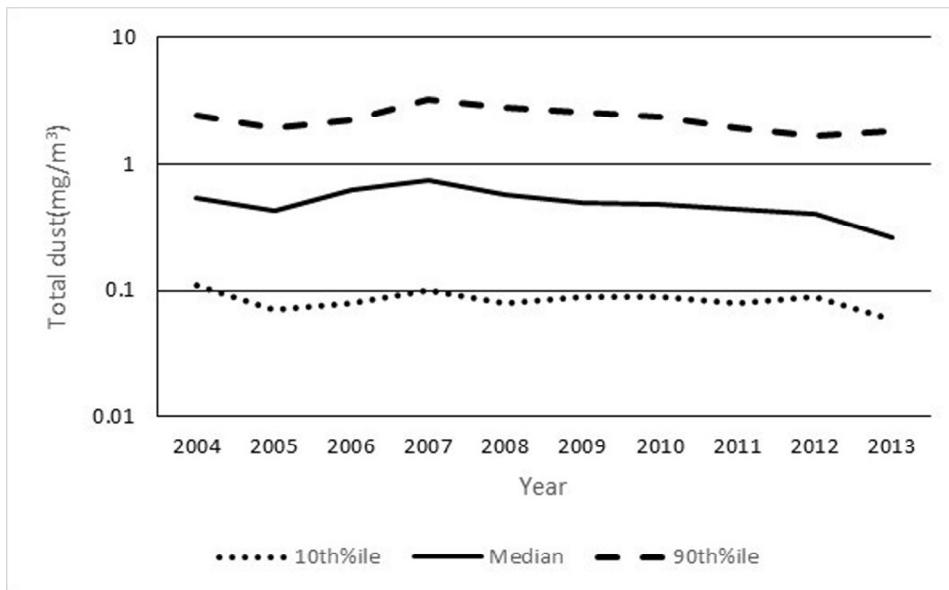

Fig. 1 The number of subjects analyzed based on the inclusion criteria of this study

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6-7
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8-10
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10-15
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*10-15*
Bias	9	Describe any efforts to address potential sources of bias	9, 10-13
Study size	10	Explain how the study size was arrived at	8-9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	15-17
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	17
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	8-9
		(*e*) Describe any sensitivity analyses	22-23, 48-50
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8-9, 19
		(b) Give reasons for non-participation at each stage	8-9, 19
		(c) Consider use of a flow diagram	51(Fib.1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	19-20, 38-39, 52(Fig.2)
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	19
Outcome data	15*	Report numbers of outcome events or summary measures over time	21-22, 43-47
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	21-22, 43-47
		(*b*) Report category boundaries when continuous variables were categorized	12, 14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	22-23, 43-45, 48-50
Discussion			
Key results	18	Summarise key results with reference to study objectives	27, 31
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	29-30
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	32

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.